Cork Taint in Wine: Scientific Knowledge and Public Perception — A Critical Review

C. Silva Pereira,1 J. J. Figueiredo Marques,1,2 and M. V. San Romão1,3*  

1Instituto de Biologia Experimental e Tecnológica/Instituto de Tecnologia Química e Biológica-Universidade Nova de Lisboa, Apt. 12, 2781-901, Oeiras, Portugal;  
2Estação Agronómica Nacional, 2784-505 Oeiras, Portugal; 3Estação Vitivinícola Nacional, 2565-191 oOis Portos, Portugal

ABSTRACT: The manufacturing process of cork stoppers includes a stabilization period of the cork slabs, following boiling, during which mold growth completely covers the cork slabs. This process has been used traditionally for several decades; however, due to the possibility of certain molds isolated from cork to produce off flavor compounds, especially 2,4,6-trichloroanisole and 2,3,4,6-tetrachloroanisole, recently cork stoppers are being unsoundly targeted with the accusation of inducing cork taint in wine. This article reviews the manufacturing process of cork stoppers, the diversity of microorganisms associated with cork, and finally the diversity and origins of the compounds associated with cork taint in wine, focusing on those currently considered as more important. Some important results recently obtained by the authors are also included. The current idea of suppressing mold growth during cork stopper manufacturing is discussed, as well as the erroneous idea of imputing, directly and exclusively, to cork the responsibility of the so-called cork taint in wine.

KEY WORDS: cork taint, molds, chemical compounds, chloroanisol.

I. INTRODUCTION

Portugal is the major world producer of cork, and the industrial manufacturing of cork stoppers has a significant impact on the Portuguese economy. The recent introduction within the market of synthetic stoppers, which may interfere with wine quality, is endangering the Portuguese cork stopper producers, even if some1 sustain that cork stoppers cannot be replaced in its use of sealing quality wine during conservation and aging.

The occurrence of taint in bottled wine, causing unpleasant alterations in wine flavor or aroma, is responsible for economical losses in the wine industry. The most important off-flavor is the so-called cork-taint, clearly distinguished by the contamination of wine with a musty/moldy aroma, and reported to be affecting 0.5 to 6% of total bottled wines,2 and 3 to 4% of French bottled wines.3 This problem has earned importance and become a public concern in recent years, due to advertising and commercial forces. The economical interests involved create difficulties to the research in this matter, because public opinion is exposed to disperse and unclear information published by the media.

The true cork-taint is rare and easily recognized by the wine specialists.4-6 Riboulet, after analyzing a great number of works deal-
ing with cork-taint, attend to the ambiguous meaning of this expression, but in the subsequent years the designation cork taint continued to be used indiscriminately. Recently, the research team of the EC project QUERCUS defined a methodology to be used systematically in wine sensory analysis in order to avoid confuse interpretations leading to the classification of diverse taints with the unique name of cork-taint and to discriminate sensory alterations with distinct origins. A broad set of aromas was defined and presented as a pie chart. It includes the five family of taints that can be originated by the cork stopper and detected in wine: vegetal, conifer, moldy, moss, and chemical. This allows the exclusion of untrue cork taint and constitutes a useful common ground for classification.

II. THE “CORK TAINT” IN WINE

Several authors considered cork the major origin of the cork taint problem. In their review, Rapp et al. consider two main theories as to how these flavors may arise: by reaction of the chemical compounds generated during manufacturing of cork stoppers (lignin sulfonic acids) with some compounds present in wine, or as metabolites produced during the growth of microorganism on cork. This strategy of discussing the problem of cork taint formation, although it is based on acceptable hypothesis, seems unsound. The cork manufacture industry is suffering so from the accusation that it uses a manufacturing process that endangers the quality of cork, resulting in the production of cork taint in bottled wines. The manufacturing process is based on traditional methods of processing dependent on experiential knowledge, and the research on this subject is still unable to explain with precision all the implications of each step. The different types of natural cork stoppers are not considered separately when its quality is being put in question. The manufacture process of agglomerate cork stoppers should be considered apart because its manufacture generally uses cork of reduced quality, gums, and other chemical compounds.

In order to clarify this problem, a critical analysis of the manufacturing process of cork stopper, of the diversity of microorganism associated with cork, as conditioned by the industrial process and factory environment, and finally the diversity of compounds associated with cork taint is necessary, focusing on those currently considered as more important.

During the manufacturing process, and especially in the maturation step after cork boiling, the microbial growth on cork slabs is obvious, with molds completely covering the maturing cork slabs. Studies of the microbial diversity associated with the various steps of stoppers manufacturing, performed in different industrial installations, show that the different molds appearing in the process cannot be only associated with cork-borne microorganism being strongly influenced by installation design and maturation period (unpublished results). Moreau also suggested this hypothesis in similarity to what was observed by Pelhate in his study concerning the contamination of cereals during storage. This mechanism was also pointed to as an explanation for the observed colonization in other traditional industries involving microbial activity, as in the case of wine production. Cork is not an inert biological material, and its possible contribution to the aromatic character of wine was analyzed by Boidron et al., who extracted from cork more than 70 different volatile compounds. These authors have suggested that a healthy cork transfers to wine compounds that favor its complex aromatic aroma.
III. OFF-FLAVOR COMPOUNDS

The first report about the presence of a taint in bottled wine is thought to be from 1904. During the decade of 1970s, the works of Gerber, concerning *Actinomyces*, and Kaminski et al., concerning *Aspergillus* and *Penicillium* sp., have proven the ability of these microorganisms to produce volatile compounds with a moldy odor, which could impart in wine sensory alterations. A wide variety of odor compounds are common products of mold or bacteria metabolisms. Some microorganisms have the capability to perform chemical breakdown of lignin, producing guaiacol that when present in wine it can produce a flavor modification by combination with other compounds. It was suggested that the odor modification induced by guaiacol alone is not the characteristic cork taint. Riboulet and Lefebvre et al., reported the importance of *Streptomyces* sp. in the production of this compound. Other possible metabolites of molds produced during degradation of lipids are 1-octeno-3-ona and 1-octeno-3-ol. Lee and Simpson reported that both compounds can induce a mushroom-like flavor in wine. Geosmin and 2-methylisoborneol (2-MIB) were reported to cause cork taint in water supplies, and other aquatic and marine foods. These compounds have been identified as metabolites of *Actinomycetes* (in particular *Streptomyces*) and *Cyanobacteria*. Contamination of cork with these compounds may occur from contact between the cork and the contaminated water or soil. Some molds isolated from cork, an *Aspergillus* sp., a *Rizoctonia* sp, and several *Penicillium* spp. and *Trichoderma* spp., can produce geosmin and 2-MIB aromas.

A. Chlороanisoles

Several authors consider chlороanisoles as the main responsible cause for the occurrence of cork taint in wine. Buser et al. were the first authors to report the correlation of cork taint in wine with the presence of 2,4,6-trichloroanisole (TCA), and suggested that the detected TCA could possibly arise from the chlorination of lignin degradation products during chlorine bleaching of corks. Châttonet (1994) confirmed the results of Buser et al. correlating the more intense odor in wines affected by cork taint with the presence of TCA and 2,3,4,6-tetrachloroanisole (TeCA).

Nowadays, the term “cork taint” is somehow automatically confused with the presence of the compound TCA, which is the main responsible factor for cork taint in wine, although not exclusively so. Recently, Ribéreau-Gayon et al. suggested that the designation “cork taint”, associated with TCA presence, should be replaced by “moldy taint”. However, this suggestion is still not being used in literature. In the opinion of Ribéreau-Gayon et al. the true cork taint is extremely rare. It corresponds to a very unpleasant putrid odor, still of unknown origin, which should not be confounded with the musty/moldy odor of TCA. This putrid odor can be produced during the growth of *Armillaria mellea*, normally designated by “yellow spot”, that sometimes infects the bottom of oak trees, namely, in wet environments. This defect in cork is easily identified, and so the affected cork is currently not used for the manufacture of cork stoppers.

The correlation of cork taint with a single molecule in a complex matrix like wine is impossible, because several compounds can be contributing to the same effect, sometimes in a synergetic way, and the contribution of each compound to the resulting defect is impossible to define. The chlороanisoles possess very low odor thresholds in wine, but the concentration to determine a defect is affected by the wine characteristics and composition. The perception levels for...
chloroanisoles is 0.03 ng/l, and 4 ng/l for TCA and TeCA, respectively. However, the concentration considered necessary to produce a defect in wine is higher than these values, and an average value can be defined as 10 and 25 ng/l for TCA and TeCA, respectively (Riboulet, personal communication).

The contamination of food and related products with TCA is well reported in the literature. This compound was already reported to affect chickens, dried fruits, and Brazilian coffee. In the case of the musty taint in dried fruits contaminated during ship transportation, the taint was traced to the presence of TCA and TeCA. The package material (cardboard) was made of carton manufactured from fibreboard with high content of recycled wastepaper that contained relatively high levels of chlorophenolic compounds. TCA and TeCA were produced from chlorophenols due to microbial activity, which was favored during the oscillation of humidity and temperature. Simpson and Lee used a similar explanation for the chloroanisole contamination of Champagne cork stoppers during its transport to Australia. It was assumed that the shipping floors had been treated with a commercial wood preservative containing 2,4,6-trichlorophenol (TCP) as its active ingredient, and that microbial activity within the treated zones led to TCA formation that was absorbed by cork stoppers. After sealing the bottles the TCA diffused into wine, inducing the cork taint defect. These two examples clearly prove that the production of TCA can occur without cork involvement. The molds detected were probably part of the resident molds of the ships, so the microorganisms responsible for the conversion of chlorophenolic compounds to the correspondent chloroanisole had no relation with cork microbiota. In the opinion of Simpson and Lee, the cargo hold of a ship is a potential source for microbial and chemical contamination. This same kind of event has already been reported in the winery environment. Some cellars were found to have the atmosphere contaminated with high concentrations of chlorophenolic and chloroanisol compounds, which have induced cork taint even in wine conserved in wood barrels that have never contacted cork. The microbiota found in this case was the one resident in the cellar environment with Penicilliums sp. as the most frequent genus. Penicillium roqueforti, which is normally considered epidemic in the winery environment, was the dominant mold species. This is not surprising, because the Penicillium species are equally spread in all ecosystems and have been reported to contaminate several different types of materials, possessing metabolic capabilities that allow them to degrade very complex substrates as cellulose, lignin, and suberin.

The knowledge that cork taint arising in wine can have very different origins, completely apart from cork, have led to the idea that the type of chloroanisole present in the spoiled wine could define the origin of such a compound. These authors proposed that TCA and TeCA detected in wine was due to the cork stopper manufacturing process and wine industry, respectively. However, this proposal does not consider all possible origins for TCA production. The ability of producing chloroanisoles as a detoxification process, when in the presence of sublethal concentrations of chlorophenolic compounds, seems to be an ordinary biochemistry event in molds, and soil microorganism, although with marked differences in their methylation ability. Also, lethal concentrations of chlorophenolic compounds differ greatly for different mold species. The degradation of chlorinated aromatic compounds is a very interesting subject for study, in bacteria and molds, due to the ecological importance of finding potential microorganisms to use in decontamination programs.
B. Chlorophenols

The chlorophenolic compounds were used industrially as fungicides, biocides, and herbicides intermediates, especially pentachlorophenol (PCP), which was the principal chlorophenol used as biocide. PCP possesses significant toxicological proprieties and some authors have reported its use to control insect pest in the bottom of cork oak trees. Fortunately, in 1991 a directive from the European Community has severely restricted the use of PCP and related chlorophenols, and in the U.S. PCP is also now used rarely as fungicide or biocide. Nevertheless, some authors reported high levels of persistence of this class of compounds in environment (soil, ground water, etc.), constituting a priority preoccupation in the U.S. Some work also suggested the importance of the production of chlorinated aromatic compounds by wood degrading fungi, but it is still not clear if the production levels in natural environment is significant.

The level of chloroanisoles and chlorophenols in the Portuguese Oak Forest is not known, but Rigaud et al. has shown that it is possible to detect TCA, TeCA, pentachloroanisole (PCA), and the related chlorophenols in cork slabs collected from Portuguese Oak tree forests before stopper manufacture. These authors correlated the appearance of such compounds to the presence of PCP. Duncan et al. analyzing cork samples collected from Portuguese Oak Forest, observed that TCA could only be detected in 1 to 2% of the analyzed samples, and that the concentrations found were far below the perception levels for TCA in wine. The detected TCA was particularly concentrated in the bottom of the tree, which traditionally is not used to produce cork stoppers. These authors also reported that the boiling process reduces the concentration of the detected TCA to more than half. This result can support the opinion of some researchers that advise the frequent change of the water used to boil cork slabs. Rigaud et al. also reported that the incidence of TCA and PCA in cork slabs is concentrated predominantly in the external part and that the inner parts, used to punch cork stoppers, presented lower levels of such compounds.

IV. CORK STOPPERS MANUFACTURE

The traditional manufacturing process of cork stoppers has suffered several modifications introduced with the goal of obtaining a more uniform end product and increasing the efficiency of the production. The implications of such alterations in cork quality are still not clearly understood. Only hypothetically can it be considered that these modifications are contributing to an increase in the percentage of spoiled wines. The duration of field stabilization, traditionally 1 year, has been reduced recently to 6 months, or even less. Riboulet evoked the importance of physical stabilisation in the forest, as this period can allow the cleaning of off-flavor compounds due to rain washing. The influence of this change in cork quality is unknown, and information about cork stabilization and general field conditions is missing. This subject should be of priority importance to both researchers and industrialists.

The traditional manufacturing process includes several treatments in cork stoppers, which can be summarized as: dimensional correction, automated choice by classes of quality, washing and bleaching, drying, and the final treatments (waterproofing and labeling). All these steps are within well-defined conditions, which guaranties their contribution to the formation of cork taint should be negligible. However, the complexity of cork taint origins is increased if it is considered that the possible formation of
chlorophenolic compounds due to chlorination of lignin degradation products, produced during growth on cork, is involved. Traditionally, chlorine was used at the washing and bleaching step. Zehnder et al. reported that the incidence of TCP in cork stoppers treated with chlorine was higher than in those treated with peroxide, but Amon et al. proved that in the absence of microbial growth, the TCA concentration was similar to that in untreated corks. Nowadays almost all producers have replaced chlorine treatment by hydrogen peroxide treatment. However, it is possible that few small producers, who are less informed, can continue with the old processing, but the use of peroxides is strongly recommended in the “Code International des Pratiques Bouchonnieres”.

Lignin degradation products can also appear in wood, which if it undergoes chlorination can produce TCP, for example, in wood barrels used in wine conservation. Saxby reported that wood treated with 5% sodium hypochlorite resulted in chloroanisoles formation at µg/g level. Both producers and users of cork stoppers must realize the importance of using waters not treated with chlorine.

Simpson and Lee established a possible explanation for the origin of chlorophenolic compounds detected during off-flavor analysis of food and related products, and suggested that compounds containing three chloride atoms, or less, are typical products of phenol reaction with chloride. On the other hand, chlorophenolic compounds with more than three chloride atoms probably appear due to the use of PCP-related pesticides. Microbial degradation of PCP by soil bacteria yields a big range of chlorophenolic compounds, rarely with less than 3 chloride atoms. PCP can also undergo photochemical reduction to TeCA and 2,3,5,6-tetrachlorophenol. The concept proposed by Simpson and Lee should be used carefully because information about mold metabolism of PCP is missing. It is obvious that the methylation to chloroanisoles is not the sole pathway for PCP degradation, and the production of intermediate compounds with less chloride atoms could occur, due to reductive dechlorination.

Both chloroanisoles and chlorophenols can induce off-flavors in wine, although with such big differences in their perception level (TCA and TCP it is 0.03 ng/l and 2 µg/l, respectively) those chlorophenolic compounds can rarely be considered directly responsible for cork taint. Only the microbiological methylation of chlorophenol to chloroanisole produces a compound with a lower perception level capable of inducing cork taint. It is necessary to review the diversity of microorganisms that can appear associated with cork during the manufacturing of cork stoppers, and, following this, it is important to clarify which microorganisms within this microbiota show the ability to produce chloroanisoles.

V. MICROBIAL DIVERSITY ASSOCIATED WITH CORK

The microflora associated with cork during its manufacturing process, despite the number of published studies concerning this issue, is difficult to define. Published work differs in the cork sampling methods, in the methodology used for isolation of the microorganisms, and in the conditions used for sample transport that are rarely reported. Only little work exists that is concerned with the sampling analysis of the industrial environment. Lacey determined the following as the principal molds (found in 75% of air samples) Penicillium frequentans (P. glabrum), Penicillium granulatum (both species were widespread inside the industry compartments), Mucor plumbeus, and Monilia sitophila (Chrysonilia sitophila), which were less abundant and restricted to
the compartments where physical stabilization (maturation) after reboiling was carried out. Danesh et al.\textsuperscript{58} isolated \textit{C. sitophila}, \textit{P. glabrum}, \textit{M. plumbeus}, and \textit{Trichoderma longibrachiatum}, with the dominance of \textit{C. sitophila} in all the different processing areas, especially in the cork slabs maturation room. Recently, in some similar studies the dominant presence of a \textit{Penicillium} sp. (90\% was found), corresponding the remaining 10\% to an \textit{Aspergillus} sp.\textsuperscript{59} The presence of \textit{Aspergillus} that was considered by Moreau\textsuperscript{60,61} as a pioneer species in cork is not confirmed in all the following work.

The microbial diversity on cork is not static or constant along time. Instead, it results in different successions of species with different origins and ecologies.\textsuperscript{62} Pereira et al.\textsuperscript{63,64} evaluated the diversity of mold species associated with the different steps in the manufacturing process within two cork stoppers factories and observed the presence of \textit{C. sitophila} as the dominant mold covering cork slabs during the maturation period. Among the other isolated mold species, the most frequently found were penicilia. The observed presence of a particular species in each factory installation suggested that the environment surrounding cork in those spaces is a determinant factor for cork recolonization.

Other possible origins of microorganisms found to be associated with cork is the result of further recolonization during cork stopper transportation, or even within the winery environment. In fresh harvested cork, Simpson and Lee\textsuperscript{20} found \textit{Penicillium} sp., which dominated among the molds, followed by \textit{Trichoderma} and \textit{Acremonium}. The same authors reported the dominant presence of \textit{Penicillium} sp., \textit{Trichoderma}, \textit{Cladosporium}, and \textit{Rhizoctonia} on cork stoppers imported from Australia, and pointed out that most of these molds have strong to moderate growth at 10 to 20\°C, as well as at low water activity (aw). The almost universal presence of \textit{Penicillium} sp. in imported corks has been reported.\textsuperscript{13,62,65} On the other hand, the lack of work reporting the presence of \textit{C. sitophila} suggests that the origin of the isolate species can be distinct from cork and may well be other than the stopper factory.

Moreau\textsuperscript{9} and Lefebvre\textsuperscript{13} reported the presence of substantial mycelium growth inside cork lenticels and suggested that its development could be encouraged during an increase in the humidity levels on corks. It seems that only some mold species within the ones found in association with cork during its manufacture can retain cellular viability inside cork structure. Danesh et al.\textsuperscript{58} have slowly increased the humidity level of finished cork stoppers through the injection of a humid atmosphere until it reaches an adequate level for fungal development, but no microbial growth was observed. Using microbiological studies on cork samples throughout the different steps of cork stoppers manufacture (not boiled cork, boiled cork, not treated, treated, and finished cork stoppers), it was concluded that after the first treatments applied to cork stoppers the detectable number of fungi had decreased to zero.\textsuperscript{59} The germination of dormant molds inside cork lenticels of a cork stopper sealing a bottle of wine is an unlikely event. Almost all the mold species already detected in cork are aerobic species, and so acidity and oxygen limitation inside a bottle of wine are very unfavourable conditions for their growth.\textsuperscript{9,66,67} Nevertheless, Jäger\textsuperscript{68} using scanning electronic microscopy (SEM) analysis on a cork stopper, collected from a 61-year-old bottle, observed that the hiphæ had penetrated 70\% of the cork, without reaching the inside surface facing the wine. Inside the inner cavities of cork tissues, these authors found mostly, bacteria and yeast in the lenticels, protected within a polysaccharide layer, and only few hyphae, which had collapsed and that failed to present any evidence of physiological activity. The cork stoppers are deliberately punched at right
angles to the growth of the cork tree, so that all lenticels are oriented perpendicularly to the bottled wine. This strategy maximizes the sealing proprieties and decreases the chance of internal mycelia growth to reach the wine surface. On the other hand, mold growth on the surface of a finished stopper was reported to be unlikely, because the type of chemicals used to waterproof cork stoppers reduce the contact of molds with the available cork substrate.

VI. ROLE OF MOLD GROWTH ON THE QUALITY OF CORK

The role of mold development in the physical and chemical proprieties of cork is unknown. It was suggested that molds could have a beneficial effect, resulting in the softening of the cork material, the washing of the cut surfaces, and the collapsing of the internal lenticular channels. This hypothesis is in accordance with the beliefs of the traditional producers, however, it was observed, using SEM, that the depth of the mold attack growing on cork, after long periods from 2 to 8 months, was only around 8 to 15 cellular levels. Once cork stoppers are made cutting cylinders in the middle zone of cork strips, it was suggested that mold development on cork does not reach the cork zones used to punch off stoppers.

VII. METABOLIC PRODUCTION OF CHLOROANISOLES

The responsibility of cork taint appearance in wine cannot be attributed to all the molds associated with cork during stopper manufacturing. Only those with the metabolic ability to produce chloroanisoles, especially TCA, can be blamed. Lefebvre et al. and Rigaud et al. reported the contribution of cork microorganism to the arising of off-flavor compounds in wine. The first authors reported that the characteristic cork taint in wine was induced by A. versicolor growth, and suggested that the contribution of P. glabrum to the defect could not have a privileged position, because this mold was consistently associated with their cork samples. Simpson and Lee reported that cork stoppers contaminated with P. glabrum, P. granulatum, and Trichoderma sp. could impart typical cork taint to wine. Danesh et al. (1997) suggested that the role of C. sitophila to cork taint should be minimal, due to its systematic presence in the manufacturing process.

Silva Pereira et al. reported that some molds, which were isolated from maturing cork slabs, possess low potential for chloroanisole production, with reduced efficiency, notable in the case of C. sitophila. Under the experimental conditions used, C. sitophila presented efficiency for TCP methylation of only 0.03%.

The mold ability to produce chloroanisoles by direct methylation of the correspondent chlorophenol is reported to have no direct correlation with the amount of TCP degraded. Simpson and Lee reported that the tested molds, isolated from cork, metabolize 47 to 100% of TCP present, but TCA production was around 0 to 23%. Silva Pereira et al. tested nine different molds, which were isolated from maturing cork slabs. The TCP degradation levels were comprised from 6 to 97%, but the yield of TCA production only varied from 0.03% to 9.5%. These authors also reported that no direct correlation exists between the percentage of TCP degraded and yield of TCA production. C. sitophila has degraded more than 80% of the available TCP, but the yield of TCA production was 0.03%, in opposition to a related Penicillium sp. that gave the lowest TCP level of degradation, but was the mold that more efficiently methylated TCP to TCA. These results are in agreement with previous
observations, as *C. sitophila* was also not able to produce cork taint compounds (TCA, guaiacol, and 1-octeno-3-ol) during its growth on cork. 37,73,77 This mold also inhibited the growth of other colonizing species on maturing cork slabs during a period up to 30 days. 37

In addition to the microbial production of TCA by direct methylation of TCP, Maujean et al. 24 described the ability of some molds to produce TCA, as a microbial detoxification process to minimize the amount of chloride present in their surrounding. These authors isolated a *Penicillium* sp. from a Champagne cork stopper and from a polluted cellar, which shows the ability to biosynthesize the phenolic nucleus using the pathway of pentoses leading to shikimic acid. The phenolic nucleus evolves to TCP through a chlorination process, which *in situ* undergoes an esterification, assisted by folic acid and methionin (methionin can arise from the casein use to assemble champagne stoppers), producing TCA.

Jäger et al. 68 mentioned the role of some yeasts isolated from granulated cork stoppers that were able to produce TCA in a liquid medium of diluted wine supplemented with TCP. These authors concluded that this result in wine media did not prove that these yeasts could play an important role in the appearance of cork taint in wine after bottling. However, further research is justified, because the isolated yeasts also grow in medium of wine (nondiluted) and pulverized cork. The level of TCA production could be questionable, because only one defined time of growth (3 weeks) was tested. Nevertheless, the results of Armenante et al. 42 suggested that TCP degradation is not detected after prolonged incubation times under depletion of nutrients.

Comparing the relevance of molds, yeasts and bacteria on the occurrence of cork taint in wine, especially during the cork stopper manufacture, it seems obvious that the last ones lose importance. Moreover, the microbiological control of cork stoppers is well defined by quality rules, 74 and using these procedures to define the microbial diversity inside cork industry it appears clear that the community of yeasts and bacteria is of minor importance. 62

The interrelationship between microbial enzymes, endogenous enzymes, and off-flavor can be complex. 75 The interrelationship between all the possible enzymatic pathways for off-flavor production by all the individuals within a microbial population growing on cork, in any step of its manufacture or use, is far from being completely analyzed.

**VIII. QUALITY OF THE FINISHED CORK STOPPERS**

The microorganisms growing on cork appear as a succession of different populations throughout time. At each time a particular population is favored in opposition to another, partially because of the endogenous characteristics of cork but also because of the influence of environmental conditions, such as the available inoculum and the humidity and temperature levels. 9,62

The current legislation imposes the control of the cultivable microbial numbers on finished cork stoppers. 74 To perform the analysis, the cork sample is submerged in a liquid extraction medium. This medium, after shaking overnight, is used to inoculate a rich, nonselective, solid medium. The result obtained is an indicator of the microbial populations on cork stoppers, within favorable conditions of microbial development, but it refers only to the superficial contamination. Nevertheless, this information should be considered enough, as several works reported the improbability of further microbial growth on a finished cork stopper, 59,70 especially after contact with wine in its use of sealing wine bottles. 9,66-68
IX. STORAGE AND USE OF CORK STOPPERS

During the storage of cork stoppers it is strongly advisable to use conditions that ensure that the humidity levels of packaged cork are kept between 6 to 9%, therefore eliminating the possibility of microbiological activity. Some authors suggested that packaging should be performed under an atmosphere of SO₂, a gas that inhibits the growth of the microorganisms generally found in association with cork during its manufacturing process.

The quality of the packing materials has been studied, and it was reported that some permeable package materials allow the diffusion of volatile compounds, as TCA, that can be absorbed by the packaged cork. It is clear that the storage conditions for finished cork stoppers, until their use, are crucial.

Chatonnet et al. found chloroanisoles compounds, in wines conserved in wood barrels, which have never been in contact with a cork stopper. Inside the wineries the atmospheric contamination with chloroanisoles had occurred during microbial conversion of chlorophenols, which enter the composition of some paints used in the walls of the cellar, and also in some wood preservatives used to clean the wood barrels. Bertrand and Barrios also found cellars highly polluted with wood preservative compounds formulated with PCP. The analyzed cork stoppers presented high levels of contamination with PCP and PCA, concentrated especially in the cork zones facing the exterior of the bottle.

Cork is a biological material with a specific cellular composition and an abundant proportion of lipids, which makes it especially sensitive to contamination with volatile compounds, such as the TCA and TCP present in the surrounding atmosphere. However, the ability of cork to absorb these compounds is also dependent on their volatility, which is strongly influenced by temperature and humidity level.

If hypothetically considered that the cork stopper is contaminated with TCA, its presence in the cork stopper is not the only relevant factor for the appearance of cork taint in wine. The extraction ability of wine, which is especially dependent on its aromatic composition, will strongly influence the concentration of TCA that will be transferred from the cork to wine. Tanner and Zanier reported that after bottling, and during aging and the conservation of wine, 50% of the TCA present in a cork stopper could move into wine. However, Chatonnet et al. only found in wine less than 6% of the TCA concentration found in the contaminated cork stopper. The published works concerning the possible contamination of cork and cork stoppers by absorption of chloroanisoles are incomplete, because the total complexity of the system under analysis was not integrally reproduced. The parameters that influence the kinetic of TCA transference from cork to wine are very complex and difficult to reproduce in laboratory.

X. CONCLUSION

It is clear from this review that the contamination of cork stoppers or wine by TCA and other chloroanisoles can have several origins. Using good manufacturing practices, with an accurate control of cork during the processing steps, and storing cork stoppers with impermeable packaging, in conditions of low humidity, contamination with TCA should be improbable. After these guarantees, the TCA detected in wines will not be a responsibility of the cork producers. The users of cork stoppers should be unquestionably blamed for this contamination, occurring probably because of their deficient procedures of transportation, storage, and handling. The main difficulty in the separa-
tion of true cork taint from other taints is that several off-flavors in wine have organoleptic characteristics similar to cork taint, but their microbiological and metabolic origin is different and cork per se have not interfered in those processes.

Traditionally, cork slabs were considered to be of good quality for stopper manufacturing when they were completely covered with white or salmon molds, which corresponds reasonably well to the macroscopic aspect of Chrysonilia sitophila growth on cork. Some of the facts presented in this article have induced producers to think that mold development should be suppressed during the manufacturing process, but there is no scientific support to justify this tendency. For the producers of cork stoppers, and due to the possibility of microbial production of off-flavors, the critical phase within the cork stoppers manufacture is the maturing step of cork slabs. It is important for the cork industry to establish strict rules of process separation, especially in the compartment where the maturing stage of slabs takes place, which highly influence the microbial population that will establish as resident in the industrial space. It is advisable to separate the maturation compartments from the area where the cork slabs are normally cut and chosen before stopper manufacture, and to use the adequate sanitary conditions, which reduce spore dispersion. These procedures will reduce the diversity of species growing on boiled cork slabs during the “maturing step”. Visual control of the levels of mold development over the maturing slabs is also feasible because the macroscopic aspect of C. sitophila is easily recognized and distinguishable from other contaminant molds that endanger cork quality. Moreover, C. sitophila was proven to have the capability to restrict, at least during a period of 30 days, the growth of other mold species, which can compromise cork stopper quality. These authors suggested that the controlled growth of C. sitophila could be exploited by the industry to establish a “clean” process of stopper manufacturing. However, C. sitophila dominance inside the industry space and on maturing cork slabs is endangered when prolonged maturation periods are used, what can be attributed to the reduction of the humidity in cork slabs that favors better adapted mold species.

According to Land “the most sensitive few people are about 2000 times more sensitive that the least sensitive few”. This explains the different responses between people to a certain level of cork taint in wine, but allows a criticism to part of the published works that used sensory analysis to evaluate cork taint. The threshold levels for off-flavor compounds, established during expert sensory analysis, seem to be far above the threshold level for a normal consumer. The publicity around this subject is being partially controlled by economical forces, and the accusations, that question cork quality seem to be related to the endeavor of introducing an artificial cork stopper into the market.

The subject of cork taint involves microbiologists, chemists, ecologists, biochemists, industrials, and consumers. It is obvious from this review that the flow of information between all these different areas is not occurring. Some important works in a specific field described that molds colored grey and green cover maturing cork slabs. This seems to be in contradiction to the results reported in the work performed inside the cork industries. Other contradictory examples could be quoted, like the works of Lee and Simpson, who blame almost exclusively cork production for the responsibility of cork taint’s appearance in wine, suggesting that mold growth should be suppressed during cork stopper manufacturing. The recommendation of these authors did not consider the possible importance of mold growth during physical stabilization of cork, and that the
traditional procedures used for decades only recently are being accused of endangering cork and wine quality.

The true cork taint, which originates in defective cork, is rare. Negligent storage, transport, and handling, of both cork stoppers and wine, generate the other related off-flavors. All these defects can be reduced or eliminated.

A replacing material that express all the specific characteristics of a quality cork stopper, which allows the perfect sealing and aging of quality wines is still to come.¹

REFERENCES


78. Bertrand, A. and Barrios, M. L., Contamination des bouchons par les produits de
traits de palletes de stockage des bouchons, Rev. Fr. Oenologie, 149, 29, 1994.


