THE INFLUENCE OF SELECTED OENOLOGICAL PRACTICES ON THE SENSORY IMPACT OF VOLATILE PHENOLS IN RED WINES

Antonina BARBOSA[1,2], Tim HOGG[1] and José António COUTO[1]*

1 : CBQF/Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr António Bernardino de Almeida, 4200-072 Porto, Portugal
2 : Falua – Sociedade de Vinhos SA, Zona Industrial Lt 56, 2080-221 Almeirim, Portugal

Abstract

Aims: Volatile phenols (4-ethylphenol and 4-ethylguaiacol) affect wine quality by imparting, above certain concentrations, aroma defects. This work aimed to evaluate the effect of some common oenological practices on the concentration of volatile phenols and the sensory impact of these odour-active compounds in wines.

Methods and results: The studied oenological practices were based on the addition of gum arabic, mannoproteins, yeast cell walls, lees, oak chips, dairy cream and potassium caseinate and on the application of a bubbled nitrogen stream. These treatments were applied to naturally contaminated wines containing volatile phenols at concentrations above detection threshold. The treated wines were characterised by chemical and sensory analysis. The most effective treatments in the reduction of the concentration of volatile phenols were the addition of fine lees, cream and potassium caseinate. Apart from being able to partially remove these compounds (approximately 35 % removal), fine lees contributed to a greater "freshness" and complexity of the wines. Although the addition of cream did decrease the sensory impact of volatile phenols, it did not benefit the wines as these were strongly marked by dairy aromas. The use of oak chips, although it did not have a notable effect on the removal of volatile phenols, led to a significant reduction in the perception of these compounds.

Conclusions: It is demonstrated that certain oenological practices may decrease the impact of volatile phenols in red wines.

Significance and impact of the study: Solutions, particularly applicable in a winemaking environment, are suggested to the wine industry.

Key words: lees, oenological practices, sensory impact, volatile phenols, red wine

Résumé

Objectifs: Les phénols volatils (4-éthylphénol et 4-éthylguaiacol) affectent la qualité du vin en conférant, au-dessus de certaines concentrations, des défauts aromatiques. Ce travail visait à évaluer l'effet de certaines pratiques œnologiques sur la concentration de phénols volatils et l'impact sensoriel de ces molécules odorantes dans les vins.

Méthodes et résultats: Les pratiques œnologiques étudiées étaient basées sur l'ajout de gomme arabique, mannoprotéines, parois cellulaires de levure, lies, copeaux de chêne, crème et caséinate de potassium, ainsi que sur l'application d'un courant d'azote à bulles. Ces traitements ont été appliqués aux vins naturellement contaminés contenant des phénols volatils dans des concentrations supérieures au seuil de perception. Les vins traités ont été caractérisés par analyse chimique et sensorielle. Les traitements les plus efficaces dans la réduction de la concentration de phénols volatils ont été l'ajout de lies fines, la crème et le caséinate de potassium. En plus d'induire une réduction d'environ 35 % de ces composés, les lies fines ont contribué à une plus grande « fraîcheur » et complexité des vins. Malgré une diminution de l'impact sensoriel des phénols volatils, l'ajout de la crème ne profitait pas aux vins, qui étaient fortement marqués par des arômes lactiques. L'utilisation de copeaux de chêne, bien qu'elle n'ait pas un effet notable sur l'élimination des phénols volatils, a conduit à une réduction significative de la perception de ces composés.

Conclusions: Il est démontré que certaines pratiques œnologiques peuvent diminuer l'impact de phénols volatils dans les vins rouges.

Signification et impact de l'étude: Des solutions, en particulier applicables dans un environnement de vinification, sont proposées pour l'industrie du vin.

Mots clés: lies, pratiques œnologiques, impact sensoriel, phénols volatils, vin rouge

manuscript received 31th May 2010 - revised manuscript received 14th September 2011

*Corresponding author : jcouto@porto.ucp.pt

J. Int. Sci. Vigne Vin, 2012, **46**, n°2, 131-138
©Vigne et Vin Publications Internationales (Bordeaux, France)

INTRODUCTION

Volatile phenols (4-ethylphenol and 4-ethylguaiacol) are aromatic compounds mainly produced by the yeasts *Brettanomyces*/*Dekkera*. Above certain concentrations, these compounds impart aromas normally described as "horse sweat", "animal", "leather", "medicinal", "spicy", etc. (Heresztyn, 1986, Chatonnet *et al*., 1995, Edlin *et al*., 1995, Chatonnet *et al*., 1997). The presence of these compounds in wine is currently of great concern among wine producers and is being considered a key point in the control of wine quality. In red wine, the detection threshold of ethylphenols has been reported to be 426 μg/L for a 10:1 mixture of 4-ethylphenol and 4-ethylguaiacol (Chatonnet *et al*., 1992). However, the threshold concentrations of these compounds can vary substantially, the perception being greatly influenced by the wine style, the grape variety used and the consumer's perceptive ability (Ribéreau-Gayon *et al*., 2000, Phister and Mills, 2004, Fugelsang and Edwards, 2007, Oelofse *et al*., 2008).

The precursors of volatile phenols are natural constituents of grape juice and wine – the hydroxycinnamic p-coumaric and ferulic acids (Heresztyn, 1986, Chatonnet *et al*., 1995). The transformation of these compounds involves a sequence of two enzymatic reactions. In the first reaction, the hydroxycinnamic acid is decarboxylated into the corresponding vinyl derivative (4-vinylphenol from p-coumaric acid or 4-vinylguaiacol from ferulic acid); in the second reaction, a reductase converts the vinyl into the corresponding ethyl compound (4-ethylphenol or 4-ethylguaiacol) (Heresztyn, 1986).

The origin and the sensory impact of these compounds, as well as the characterisation, detection and control of *Brettanomyces*/*Dekkera* have been well studied over the past 15 years. Nevertheless, the extensive information that has been accumulated on this subject has not been sufficient to enable producers to eradicate the contamination by spoilage yeasts and the subsequent development of spoilage characteristics. Thus, defence strategies based on the prevention of contamination, the control of microbial growth or the elimination of microorganisms from wine, whilst contributing greatly to the reduction in the incidence of spoilage, cannot always be counted on to achieve the desired effect. Alternatively, curative measures based on the removal of the odour-active compounds and/or on the reduction of the sensory effect by masking agents may provide methods for the recovering of tainted wines to acceptable quality levels. Whilst potential remedial measures can be found in the scientific literature, apparently nothing has been demonstrated in practical conditions.

Some studies have demonstrated that yeast cell walls have a good capacity to adsorb phenolic compounds, specifically anthocyanins and compounds responsible for the browning of white wines (Razmkhab *et al*., 2002, Morata *et al*., 2003 and 2005). Differences in anthocyanin adsorption capacity have been observed between the different yeast strains tested, possibly due to variation in the composition of yeast cell walls. Chassagne *et al*., (2005) studied the capacity of Saccharomyces cerevisiae yeast lees to sorb 4-ethylguaiacol and 4-ethylphenol. Active dry yeast was found to be more effective when volatile phenols were diluted in red wine than in synthetic medium. Wine yeast lees showed a higher affinity for volatile phenols than active dry yeast. The effect of yeast lees on volatile phenol sorption was found to be sensitive to yeast autolysis level and to physicochemical parameters, such as ethanol content, temperature and pH. The clarification of wines with casein or potassium caseinate was also mentioned as capable of reducing the concentration of volatile phenols (Ruiz-Hernández, 2003). We note that this study has based its conclusions on sensory analysis only and has used wines with low levels of ethylphenols. More recently, Ugarte *et al*., (2005) used a two-step process that uses reverse osmosis and an ion-exchange treatment column to remove *Brettanomyces*-induced odour-active compounds. Wines subjected to this treatment showed a 50-70 % reduction in the concentration of 4-ethylphenol and 4-ethylguaiacol. The colour, body and ethanol concentration of the wines did not change significantly, but a significant reduction in the concentration of certain aromatic compounds (higher alcohols, esters, acids, and phenolic compounds) was detected. Thus, caution should be taken due to the risk of stripping the wine of desirable flavour/aromatic components.

The objective of this work was to evaluate the ability of some common oenological practices to decrease the concentration of 4-ethylphenol and 4-ethylguaiacol and/or to reduce the negative sensory impact of these odour-active compounds. It is aimed to find solutions particularly applicable in a winemaking environment, thus presenting important advantages to the wine industry.

MATERIALS AND METHODS

1. Wines

The red wines used in this work (from the Tejo Demarcated Region, Portugal, 2005 vintage) contained levels of volatile phenols above the odour threshold due to natural microbiological contamination. Upon sensory detection of the characteristic phenolic aroma, 4-ethylphenol and 4-ethylguaiacol were quantified by GC-FID (gas chromatography-flame ionisation detection). The wine used in assays 1 and 2 contained approximately 3200 μg/L of 4-ethylphenol, while the 3 wines used in assay 3 contained approximately 1250 μg/L (DB14), 1600 μg/L (CC8) and 2000 μg/L (AC3) of 4-ethylphenol.

©Vigne et Vin Publications Internationales (Bordeaux, France)

Table 1 - Basic parameters of the wines used

Wine	Alcoholic strength (%v/v)	Volatile acidity (g/L of acetic acid)	Total acidity (g/L of tartaric acid)	pH
Assays 1 and 2	12.4	0.77	5.2	3.66
Assay 3 – CC8	14.5	0.70	5.4	3.70
Assay 3 – AC3	14.5	0.55	5.4	3.75
Assay 3 – DB14	14.0	0.65	5.8	3.60

After the detection of volatile phenols, free SO_2 was adjusted to 60 mg/L in all wines. Basic parameters are shown in Table 1.

2. Treatments

The following treatments, each performed in triplicate, were applied to wines in dark green 1.5 L glass bottles. Wines were stored at room temperature before and after being subjected to the different treatments. Appropriate controls, i. e., wine simply held or agitated for the same duration as the treatments, were prepared.

a. Assay 1

Gum arabic : 4 ml of Filtrostabil (Martin Vialatte Oenologie, Epernay, France) was added to 1.5 L of wine (267 ml/hL) (recommended dose : 30 – 200 ml/hL). The wine was stirred with a magnetic stirrer for 5 minutes and racked 1 week later.

Atomised egg albumin : 0.6 g of Ovocol (Martin Vialatte Oenologie, Epernay, France) was dissolved in 6 ml of water (15 ºC) and then added to 1.5 L of wine (40 g/hL) (recommended dose : 10 g/hL) under magnetic agitation for 5 min. The wine was racked 1 week later.

Red wine fine lees 2005: Lees collected from different wines after malolactic fermentation were mixed, then pH was adjusted to 2.7 (with tartaric acid) and total SO2 was adjusted to 500 g/hL. Lees were agitated 3 times/week in the first 6 months and once a week thereafter. Fifteen mL of lees was added to 1.5 L of wine (1 % v/v) under magnetic agitation for 5 min. The wine was racked 1 week later.

Oak chips : 5 g of American oak chips (Axon corporation, USA), with strong toasting, was added to 1.5 L of wine (333 g/hL). The wine was racked after 18 days of contact.

Dairy cream : 6 g of cream (Parmalat, Sintra, Portugal) was added to 2 l of wine (300 g/hL) under agitation with a Ufesa Brio 300W homogeniser (Ufesa, Spain) for 2 min. The wine was racked twice, 24 h and 12 days later.

Mannoproteins : 1.5 g of Mannostab (Laffort, Bordeaux, France) was added to 1.5 L of wine (100 g/hL) (recommended dose : 10 – 30 g/hL) under magnetic agitation for 5 min. The wine was racked 1 week later.

Nitrogen : A stream (100 L/min) of nitrogen gas was flushed in 4 L of wine for 30 min.

b. Assay 2

Yeast cell walls : 4.5 g of Extraferm (DSM, Heerlen, The Netherlands) was added to 1.5 L of wine (3 g/L) (recommended dose : 0.2 – 0.8 g/L) under magnetic agitation for 5 min. The wine was racked 1 week later.

Autolysed yeasts : 4.5 g of Oenolysat (Oenofrance, Bordeaux, France) was added to 1.5 L of wine (3 g/L) (recommended dose : 0.1 – 0.5 g/L) under magnetic agitation for 5 min. The wine was racked 1 week later.

Red wine fine lees 2006: Before use, lees collected after malolactic fermentation were treated as described above for red wine fine lees 2005. Thirty mL of lees was added to 1.5 L of wine (2 % v/v) under magnetic agitation for 5 min. The wine was racked 1 week later.

Red wine heavy lees 2006: 30 mL of lees collected after alcoholic fermentation (Falua, Almeirim, Portugal) was added to 1.5 L of wine (2 % v/v) under magnetic agitation for 5 min. The wine was racked 1 week later.

c. Assay 3

Oak chips : Oenotannin DC 310 (Oenofrance, Bordeaux, France), with strong toasting, was added to 3 L of wine to final concentrations of 1 g/L and 3 g/L. The wine was racked and filtered (Whatman no 1 filter paper) after 10 days of contact.

Yeast cell walls : Extraferm (DSM, Heerlen, The Netherlands) was added to 1.5 L of wine to final concentrations of 1 g/L and 3 g/L under magnetic agitation for 5 min. The wine was racked and filtered (Whatman no 1 filter paper) 1 week later.

White wine fine lees 2006: Before use, lees collected after alcoholic fermentation were treated as described above for red wine fine lees 2005. Lees were added to 1.5 L of wine to final concentrations of 1 % and 3 % (v/v).

©Vigne et Vin Publications Internationales (Bordeaux, France)

Dairy cream: Cream (Parmalat, Sintra, Portugal) was added to 1.5 L of wine to final concentrations of 1 g/L and 3 g/L under magnetic agitation for 5 min. The wine was racked and filtered (Whatman no 1 filter paper) 1 week later.

Potassium caseinate: 3 g of potassium caseinate (A. Freitas Vilar, Lisbon, Portugal) was dissolved in 20 mL of water at room temperature and then added, drop by drop, to 1.5 L of wine (2 g/L) under magnetic agitation for 20 min. The wine was racked and filtered (Whatman no 1 filter paper) 72 h later.

3. Chromatographic analysis of volatile phenols

Wines were centrifuged (3000 g, 10 min) and the volatile phenols were analysed according to the method developed by Bertrand (1981). Fifty μL of 3-octanol (internal standard, 1.073 g/L) was added to 50 mL of wine. This mixture was successively extracted with 4.0 mL, 2.0 mL and 2.0 mL of ether/hexane (1:1, v/v) by stirring for 5 min. The organic phases were collected, mixed, and concentrated under a stream of nitrogen to approximately one-third of the original volume. Two μL of the extract was injected into a Perkin Elmer GC-FID (Shelton, CT, USA). The column used was a FFAP type (BP 21, 50 m x 0.25 mm x 0.2 μm) from SGE (Austin, Texas). The injector (split/splitless) was heated to 250 ºC with a split flow of 30 mL/min and a splitless time of 0.3 min. The carrier gas flow was adjusted to 1 mL/min. The oven temperature was maintained at 40 ºC for 5 min after the injection and was then increased to 220 °C at a rate of 2 ºC/min. This temperature was maintained for 20 min. The standard error of the method is approximately 10 μg/L.

4. Sensory analysis

The effect of the various treatments was evaluated by sensory analysis through multiple comparison tests. Tastings were conducted in a specialised white sensory evaluation laboratory at room temperature (19 ± 2 °C). The wines were stored in a dark cabinet at room temperature. The bottles were open 15 min prior to each session and panellists were served approximately 40 mL of wine per sample. Panellists were asked to score on a -3 to +3 scale the intensity of volatile phenols in comparison to the control wine (non treated, score = 0). Anchor terms were placed at the extreme ends of the scale line (-3: less volatile phenols than the control wine; +3: more volatile phenols than the control wine). The sensory analysis panel was composed of 7 trained judges with experience in wine sensory evaluation, in particular in the detection of the characteristic phenolic aroma.

The data obtained were converted to a 0 to 6 scale, in which the control received the fixed score of 3. Results were submitted to statistical analysis: analysis of variance (ANOVA double factor with no repetition, 95 % probability) and Tukey test.

RESULTS AND DISCUSSION

The wine used in assay 1 contained 3199 and 422 μg/L of 4-ethylphenol and 4-ethylguaicol, respectively, due to natural microbiological contamination. Table 2 shows the effect of several oenological practices on the concentration of these compounds. It can be seen that the treatments with fine lees 2005 and cream led to a reduction in the concentration of volatile phenols by about 35 %. The effect observed for cream (used in wines, in the past, as a deodorant agent) might be related to the presence of casein in its composition (approximately 3.3 g/100 mL; Food Standards Agency, 2002). Besides the capacity to remove iron, thus protecting against ferric instabilities, this protein binds with high affinity to polyphenols, contributing to the reduction in the browning of white wines (Bertrand *et al*., 2000). Ruiz-Hernández (2003) noticed a reduction in the phenolic character of wines treated with casein or potassium caseinate. Yeast cell walls, which are essentially composed of polysaccharides (ß-glucans) and glycoproteins (mannoproteins), have been shown to be able to adsorb volatile phenols (Razmkhab *et al*., 2002: Morata *et al*., 2003 and 2005; Chassagne *et al*., 2005). Based on this attribute, two treatments were tested in this work: addition of fine lees and mannoproteins. While the application of fine lees led to a 30-35 % reduction in the concentration of volatile phenols, the mannoproteins did not exhibit any effect. This result suggests that mannoproteins, at least when isolated from the cell walls, are not capable of removing volatile phenols and that other components of lees may be responsible for the sorption of these compounds. The application of mannoproteins is intended for the stabilisation of phenolic compounds, tartaric salts and proteins but not formally for the removal of volatile phenols (Bertrand *et al*., 2000, Caridi, 2006). Fine lees may contain yeasts at different levels of autolysis (Delteil, 2002) and, according to Chassagne *et al*., (2005), autolysed yeast biomass has a higher uptake capacity of volatile phenols than non-autolysed yeast.

The wines treated with gum arabic (Filtrostabil), egg albumin (Ovocol) and oak chips did not undergo significant changes in the concentration of volatile phenols, suggesting the inability to establish molecular interactions with these compounds. The application of nitrogen to evaluate an eventual loss of volatile phenols by stripping did not generate any significant effect.

The impact of the different treatments on the sensory perception of the spoilage characteristics of volatile phenols was also studied. The panellists scored the samples

©Vigne et Vin Publications Internationales (Bordeaux, France)

Table 2 - Concentration of 4-ethylphenol and 4-ethylguaiacol (μg/L) in the control and in the treated wines of assay 1 (values are the average of 3 experiments ± standard error)

	4-ethylphenol		4-ethylguaiacol	
	Average ± SE (μg/L)	Removal (%)	Average ± SE (μg/L)	Removal (%)
Control	3199 ± 32		422 ± 5	
Gum arabic (Filtrostabil, 267 mL/hL)	3242 ± 51	0	436 ± 19	0
Egg albumin (Ovocol, 40 g/hL)	3179 ± 158	1	377 ± 52	11
Red wine fine lees 2005 (1%)	2083 ± 205	35	286 ± 25	32
American oak chips (333 g/hL)	3186 ± 89	0	414 ± 22	2
Nitrogen (30 min)	3235 ± 59	0	442 ± 14	0
Cream (300 g/hL)	2052 ± 64	36	220 ± 46	48
Mannoproteins (Mannostab, 100 g/hL)	3206 ± 34	0	445 ± 49	0

in terms of perceived intensity of volatile phenols in comparison to the control (non treated wine). Figure 1 shows that the wines treated with fine lees, oak chips and cream were considered significantly less intense than the control in terms of volatile phenols. The results obtained for treatments with fine lees and cream are in agreement with the quantification analysis of volatile phenols (Table 2). The wine treated with oak chips, despite the absence of reduction in the concentration of volatile phenols, was considered the one in which the characteristic spoilage aromas were less evident. The masking effect caused by the woody aromas is most likely responsible for this result. It is important to note that the wine treated with fine lees was described as more fresh and fruity than the control wine (data not shown). On the other hand, unpleasant dairy notes were detected in the wine treated with cream.

Since the above results highlight the potential of lees in reducing volatile phenols in wine, some commercially available products based on autolysed wine yeasts were tested (assay 2). The treatments used and their effects are depicted in Table 3. Again, the ability of fine lees to reduce the concentration of volatile phenols is shown. This effect was also found for the heavy lees, although with a lower percentage removal, which may be linked to the fact that heavy lees contain a lesser proportion of intact yeast material (being obtained immediately after the end of alcoholic fermentation when the major parts of yeasts are still in suspension). According to Delteil (2002), heavy lees are composed of vegetal particles, agglomerations of tartaric crystals, yeasts, colouring matter and precipitated tannins and flakes derived from reactions between proteins, polysaccharides and tannins during maceration. These particles sediment within 24 h and display sizes varying from 100 μm to a few mm. Yeast cells may be entrapped in the agglomerates, thereby becoming less available to sorption interactions with other compounds. Fine or light lees are composed of particles that remain suspended 24 h after the wine has been moved (draining, racking, stirring, pumping, etc.) and are constituted of yeast alone (towards the end of alcoholic fermentation) or yeast and lactic acid bacteria (towards the end of malolactic fermentation) at a concentration of 30 to 100 g/L of wine (Delteil, 2002). The Extraferm (yeast cell walls) was found to have a similar effect as the heavy lees. The lower capacity in comparison to fine lees may be due to the fact that the sorption efficiency of isolated cell walls is lower than the equivalent amount of complete yeast cells (Razmkhab *et al*., 2002). Chassagne *et al*., (2005) found a larger affinity of volatile phenols for wine yeast lees than for active dry yeasts or yeasts obtained from the fermentation of synthetic musts. Oenolysat, mainly composed of compounds released from the hydrolysis of the cell walls, did not show any influence. This is not in agreement with Chassagne *et al*., (2005), who observed that autolysed yeasts have high volatile phenol uptake capacity.

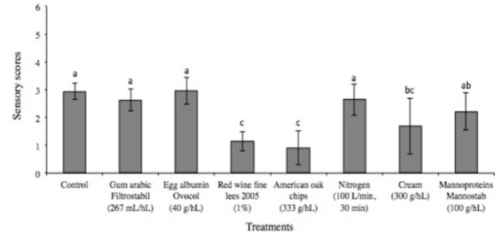


Figure 1 - Perceived intensity of volatile phenols in the control and in the treated wines of assay 1 (3 – equal to control; 0 to 3 – less intense than the control; 3 to 6 – more intense than the control).
Different letters above the columns indicate samples significantly different as revealed by the Tukey test (at 95 % probability). Values are the average of 3 experiments and the small bars in the columns represent the standard errors.

J. Int. Sci. Vigne Vin, 2012, **46**, n°2, 131-138
©Vigne et Vin Publications Internationales (Bordeaux, France)

Table 3 - Concentration of 4-ethylphenol and 4-ethylguaiacol (*μ*g/L) in the control and in the treated wines of assay 2 (values are the average of 3 experiments ± standard error).

	4-ethylphenol		4-ethylguaiacol	
	Average ± SE (*μ*g/L)	Removal (%)	Average ± SE (*μ*g/L)	Removal (%)
Control	3413 ± 66		437 ± 23	
Yeast cell walls (Extraferm, 3 g/L)	3145 ± 213	8	407 ± 11	7
Autolysed yeasts (Oenolysate, 3 g/L)	3441 ± 143	0	439 ± 14	0
Red wine fine lees 2006 (2%)	1818 ± 192	47	319 ± 86	27
Red wine heavy lees 2006 (2%)	3145 ± 133	8	405 ± 30	7

The impact of the different treatments on the sensory perception of the volatile phenols was also studied. Figure 2 shows that the wines treated with lees, either fine or heavy, were significantly different from the control. Concerning the wine treated with fine lees 2006, the result was in accordance with the chemical analyses (Table 3). All tasters noticed an increase in the fruity character of this wine, perhaps due to the release of aromatic compounds into the wine. This can also explain the result obtained in the wine treated with heavy lees 2006.

The most efficient treatments (fine lees, oak chips, cream and Extraferm) found in assays 1 and 2 were applied in two concentration levels to 3 different wines (assay 3) : DB14, CC8 and AC3 with 1256, 1595 and 2029 *μ*g/L of 4-ethylphenol, respectively. This part of the work aimed to ascertain whether the efficacy of the treatments depends on the dose applied and on the level of volatile phenols in wines. A treatment with potassium caseinate was also performed to evaluate whether or not casein has a similar effect as cream. The results of the effect on the concentration of volatile phenols are shown in Table 4. As can be observed, the treatments with lees, cream and potassium caseinate showed the highest influence on the concentration of 4-ethylphenol and 4-ethylguaiacol. Contrasting with assay 1, the treatment with oak chips caused a small decrease in the concentration of volatile phenols, probably due to a sorption effect of wood components. Barrera-Garcia *et al*., (2008) showed that components of plant cell walls (cellulose, hemicellulose and lignin) have the ability to sorb phenolic compounds such as 4-ethylguaiacol and 4-vinylguaiacol. As also found in assay 2, the Extraferm had a lower effect than the lees. The percentage removal of 4-ethylphenol by the white fine lees 2006 varied between 25 and 36 %: the higher the concentration of 4-ethylphenol in the wine, the higher the percentage of removal. The quantity of lees used (1 and 3 %) did not influence the amount of 4-ethylphenol eliminated. A similar behaviour was found in the wines treated with cream. Potassium caseinate was able to remove volatile phenols, supporting the hypothesis that casein plays a role in the observed effect caused by cream. In spite of contributing more casein, the removal effect of potassium caseinate was not considerably higher than that of cream.

The impact of the different treatments on the sensory perception of the volatile phenols was evaluated (Table 5). Treatments with letter c are the most significantly different from the control, followed by those with letter b. The wines treated with Extraferm and potassium caseinate, although showing a reduction in the amount of volatile phenols, were not significantly different from the control in the sensory analysis. The results obtained strongly indicate that the application of cream, besides diminishing the concentration of volatile phenols, overlaps the typical aroma of these compounds. The panellists were unanimous in considering theses wines as strongly marked by dairy aromas. The wines treated with fine lees and oak chips were considered the most different from the control in the 3 wines studied. In the case of oak chips, this was mainly due to an overlapping effect of aromas (woody aromas over phenolic aromas), which was stronger with 3 than with 1 g/L. With fine lees, this was mainly due to the partial elimination of volatile phenols. Moreover, the fruity character was, once more, emphasised by the tasters with both concentrations of lees used, reinforcing the idea of release of aromatic compounds into the wine. In wine,

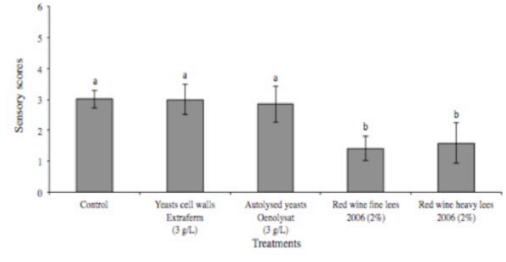


Figure 2 - Perceived intensity of volatile phenols in the control and in the treated wines of assay 2 (3 – equal to control; 0 to 3 – less intense than the control; 3 to 6 – more intense than the control).

Different letters above the columns indicate samples significantly different as revealed by the Tukey test (at 95 % probability). Values are the average of 3 experiments and the small bars in the columns represent the standard error.

©Vigne et Vin Publications Internationales (Bordeaux, France)

Table 4 - Concentration of 4-ethylphenol and 4-ethylguaiacol (μg/L) in the control and in the treated wines of assay 3 (values are the average of 2 experiments ± standard error)

	Wine DB14		Wine CC8		Wine AC3	
	4-ethylphenol Average ± SE (μg/L)	4-ethylguaiacol Average ± SE (μg/L)	4-ethylphenol Average ± SE (μg/L)	4-ethylguaiacol Average ± SE (μg/L)	4-ethylphenol Average ± SE (μg/L)	4-ethylguaiacol Average ± SE (μg/L)
Control	1256 ± 6	241 ± 2	1595 ± 21	296 ± 6	2029 ± 72	334 ± 6
Oak chips (1 g/L)	1261 ± 89	207 ± 5	1435 ± 9	300 ± 8	1891 ± 17	321 ± 3
Oak chips (3 g/L)	1075 ± 295	205 ± 48	1405 ± 8	283 ± 11	1832 ± 91	319 ± 45
Yeast cell walls (Extraferm, 1 g/L)	1189 ± 71	220 ± 14	1277 ± 184	256 ± 16	1795 ± 8	289 ± 27
Yeast cell walls (Extraferm, 3 g/L)	1054 ± 188	194 ± 37	1480 ± 87	285 ± 18	1722 ± 83	205 ± 88
White wine fine lees 2006 (1%)	902 ± 82	135 ± 49	1064 ± 80	225 ± 14	1297 ± 209	243 ± 119
White wine fine lees 2006 (3%)	943 ± 58	166 ± 8	1115 ± 31	234 ± 6	1424 ± 48	342 ± 9
Cream (1 g/L)	899 ± 23	156 ± 0	1109 ± 212	219 ± 23	1560 ± 295	306 ± 13
Cream (3 g/L)	1086 ± 235	191 ± 40	1165 ± 175	231 ± 21	1547 ± 175	296 ± 17
Potassium caseinate (2 g/L)	843 ± 1	140 ± 6	1186 ± 118	267 ± 47	1660 ± 58	271 ± 11

when naturally released at the right level, amino acids and nucleic acids (known in the food industry as flavour enhancers) participate in amplifying the intensity of taste sensations and complex aromatic aftertaste (Delteil, 2002).

CONCLUSIONS

The results obtained in this work demonstrate that certain common oenological practices are capable of reducing the adverse effect of volatile phenols, thus improving the quality of the affected wines. The negative impact of volatile phenols was reduced due to its partial elimination and/or the masking effect caused by compounds released into the wine. The most beneficial treatments were the application of lees and oak chips. It would be interesting to test the combination of both in future works; the combined effect would permit the reduction of the amount of oak chips used, thus avoiding any excessive influence of wood on the aroma of the wine. It has also been clearly demonstrated that not all treatments capable of reducing the impact of volatile phenols exhibit an overall positive influence on the quality of the wine, as it happened with the application of cream.

Prevention is the best strategy to control wine spoilage by *Brettanomyces*/*Dekkera*, e.g., by the application of high levels of hygiene and avoiding cross contamination. It is also important to apply reliable methods for the detection of contaminants before the wine quality is severely altered. There are a number of ways to hinder the growth of *Brettanomyces*/*Dekkera*, e.g., through the use of appropriate antimicrobial agents (SO_2, dimethyldicarbonate, etc.) and storage under adequate conditions. If these control strategies do not produce the desired effect, as it seems to happen quite frequently, the last alternative is the application of curative measures. The present work contributes to the knowledge of common oenological practices that show the ability to reduce or eliminate the negative sensory impact of volatile phenols in wines. These solutions are applicable in a winemaking environment, thus presenting important advantages to the wine industry.

Acknowledgments: The authors would like to thank FCT (Fundação para a Ciência e Tecnologia) for funding this research via project POCTI/AGR/61331/2004. We are grateful to Maria João Monteiro and Susana Teixeira for expert assistance in the sensory analysis and to Mário Andrade for helpful advice and stimulating discussions.

REFERENCES

Barrera-Garcia V.D., Gougeon R.D., Karbowiak T., Voilley A. and Chassagne D., 2008. Role of wood macromolecules on selective sorption of phenolic compounds by wood. *J. Agric. Food Chem.*, **56**, 8498-8506.

Bertrand A., 1981. Formation des substances volatiles au cours de la fermentation alcoolique. Incidence sur la qualité du vin. *In : Colloque de la Société Française de Microbiologie, Reims*, pp. 251-267.

Bertrand A., Canal-Llaubères R.-M., Feuillat M., Hardy G., Lamadon F., Lonvaud-Funel A., Pellerin P. and Vivas N., 2000. Chapitre VIII : Produits de correction, clarification et stabilisation. *In : Produits de traitement et auxiliaires d'élaboration des moûts et des vins.* Éd. Féret, Bordeaux, pp. 98-124.

Caridi A., 2006. Enological functions of parietal yeast mannoproteins. *Antonie van Leeuwenhoek*, **89**, 417-422.

©Vigne et Vin Publications Internationales (Bordeaux, France)

Table 5 - Perceived intensity of volatile phenols in the control and in the treated wines of assay 3 (3 – equal to control; 0 to 3 – less intense than the control; 3 to 6 – more intense than the control) (values are the average of 3 experiments ± standard error).

Wine DB14		Wine CC8		Wine AC3	
Treatment	Average ± SE	Treatment	Average ± SE	Treatment	Average ± SE
Control	3.0 ± 0.3a	Control	2.9 ± 0.2a	Control	3.0 ± 0.2a
Potassium caseinate (2 g/L)	2.5 ± 0.5ab	Potassium caseinate (2 g/L)	2.9 ± 0.3a	Yeast cell walls (Extraferm, 1 g/L)	2.9 ± 0.7a
Yeast cell walls (Extraferm, 1 g/L)	2.5 ± 0.5ab	Yeast cell walls (Extraferm, 3 g/L)	2.8 ± 0.4a	Potassium caseinate (2 g/L)	2.8 ± 0.6a
Oak chips (1 g/L)	2.2 ± 0.2ab	Yeast cell walls (Extraferm, 1 g/L)	2.6 ± 0.6a	Cream (1 g/L)	2.5 ± 1.3a
Yeast cell walls (Extraferm, 3 g/L)	1.8 ± 0.4b	Oak chips (1 g/L)	1.5 ± 0.6b	Cream (3 g/L)	2.0 ± 1.4ab
Cream (1 g/L)	0.8 ± 0.9c	Cream (1 g/L)	1.4 ± 0.5b	Oak chips (1 g/L)	1.7 ± 0.8ab
White wine fine lees 2006 (1%)	0.7 ± 0.7c	White wine fine lees 2006 (1%)	0.9 ± 0.4bc	Oak chips (3 g/L)	1.0 ± 0.7bc
Cream (3 g/L)	0.6 ± 0.5c	Cream (3 g/L)	0.7 ± 0.5c	Yeast cell walls (Extraferm, 3 g/L)	0.6 ± 0.8bc
Oak chips (3 g/L)	0.5 ± 0.5c	White wine fine lees 2006 (3%)	0.6 ± 0.4c	White wine fine lees 2006 (1%)	0.2 ± 0.3c
White wine fine lees 2006 (3%)	0.4 ± 0.4c	Oak chips (3 g/L)	0.6 ± 0.4c	White wine fine lees 2006 (3%)	0.1 ± 0.2c

Different letters indicate samples significantly different as revealed by the Tukey test (at 95% probability). In each wine, treatments are placed in order of perceived intensity, from the highest to lowest.

Chassagne D., Guilloux-Benatier M., Alexandre H. and Voilley A., 2005. Sorption of wine volatile phenols by yeast lees. *Food Chemistry*, **91**, 39-44.

Chatonnet P., Dubourdieu D., Boidron J.N. and Pons M., 1992. The origin of ethylphenols in wines. *J. Sci. Food Agric.*, **60**, 165-178.

Chatonnet P., Dubourdieu D. and Boidron J.N., 1995. The influence of *Brettanomyces/Dekkera* sp. yeasts and lactic acid bacteria on the ethylphenol content of red wines. *Am. J. Enol. Vitic.*, **46**, 463-468.

Chatonnet P., Viala C. and Dubourdieu D., 1997. Influence of polyphenolic components of red wines on the microbial synthesis of volatile phenols. *Am. J. Enol. Vitic.*, **48**, 443-448.

Delteil D., 2002. Working with lees: key elements to wine maturing. *Australian Grapegrower and Winemaker, 30th Annual Technical Issue* **461**, 104-108.

Edlin D.A.N., Narbad A., Dickinson J.R. and Lloyd D., 1995. The biotransformation of simple phenolic compounds by *Brettanomyces anomalus*. *FEMS Microbiology Letters*, **125**, 311-316.

Food Standards Agency, 2002. McCance and Widdowson's *The Composition of Foods*, Sixth Summary Edition. Cambridge: Royal Society of Chemistry.

Fugelsang K.C. and Edwards C., 2007. *Wine Microbiology: Practical Applications and Procedures*, Second Edition. The Chapman & Hall Enology Library, New York.

Heresztyn T., 1986. Metabolism of volatile phenolic compounds from hydroxycinnamic acids by *Brettanomyces yeast*. *Archives of Microbiology* ,**146**, 96-98.

Morata A., Gómez-Cordovés M.C., Suberviola J., Bartolomé B., Colomo B. and Suárez J.A., 2003. Adsorption of anthocyanins by yeast cell walls during the fermentation of red wines. *J. Agric. Food Chem.*, **51**, 4084-4088.

Morata A., Gómez-Cordovés M.C., Colomo B. and Suárez J.A., 2005. Cell wall anthocyanin adsorption by different *Saccharomyces* strains during the fermentation of *Vitis vinifera* L. cv Graciano grapes. *European Food Res. Technol.*, **220**, 341-346.

Oelofse A., Pretorius I.S. and du Toit M., 2008. Significance of *Brettanomyces* and *Dekkera* during winemaking: A synoptic review. *South African J. Enol. Vitic.*, **29**, 128-144.

Phister T.G. and Mills D.A., 2004. Novel methods to detect *Brettanomyces* (*Dekkera*) in wine. *In : Technical abstracts, 55th Annual Meeting*, San Diego, CA. Davis, CA: American Society for Enology and Viticulture, p. 30.

Razmkhab S., Lopez-Toledano A., Ortega J.M., Mayen M., Merida J. and Medina M., 2002. Adsorption of phenolic compounds and browning products in white wines by yeasts and their cell walls. *J. Agric. Food Chem.*, **50**, 7432-7437.

Ribéreau-Gayon P., Glories Y., Maujean A. and Dubourdieu D., 2000. *Handbook of Enology Volume II, The Chemistry of Wine : Stabilization and Treatments*, Second Edition. John Wiley & Sons Ltd, UK.

Ruiz-Hernández M., 2003. Casein for correction of defects caused by *Brettanomyces* and *Dekkera*. *Semana Vitivinícola*, 58, 1462-1463.

Ugarte P., Agosin E., Bordeu E. and Villalobos J.I., 2005. Reduction of 4-ethylphenol and 4-ethylguaiacol concentration in red wines using reverse osmosis and adsorption. *Am. J. Enol. Vitic.*, **56**, 30-36.

©Vigne et Vin Publications Internationales (Bordeaux, France)