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Research regarding wine faults due to filamentous fungi

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Abstract *- In this paper, we invest cork stoppers' faults due to microbial symbioses with the cork. Based on that, fungi species of Penicillium and Phanerochaete were isolated from cork stoppers from wine bottles with a musty off-odor. Isolates of Penicillium chrysogenum, Penicillium expansum, and Phanerochaete spp. were further tested for their ability to induce wine faults related to the cork taint of wine. Phanerochaete genus significantly affects Merlot wine by producing high volatile and total acidity and Syrah wine by producing low alcohol, residual Sugar, Ash, and Zn. Moreover, Phanerochaete isolates significant decrease values of residual Sugar, Ash, and Zn in Cabernet wine suggested that in all wine treatments, Phanerochaete strain confirmed the ability of the fungus to induce wine faults.*

Keywords *- Cork stoppers, Cork taint, Fungi, Wine cross-contamination.*

I. INTRODUCTION

Cork stoppers have been using for many years for sealing wine bottles. These cork stoppers or cork closures are cylindrical and fully inserted in the wine bottle. Those cork stoppers' main properties were a) avoid any leakage occurs and b) allow an air permeability (oxygen transfer) to the wine in the bottle. Generally, a cork is a plant product. Still, our day's corks are usually classified into products of a) natural cork (single piece of cork) b) products of aggregated cork, made from small particles of cork and glued together and c) premium agglomerated cork, have a core made from particles and at each end have a thick solid cork disk. Cork origin material is the bark of *Quercus suber* L., commonly known as cork oak tree.

As a natural product, all types of cork stoppers were infected by micro-organisms, especially from different genera of fungi. The literature showed that *Monilia scatophilia* (*Chrysonilia sitiphila*) reported as dominant a fungus of cork stoppers, and *Penicillium* species occurred as cork-dominant moulds during the manufacturing process [21], [5].

The occurrence of the fungi mentioned above and other fungi genera such as the *Trichoderma Acremonium, Cladosporium, Fusarium, Mortierella, Mucor, Paecilomyces,* and *Verticillium* [1], have been reported as causing an unpleasant alternation in wine flavor called cork-taint [21], [1], [5].

Generally, cork-taint affects wine consumers' acceptability and significantly decreases the wine value. Hence, from our observations of local winemaking companies in Greece, we can say that cork-taint refers to a wine cork fault with a characteristic taste or smell that occurred after bottling. But the main impact component regarded the primary cause of cork-taint is 2,4,6-TCA anisole (2,4,6 Trichloroanisole), a chemical compound causes of cork-taint in bottled wine. Besides that, the literature reported [11], [21] that common molds such as *Aspergillus* and *Penicillium* genera can produce 2,4,6-trichloroanisole by methylation of chlorophenols (e.g., 2,4,6-trichlorophenol). These fungi and mainly the *Penicillium* species are responsible for the cork-taint when these species grown directly on cork in the presence of 2,4,6-Trichlorophenol [1].

Microbes' ecology and especially *Penicillium* species are very complex. The literature showed that fungal growth, especially *Penicillium,* may interfere in the cork stoppers derived from the manufacturing process or have originated from the bark of cork oak trees [5].

Overall, this study's primary objectives were to study the presence of different fungal genera and species that occurred on cork stoppers used by local winemakers in Greece and explain those fungi's ability to degrade wine and involved in the so-called cork-taint of wine under laboratory conditions.

II. MATERIAL AND METHODS

A. Cork stoppers

We obtained damage cork stoppers from local winemakers in Greece from wine bottles reported with a musty off-odor in wine-related to cork stopper. 70 bottles with this off-odor were obtained from local winemakers and placed in the laboratory of Crop Protection, the University of Thessaly (formerly known TEI of Larissa). Bottles were placed in a pathogen-free room, and cork stoppers were removed from each bottle under a laminar flow cabinet to prevent contamination. All cork stoppers were further surface sterilize under a UV-C germicidal lamp of the laminar flow cabinet. The surface sterilization process was running for 10 min. After the surface sterilization process, cork stoppers were cut in half and marked as 1a and 1b, 2a and 2b ….., 70a and 70b. All half cork stoppers marked as "b, were covered with aluminum foil and autoclave in a pressure steam sterilizer at $+121^0$C over 20 minutes. All "a" half parts of cork stoppers were kept unsterilized. From sterilize and non-sterilized cork stoppers, four small pieces (fragments) of each cork were placed on the surface of potato dextrose agar (PDA, Merck, Darmstadt, Germany) plates. Finally, plates were

sealed with Parafilm and incubated in partial daylight at room temperature at 25 ^{0}C for 7 days for fungus mycelial development.

B. Fungi genera and species identification

After the given incubation time, pure fungi isolates were obtained (from PDA plates with mycelia development) by in vitro hyphal mycelium tip transfer technique. They maintained on PDA or MEA agar plates fungi genera or species identification. All fungi were identified using a) a morphological analysis on PDA or MEA solid media and b) microscopic observation of the fungal reproduction structures (e.g., for *Penicillium* genus observation was made on a) the formed of individual conidia, b) the chains of conidia, c) the structure and types of conidiophores and d) numbers of phialides).

Moreover, identifying all isolates used in this study was further confirmed using more criteria (morphological data) obtained from the fungal web site mycobank.org.

C. Wine contamination with fungi, experimental process

In this study, three wine types were used. Wine types were Merlot, Cabernet, and Syrah, all produced in Greece (Table 1).

Each wine type was divided into four Erlnmayer flasks and contaminated with different fungus, which had previously isolated from contaminated corks as described above. In details, volumes of 150 ml of each wine were added into 250ml conical flasks. Further flasks were contaminated, under sterilization conditions, with tree mycelia plugs of each fungus isolate used in this study. Treatments were flasks with wine contaminated with *Penicillium chrysogenum*; flasks with wine contaminated with *Penicillium expansum*; flasks with wine contaminated with *Phanerochaete* spp. (Table 1). Flasks with untreated wine, without any mycelia plugs, were kept as controls (Table 1). After contamination, all flasks were placed at an orbitary shaker with 150 rpm and were incubated for 12 days at 25 ^{0}C. After the incubation period, all flask contents were analyzed as follows (wine analysis and composition). Treatments were four-folds.

D. Wine analysis and composition (study parameters)

To determine the ability of the above molds isolates, *Penicillium chrysogenum*, *Penicillium expansum*, and *Phanerochaete* spp., to convert taint compounds to 2,4,6-TCA, due to the lack of a Gas Chromatography/Mass Spectroscopy (GC/MS) apparatus, a total sulfur dioxide (SO_2) and a free SO_2 was using in all wine treatments to explain the Formation of possible taint compounds, such as 2,4,6-TCA produce of the above fungi isolates when were added to the wine.

Volatile acidity (VA) was also determined to identify a possible production of a high concentration of acetic acid by fungi isolates during incubation. It is well known that microbes, e.g., strains of *Saccharomyces* can also produce large amounts of acetic acid when placed under stress, e.g., some strains *Saccharomyces* can produce large amounts of acetic acid when placed under stress, and it is well known that compounds such us 2,4,6-tribromoanisole or 2,4,6-trichloroanisole (TCA) mostly originates when micro-organisms are present in wine [1].

Carbohydrates (reducing Sugar) are also determined to obtain results in the influence of the growth rate of fungus isolates added in wine treatments.

Total Soluble Solids (TSS) were also measured to better understand the decreasing alcohol volume in wine (% Alcohol by Volume) caused by the contamination that occurred by the above fungi isolates during incubation.

Overall, at the end of the incubation period (12 days at 25, ^{0}C at an orbitary shaker with 150 rpm), wine samples were analyzed using the following analytical methods.

- Total SO2 was extracted with KOH 1N and H2SO4 25% and triatation of the remaining reagent with sodium solution 0,02N in the present with indicator starch solution.
- Free SO_2 was extracted with H_2SO_4 25% and triatation of the remaining reagent with iodine solution 0,02N in the present with indicator starch solution.
- Alcohol/Volume (%)was determined by the pycnometer method.
- Total acidity was tritiated with NaOH 0,1N with bromothymol blue as an indicator. Carbon dioxide is not included in the total acidity.
- Volatile Acidity and Carbon dioxide were first removed from the wine. Further volatile acids were separated from the wine by steam distillation and titrated using NaOH 0,1N and two drops of phenolphthalein solution.
- Residual Sugar was obtained with reagents; Fehling A (dissolved 5,464 g $CuSO_4.5H_2O$ in 100ml distilled H_2O) and Fehling B (dissolved 12,0 g KOH and 7,5 g Potassium Sodium tartare in 100ml distilled H_2O).
- Total solid and Ash. The dry solid matter estimated the total solid at 105 ^{0}C. Ash was received at 550 ^{0}C, which had previously dried at 105 ^{0}C.
- Finally, the concentration of Cu and Zn were measured by using atomic absorption spectroscopy after the digestion of each wine sample with HNO3 and HClO4 (in a 2:1 portion).

E. Statistical analysis

All experiments were repeated twice and had a complete randomized design. Data were analyzed using the Minitab statistical program [19]. One-way analysis of variance (ANOVA) was applied in all experiments, and Fisher's least significant difference (LSD) was used to detect and separate the mean treatment differences among treatments.

III. RESULTS AND DISCUSSION

A. Association of cork stoppers with filamentous fungi. Species Identification

As Figure 1 shows, 22 fungi genera was isolated from non-sterile (autoclave) cork stoppers. Furthers of fungi genera and total numbers vary with and without the autoclave process (Figure 1). More fungi genera were observed and isolated from non-sterile (autoclave) cork stoppers suggested that fungi genera were associated with corked stoppers. The most dominant fungus was the *Penicillium* genus. That genus was further separate into species level with method and techniques described by [15] and [3].

Based on Pitt's (1973) description methods for *Penicillium* species, in our study, the dominant *Penicillium* species where a) the *Penicillium chrysogenum* and b) the *Penicillium expansum.*

Apart those species, the content order of the predominant fungi genera in the non sterile (autoclave) cork stoppers were *Penicillium* $_{22\ isolates}$ > *Phanerochaete* $_{7\ isolates}$ > *Mucor* $_{4\ isolates}$ > *Sporotrichum* $_{3\ isolates}$ > *Aspergillus* $_{1\ isolate}$ > *Trichoderma* $_{1\ isolate}$ > *Verticillium* $_{1\ isolate}$ > *Fusarium* $_{1\ isolate}$ (Fig. 1).

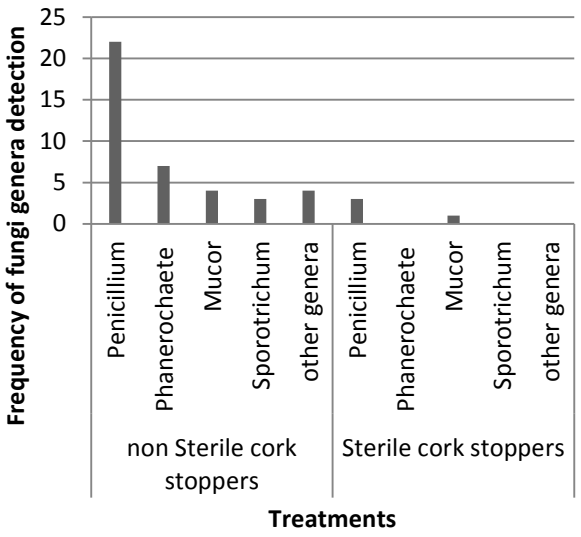

Fig. 1: Association of non-sterile and sterile cork stoppers with various fungi genera.

B. Evolution of free SO_2 at wine contaminated

It is well known that sulfur dioxide (SO_2) is an antimicrobial compound widely used in winemaking [25]. Generally, the addition of sufficient SO_2 is to reduce risks of microbial spoilage in the wine after cellar or packing of wine [12], [9].

The present study results (Table 1.) showed that in all samples of dry wine Syrah, contaminated with the three fungi isolates, and the free SO_2 concentration was significantly decreased, suggested the antiseptic role of this compound (SO_2) in Syrah wine. That decrease was related proportionally to antiseptic activity probably of the Syrah wine contains. In detail, at Syrah wine samples infected with, a) *Penicillium chrysogenum* (Wine+Pc), a 33% decrease was recorded; b) *Penicillium expansum* (Wine+Pe), a 38,8% decrease was recorded; c) *Phanerochaete* spp. (Wine+Phsp), a 44,4% decrease was recorded, respectively, compared to the untreated control (C) (Table 1).

Besides Syrah, at the Merlot wine, the free SO_2 was increased by 16,6%, at treatments contaminate only with *Phanerochaete* spp., compared with the untreated control Merlot treatment (Table 1). This is the only treatment where the free SO_2 value (44,8 mg/l) was recorded higher than the normal wine value (Control, 38,7 mg/l), suggested that the *Phanerochaete* genus acts as a "biological sulfite" fungus able to produce effective concentrations of SO_2 in Merlot wine.

Finally, it will be concluded that fungi isolates introduced in three wine types affect all three tested wine, a) by microbial growth and b) by produces secondary metabolites that affect wine quality, especially for Merlot wine.

Overall, a detecting fault in all wines treated with the *Phanerochaete* genus was observed. All wines, especially for Syrah and Merlot wine, treated with *Phanerochaete* fungus had an unusual color and produced a mushroom smell that suggested that the *Phanerochaete* genus is a fungus associated with a wine fault called cork-taint.

C. Alcohol concentration

Alcohol concentration of wine is important for the physiological effects and stability of wine's organoleptic properties [17]. From the results presented in Table 1, it is suggested that wine alcohol concentration was significantly decreased in Syrah wine treated only with *Phanerochaete* genus fungus. Overall we concluded that the reduced alcohol in Syrah wine treated with *Phanerochaete* fungus suggested a wine fault, probably a cork-taint, as a wine fault characterized by a mushroom smell.

D. Total Acidity, Volatile acidity (VA)

Based on results presented in Table 1, a significantly high variation of Total and Volatile Acidity was recorded in Merlot and Syrah wine treatments, contaminate only with the *Phanerochaete* genus confirmed that substances (secondary metabolites), produced in two wine types by *Phanerochaete* genus. A Syrah wine sample infected only with *Phanerochaete* fungus Volatile Acidity (VA) was recorded 96% higher (2,59 VA value) compared to the untreated control (1,32 VA value) (Table 1). The same results were obtained for Merlot wine treated only with the *Phanerochaete* genus (Table 1).

Concerning the *Phanerochaete* genus, a species of *P. chrysosporium* reported by [13] showed that the fungus could degrade polysaccharides such as cellulose and hemicelluloses. Moreover, authors [13] presented data that *P. chrysosporium* can degrade lignin; phenolic polymers occur in vascular plant tissues. Based on that, we can conclude or do the hypothesis that the Phanerochaete genus stain used in our research could produce 2,4,6-TCA

by O methylation of 2,4,6-trichlorophenol (2,4,6-TCP), a phenomenon described by [1]. This phenomenon (significant difference in wine treated with *Phanerochaete* genus) could be explained probably as an acid metabolism of molds in the presence of polyphenols.

E. Residual sugar and Total Solid at 105^{0}C

All wine samples (Merlot, Cabernet, and Syrah), treated only with *Phanerochaete* fungus, significantly decrease residual Sugar. Some results were observed for the Total Solid at 105 ^{0}C (TSS) (Table 1.). Syrah wine treated with *Phanerochaete* fungus presented the lowest i) residual sugar (3,4g/L) and ii) TSS (26g/L) value compared with the untreated control (Table 1.).

Basidiomycetes species such as *P. chrysosporium* have been proposed as strains produced sugar derivatives [10], [7]. Based on that our results Table 1, our research suggested that *Phanerochaete* fungus produced sugar derivatives substances (secondary metabolites) related to mushroom odor and resulted from a wine fault, probably a cork-taint by the transformation of certain phenolic compounds in carbohydrates, especially at Syrah wine (Table 1.).

F. Determination of Ash content in wines and minerals (Cu and Zn)

Determination of Ash content in wines is also an important indicator of wine quality. Ash content usually involves calcium, potassium, magnesium salts, and sulfuric (anions of SO_4^{2-}, SO_3^{2-}), phosphoric, and hydrochloric – carbonic acids. Microelements such as Cu and Zn are also important.

Besides the above, this study determines Cu and Zn minerals showed any statistical difference in total Cu contents between treatments. A significant difference was observed in total Zn contents between treatments (Table 1). Syrah wine treated with *Phanerochaete* fungus presented the lowest in percent values than untreated control (Table 1.). In detail, a 62% decrease was recorded suggested that *Phanerochaete* fungus significantly reduced the wine microelements such as Zn.

The literature shows that living mycelia of *P. chrysosporium* was used effectively to remove heavy metals of Cd, Cu, and Zn in aqueous solution [6]. In this research, authors [6] concluded that *P. chrysosporium* was a potential biosorbent micro-organism for adsorbing heavy metals. The same results, biosorption data, in our research was concluded for *Phanerochaete* fungus, indicating that the fungus can absorb other compounds such as a 2,4,6-trichloroanisole compound. The content order of the reduction of the Zn mineral in all wines, treated only with *Phanerochaete* fungus is, $Zn_{Syrah} > Zn_{merlot} > Zn_{Cabernet}$ (Table 1), provided useful evidence that the biosorption of *Phanerochaete* fungus is more active in Syrah wine.

Further, significant differences were observed in Ash contents in all treatments. All fungi significantly reduce Ash contents in all wine tested (Table 1.), confirming the above Zn obtained results.

IV. CONCLUSIONS

Several authors have reviewed cork's microbiology concluded that the cork ecosystem is a very complex microbial ecosystem. Cork is probably the best material that can effectively and safely close a bottle while allowing proper wine maturation. Literature shows that some wines (up to 7%) suffer from a defect, commonly known as cork taint attributed to the cork stopper [1]. This off-odor problem is usually perceived as a moldy, musty, and earthy aroma that can mask the natural wine aroma and lessen its quality [16].

Cork taint is a musty or mouldy off-odor in wine mainly caused by 2,4,6-trichloroanisole (2,4,6-TCA) prudence by several putative precursors, including filamentous fungi such as fungal strains belonging to the genera *Penicillium* [1]. Alvarez-Rodriquez (et al. 2002) indicated that fungi isolated from corks could produce 2,4,6-TCA, metabolize, and increase the cork's 2,4,6-TCA levels. The authors concluded that a small percentage of 2,4,6-TCP is converted to 2,4,6-TCA by *P. chrysosporium* and degraded to CO_2.

Authors [12], [1] have suggested that micro-organisms isolated from corks can produce tainting compounds cause off-odor wine fault. Hence, several chemical compounds, including anisoles, have been reported to this problem [2], [1]. Further, as reported by [8], several genera of fungi can infect grapes. Some of them can produce metabolites that delay the growth of yeasts during the fermentation process. Similar outcomes occur with lactic acid bacteria suggested that these bacteria produce amounts of acetic acid that become inhibitory of yeasts [8]. Alvarez-Rodriquez (et al. 2002), Rodríguez-Andrade (et al. 2020) concluded that 2,4,6-TCA could be synthesized from these or other precursors as a result of microbial interactions between the micro-organisms forming the complex populations found on the cork.

In this paper, we can conclude firstly that fungi species are isolated from inside cork tissues, indicated the presence of fungal spores (conidia) or hyphae, as reported by [21], [22].

Secondly, the most isolated fungi belong to the genera *Penicillium*. The same results were reported by [4].

Thirdly, all wines types (Merlot, Cabernet, and Syrah) treated with *Penicillium chrysosporium* or *Penicillium expansum* or *Phanerochaete* spp., significantly reduced sulfur dioxide (free SO_2) values, total Zn contents, and Ash contents indicated the potential degradation or detoxification activities mediated by those fungi species, suggested the hypothesis that these micro-organisms can produce substantial amounts of 2,4,6-trichlorophenol (TCP) into a synthetic medium or to reduce levels of TCA on cork reported by [21], [22], [23], [18], [24], [20], [14].

Syrah wine samples infected only with *Phanerochaete* fungus Volatile Acidity (VA) were recorded 96% higher than the untreated control. The same results were obtained for Merlot wine treated only with the *Phanerochaete* genus. In this research, the *Phanerochaete* genus is a fungus associated with a wine fault called cork-taint.

REFERENCES

[1] Alvarez-Rodriguez, M. L., L. Lppez-Ocana, J. M. Lopez-Coronado, E. Rodriguez, M. Jesus Martinez, G. Larriba, and JJR Coque. 2002. "*Cork Taint of Wines: Role of the Filamentous Fungi Isolated from Cork in the Formation of 2,4,6-Trichloroanisole by O Methylation 2,4,6-Trichlorophenol*". Appl Environ Microbiol. 68: 5860–5869.

[2] Amon, J. M., J. M. Vandepeer, and R. F. Simpson. 1989. "*Compounds responsible for cork taint in wine*". Wine Ind. Journal. 4: 62–69.

[3] Barreto, M. C., J. Houbraken, R. A. Samson, J. C. Frisvad, and M. V. San Romao. 2011. "*Taxonomic studies of the* Penicillium glabrum *complex and the description of a new species* P. subericola*"*. Fungal Diversity. 49: 22-33.

[4] Barreto, M. C., J. Houbraken, R. A. Samson, D. Brito, M. Gadanho, and M. V. San Romao. 2012. "*Unveiling the fungal mycobiota present throughout the cork stopper manufacturing process.*" FEMS Microbiol Ecol. 82: 202-14.

[5] Basilio, M.C., R. Gaspar, C. Silva Pereira, and M. V. San Romao. 2006. "*Penicillium glabrum cork colonizing isolates - preliminary analysis of their genomic similarity*". Rev. Iberoam Micol. 23: 151 – 154.

[6] Chen, G., J. Fan, R. Liu, G. Zeng, A. Chen and Z. Zou. 2012. "*Removal of Cd(II), Cu(II), and Zn(II) from aqueous solutions by live Phanerochaete chrysosporium*." Environ Technol. 33: 2653-9.

[7] De Koker, T. H., M. D. Mozuch, D. Cullen, J. Gaskell, and P.J. Kersten. 2004. *"Isolation and purification of pyranose 2-oxidase from Phanerochaete chrysosporium and characterization of gene structure and regulation*". Appl Environ Microbiol. 70 (10): 5794-800.

[8] Fleet, G. H., 2003. "*Yeast interactions and wine flavor*". International Journal of Food Microbiology. 86: 11-22.

[9] Fleet, G. H. (Ed.). 2002. "*Wine Microbiology and Biotechnology"*. Reprinted by Taylor & Francis, London.

[10] Gabriel, J., J. Volc, P. Sedmera, G. Daniel, and E. Kubatova. 1993. "*Pyranosone dehydratase from the basidiomycete Phanerochaete chrysosporium: improved purification and identification of 6-deoxy-D-glucose and D-xylosone reaction products*". Archives of Microbiology. 160 (1): 27-34.

[11] Gee, J. M., and J. L. Peel. 1974. "*Metabolism of 2,3,4,6-tetrachlorophenol by micro-organisms from broiler house liter*". Journal Gen Microbiol. 85: 237–243.

[12] Lee, T. H., and R. F. Simpson. 1993. "*Microbiology and chemistry of cork taint in wine"*. In Wine microbiology and Biotechnology. Harwood Academic Publishers. Chur: Fleet GH editor. p. 353.

[13] Liu, S., X. Li, S. Wu, J. He, C. Pang, and R. Dong. 2014. "*Fungal Pretreatment by* Phanerochaete chrysosporium *for Enhancement of Biogas Production from Corn Stover Silage"*. Applied Biochemistry and Biotechnology. 174 (5): 1907-1918.

[14] Maggi, L., V. Mazzoleni, M. D. Fumi, and M. R. Salinas. 2008. "*Transformation ability of fungi isolated from 1 cork and grape to 2 produce 2,4,6-trichloroanisole from 2,4,6-trichlorophenol*". Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 25: 265-9.

[15] Pitt, J. I., 1973. "*An appraisal of identification methods for Penicillium species: novel taxonomic criteria based on temperature and water relations"*. Mycologia. 65:1135,–1157.

[16] Rodríguez-Andrade, E.; A.M. Stchigel, J. Guarro and J.F. Cano-Lira. (2020) "*Fungal Diversity of Deteriorated Sparkling Wine and Cork Stoppers in Catalonia, Spain*". Microorganisms. 8(1): 12. doi.org/10.3390/microorganisms8010012

[17] Ribereau-Gayon, P., Y. Glories, A. Maujean, and D. Dubourdieu. 2006. "*Handbook of Enology, Volume 2: The Chemistry of Wine Stabilization and Treatments"*, by John Wiley & Sons, Ltd, West Surrex, England.

[18] Riu, M., M. Mestres, O. Busto, and J. Guasch. 2002. "*Determination of 2,4,6-trichloroanisole in wine by headspace SPME and GC-ECD*". Journal of Chromatography A. 997, 1-8.

[19] Ryan, B. F., B. L. Joiner, and J. D. Cryer, 2005. "*MINITAB Handbook: updated for release 14"*. Books/Cole-Thomson Learning, Belmont, USA. 505 pp.

[20] Sefton, M. A., and R, F. Simpson. 2005. "*Compounds causing cork taint and the factors affecting their transfer from natural cork closures to wine – a review"*. Australian Journal of Grape Wine Research. 11: 226-240.

[21] Silva Pereira, C., A. Pires, M. J. Valle, L. Vilas Boas, J. J. Figueiredo Marques and M. V. San Romao. 2000a. "*Role of Chrysonilia sitophila in the quality of cork stoppers for sealing wine bottles"*. Journal of Industrial Microbiology & Biotechnology. 24: 256-261.

[22] Silva Pereira, C., J. J. Figueiredo Marques and M. V. San Romao. 2000b. "*Cork taint in wine: scientific knowledge and public perception - a critical review*". Critical Reviews in Microbiology. 26: 147-162.

[23] Silva Pereira, C., M. Giselle, C. Soares, A. C. Oliveira, M. E. Rosa, H. Pereira, N. Moreno and M, V. San Romao. 2016. "*Effect of fungal colonization on mechanical performance of cork*". International Biodeterioration & Biodegradation. 57: 244–250.

[24] Simpson, R. F., D. L. Capone, and M. A Sefton. 2004. "*Isolation and identification of 2-methoxy-3,5-dimethylpyrazine, a potent musty compound from wine corks"*. Journal of Agricultural and Food Chemistry. 52: 5425-5430.

[25] Wedzicha, B. L., 1984. "*Toxicology. In Chemistry of Sulfur Dioxide in Foods"*, pp. 312-365. Elsevier Applied Science, London.

Table 1: Wine analysis, contaminated with different fungi species, for total SO_2, free SO_2, total acidity, volatile acidity, or other parameters

		Parameter (unit)								
Wine used	**Treatment***	**Free SO_2 (mg/l)**	**Alcohol/Volume (%)**	**Total Acidity (gr H_2SO_4/L)**	**(VA) Volatile Acidity (gr CH_3COOH/L)**	**Residual Sugar (g/L)**	**(TSS) TOTAL SOLID at 105 ^{0}C (g/L)**	**Ash at 550 ^{0}C (g/L)**	**Total Cu (mg.L^{-1})**	**Total Zn (mg.L^{-1})**
Merlot, Greece	C	38,4±0,18^c	13.5±0,01	3,14±0,01^a	0,71±0,03^c	6,6±0,12^{ab}	23±0,03^a	4±0,05^c	<0.5	0.64±0,01^d
	Wine + Pc	32±1,15^a	13,5±0,01	3,28±0.02^b	0,59±0,02^b	6,8±0,09^b	24±0,09^b	2±0,01^b	<0.5	0.45±0,02^c
	Wine + Pe	35,2±0,12^b	13,5±0,01	3,33±0,01^b	0,22±0,01^a	6,9±0,12^b	24±0,06^b	2±0,03^b	<0.5	0.34±0,01^b
	Wine+Phsp	44,8±0,31^d	13,5±0,06	3,43±0,02^c	0,94±0,02^d	6,2±0,13^a	24±0,01^b	1±0,01^a	<0.5	0.29±0,01^a
	$P_{0.05}$	>0,001	ns **	>0,001	>0,001	>0,007	>0,001	>0,001	ns	>0,001
Cabernet, Greece	C	48±0,01^c	12,5±0,06	3,82±0,02^c	1,05±0,01^c	2,8±0,01^{ab}	30±0,06^a	5±0,02^c	<0.5	1.24±0,03^d
	Wine + Pc	48±0,23^c	12,5±0,01	3,43±0,01^a	0,74±0,01^a	2,9±0,01^b	32±0,01^c	3±0,02^b	<0.5	1.07±0,02^c
	Wine + Pe	44,8±0,12^b	12,5±0,01	3,53±0,01^b	0,99±0,02^b	2,8±0,09^{ab}	31±0,44^{ab}	2±0,01^a	<0.5	0.98±0,03^b
	Wine+Phsp	41,6±0,21^a	12,5±0,06	3,53±0,01^b	1,16±0,02^d	2,4±0,20^a	31±0,07^b	2±0,02^a	<0.5	0.87±0,01^a
	$P_{0.05}$	>0,001	ns	>0,001	>0,001	>0,052	>0,002	>0,001	ns	>0,001
Syrah Greece	C	57,6±0,27^d	12.5±0,06^b	3,87±0,01^a	1,32±0,01^b	6,2±0,1^b	28±0,23^c	9±0,2^c	0.92	1.37±0,03^d
	Wine + Pc	38,4±0,01^c	12,5±0,06^b	3,92±0,01^b	1,32±0,01^b	6,2±0,31^b	27±0,12^b	3±0,06^b	<0.5	1.22±0,02^c
	Wine + Pe	35,2±0,27^b	12,5±0,01^b	3,92±0,01^b	0,85±0,01^a	6,4±0,12^b	27±0,01^b	1±0,02^a	<0.5	0.74±0,02^b
	Wine+Phsp	32±1,17^a	11,0±0,06^a	4,07±0,01^c	2,59±0,3^c	3,4±0,07^a	26±0,19^a	1±0,01^a	<0.5	0.52±0,01^a
	$P_{0.05}$	>0,001	>0,001	>0,001	>0,001	>0,001	>0,001	>0,001	ns	>0,001

* were: C: Untreated wine (Control); Wine+Pc: Wine contaminated with *Penicillium chrysogenum*; Wine+Pe: Wine contaminated with *Penicilliumexpansum*; Wine+Phsp: Wine contaminated with *Phanerochaete* spp.