Dekkera/Brettanomyces yeast — an overview of recent AWRI investigations and some recommendations for its control

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Introduction

Dekkera/Brettanomyces is a yeast that exists in two forms: Dekkera, the sexual sporulating form, and Brettanomyces, the asexual, non-sporulating form. The spoilage of wine by Dekkera/Brettanomyces yeast is well documented, with terms such as "medicinal" "pharmaceutical", "barnyard-like", "horsey", "horse sweat", "leathery", "wet dog" and "Band-aid®" having been used to describe the aroma of affected wines. Whether or not the growth of Dekkera/Brettanomyces yeast in wine and the associated aromas should always be considered as spoilage, has been hotly debated. Nevertheless, Dekkera/Brettanomyces is an organism with the potential to cause spoilage, and therefore it is desirable to understand the relationships between Dekkera/Brettanomyces growth and wine composition in order to be able to manage or control it.

Over the past four years, the Institute’s Industry Services team has received more enquiries from industry regarding Dekkera/Brettanomyces yeast and its potential for wine spoilage, than for any other subject except wine bottle closures (which during this period was also the subject of the team’s research activities). As a result of the interest in this topic a seminar relating to Dekkera/Brettanomyces control has been presented to approximately 1,500 winemakers in a number of forums, particularly Institute Roadshows. Additionally, a workshop entitled Trouble free winemaking — the identification, avoidance and management of common wine instabilities, which contains a strong emphasis on the control of microbiological instabilities including Dekkera/Brettanomyces, has been presented on 15 occasions since it was first developed in 1999.

It has become apparent after discussions with winemakers and after numerous investigations conducted by the Institute’s Industry Services team into Dekkera/Brettanomyces spoilage, that the problem in Australia is widespread. In addition, it has been observed that some of Australia’s Premium, Super Premium and Icon red wines exhibit flavour derived from Dekkera/Brettanomyces, and that the effectiveness of widely applied spoilage control measures, in some cases, is sporadic and inconsistent. It was therefore considered important that an investigation into the many possible facets of the relationship between Dekkera/Brettanomyces yeast and wine composition, including sensory attributes, be conducted.

Survey of the concentrations of 4-ethylphenol, 4-ethylguaiacol and other composition variables in Australian Cabernet Sauvignon-based wines

The major spoilage compounds associated with the growth of Dekkera/Brettanomyces yeast in wine are reported to be 4-ethylphenol, 4-ethylguaiacol (Chatonnet et al. 1992, Chatonnet et al. 1995, Singleton 1995), and isovaleric (3-methylbutyric) acid (Licker et al. 1998). However, it cannot be ruled out that other, yet to be identified compounds might also contribute to the characters associated with Dekkera/Brettanomyces spoilage. It has been suggested that 4-ethylphenol is a general ‘marker’ for the present/past growth of Dekkera/Brettanomyces yeast in wine (Fugelsang et al. 1993, Fugelsang 1997). Analytical methods for the quantitative analysis of both 4-ethylphenol and 4-ethylguaiacol in wine have previously been developed at the Institute and applied to a number of Australian red wines in a non-systematic survey (Pollnitz et al. 2000). This survey, although limited, indicated potential problems with Australian wines. Therefore, the Institute decided to focus resources in this area. As a prelude to major investigations into the relationship between Dekkera/Brettanomyces and wine spoilage, and in order to rigorously determine the extent of the problem in Australia, a survey was designed with the primary aim of examining the range of concentrations of 4-ethylphenol, 4-ethylguaiacol and isovaleric acid in Australian wines.

Evidence in the literature (Chatonnet et al. 1992, 1995), and from previous survey work conducted at the Institute (Pollnitz et al. 2000), suggested that a higher proportion of wines made from the variety Cabernet Sauvignon were likely to exhibit Dekkera/Brettanomyces spoilage compared to some other varieties. For this reason commercial Australian Cabernet Sauvignon and Cabernet Sauvignon/Merlot wines, priced in the approximate range of $15 to $120 per bottle, were surveyed in a representative manner. The wines were sourced from five regions: Barossa Valley, Coonawarra, Hunter Valley, Margaret River and Yarra Valley. In many cases, sequential vintages of wines from particular wine producers have been included in the survey. All the wines in the survey were analysed for the concentrations of 4-ethylphenol and 4-ethylguaiacol. Most of the wines were also analysed for the concentration of other compositional variables, including volatile acidity, residual sugar (glucose + fructose), pH and alcoholic strength. Concurrently, work began to develop a gas chromatography-mass spectrometry (GC-MS) method for analysis of isovaleric acid, the third major spoilage compound reported in the literature. Sensory assessments of a number of the survey wines were also conducted in order to relate any perceived spoilage characters to the actual concentrations of the reported key spoilage compounds in the wines. Finally, two 50 mL sub-samples of each wine have been sealed into glass ampoules which have been frozen at approximately –18°C, in
order that further compositional variables can be quantified at a later date.

Analytical and sensory results obtained from survey wines
By the end of July 2003, 228 wines from the vintages 1996 to 2001 had been analysed for the concentrations of 4-ethylphenol and 4-ethylguaiacol. The analytical method for isovaleric acid had only recently been completed at this time, and consequently only a relatively small number of wines (25) had been analysed for the concentration of this compound.

The results of chemical analysis of the 228 wines show that the concentrations of 4-ethylphenol and 4-ethylguaiacol were correlated ($R^2=0.74; p<0.0001$, Figure 1a), which is consistent with the findings of Chatonnet et al. (1992). Although the number of samples analysed for the concentration of isovaleric acid was relatively small, initial results obtained indicate no correlation between the concentrations of 4-ethylphenol and isovaleric acid (Figure 1b), or between the concentrations of 4-ethylguaiacol and isovaleric acid (data not shown). However, it is possible that additive sensory effects exist between these compounds, which may result, for instance, in the presence of isovaleric acid enhancing the apparent intensity of other Dekkera/Brettanomyces-derived characters in

Figure 1. a) Relationship between the concentration of 4-ethylphenol and 4-ethylguaiacol for the survey wines, and b) Relationship between the concentration of 4-ethylphenol and isovaleric (3-methylbutyric) acid for 25 of the survey wines.

Figure 2. The relationship between the concentration of 4-ethylphenol and a) mean overall 'Brett' aroma scores, b) mean Band-aid®/medicinal aroma scores, and c) mean metallic taste scores for a set of 72 wines subjected to both chemical and sensory testing.
Dekkera/Brettanomyces investigations will form a major part of the ongoing sensory relationships are elucidated, and therefore sensory wine. It is considered of the utmost importance that such 1000 µg/L of isovaleric acid.

analysis for isovaleric acid and sensory evaluation, and b) mean aroma scores for a set of 25 wines subjected to both chemical
treatment sessions, the Institute’s tasters generally agreed with
of these three compounds during sensory panel training and
“rancid” (Licker et al. 1998). When investigating the aromas (Chatonnet et al. 1995, Ribéreau-Gayon et al. 2000). The “barnyard” and “stable”, and “smoky” and “spicy”, respectively
and 4-ethylguaiacol have been described using terms including
“cheesy” when describing the aroma of isovaleric acid.

and 4-ethylphenol, and “sweaty” and
describing the aroma of 4-ethylphenol, and “sweaty” and
attributed to Dekkera/Brettanomyces yeast growth in
wine. It is considered of the utmost importance that such sensory relationships are elucidated, and therefore sensory investigations will form a major part of the ongoing Dekkera/Brettanomyces investigations at the Institute.

The aromas associated with the compounds 4-ethylphenol and 4-ethylguaiacol have been described using terms including “barnyard” and “stable”, and “smoky” and “spicy”, respectively (Chatonnet et al. 1995, Ribéreau-Gayon et al. 2000). The aroma of the compound isovaleric acid has been described as “rancid” (Licker et al. 1998). When investigating the aromas of these three compounds during sensory panel training and discussion sessions, the Institute’s tasters generally agreed with the terms indicated in the literature. However, the tasters tended to use the terms “Band-aid®” and “medicinal” when describing the aroma of 4-ethylphenol, and “sweaty” and “cheesy” when describing the aroma of isovaleric acid.

After preliminary sensory panel training and discussion sessions, where a selection of wines with a range of 4-ethylphenol concentrations was presented together with spiked reference standards, a number of attribute terms were agreed upon by the tasters. The attribute terms considered as most appropriate to differentiate and describe wines displaying varying degrees of Dekkera/Brettanomyces-derived aromas were as follows: overall fruit, overall ‘Brett’ aroma, Band-aid®/medicinal, sweaty/cheesy, and VA (volatile acidity). The most appropriate palate attribute terms selected were: fruit flavour, drying, and metallic taste. The tasters were also asked to rate and define other specific aromas or flavours detected in particular wines under the term other. During the formal sensory assessments, which were conducted in isolated booths, the tasters were asked to rate the intensity of the aroma and palate attributes. The tasters scored each of the attribute terms on a scale of 0 to 9, where 0 indicated the attribute was not perceived and 9 indicated that the attribute was of high intensity.

The results of both chemical and sensory analysis of 72 wines show that the concentration of 4-ethylphenol in the wines was correlated with the mean of the sensory panel’s scores for the attributes overall ‘Brett’ aroma ($R^2=0.65$; $p<0.0001$), Band-aid®/medicinal aroma ($R^2=0.75$; $p<0.0001$), and metallic taste ($R^2=0.71$; $p<0.0001$) (Figures 2a–c). However, the results of analysis of 25 wines show that the concentration of isovaleric acid in the wines did not appear to be correlated with the sensory panel mean scores for the attribute sweaty/cheesy aroma (Figure 3a). Non-recognition by the tasters of the sweaty/cheesy character associated with isovaleric acid does not appear to be the reason for the lack of correlation, as data for a wine that was spiked with 500 µg/L and 1000 µg/L of isovaleric acid.

It has been reported that the overall volatile phenolic aroma attributed to Dekkera/Brettanomyces yeast growth in wine is correlated with the concentration of 4-ethylphenol present (Heresztyn 1986), which is supported by the data presented in Figures 2a–c. Although these initial results suggest that the concentration of isovaleric acid does not correlate with sweaty/cheesy aromas, or 4-ethylphenol concentration, it is possible that isovaleric acid may be involved in additive sensory effects which enhance tasters’ overall perception of the intensity of other Dekkera/Brettanomyces-derived characters. Higher numbers of wines will need to be assessed by the sensory panel and analysed for the concentration of isovaleric acid, to investigate this possibility rigorously.

Table 1 shows the mean 4-ethylphenol concentrations by vintage (1996–2001) for all of the wines analysed in the survey. The data presented in this table provide some evidence that a reduction in the mean 4-ethylphenol concentration occurred in Cabernet Sauvignon-based wines produced in the five regions during the 2001 vintage. There were no significant differences (using Student’s t-Test) between the mean concentrations of 4-ethylphenol for the vintages 1996 to 2000 (data not shown). However, the mean concentration of 4-ethylphenol in wines from the 2001 vintage was 46% lower than the mean of the pre-2001 wines (Table 2). Whilst these results are encouraging, care should be taken when interpreting this data as it is possible that climatic or other factors during the 2001 growing season might account for the apparent fall in the concentration of 4-ethylphenol in this group of wines. Furthermore, this conclusion also makes the key assumption that the 4-ethylphenol concentrations did not increase during bottle storage, as the wines pre-2001 vintage were older at the time of analysis. It should also be noted that the mean concentration of 4-ethylphenol recorded in wines from the 2001 vintage remains above the 425 µg/L level which

![Graph A](image1.png)

**Figure 3.** The relationship between the concentration of isovaleric (3-methylbutyric) acid and a) mean sweaty/cheesy aroma scores for a set of 25 wines subjected to both chemical analysis for isovaleric acid and sensory evaluation, and b) mean sweaty/cheesy aroma scores for a wine spiked with 500 µg/L and 1000 µg/L of isovaleric acid.
Sensory perception thresholds\(^1\) and ratios of acetic acid bacteria and lactic acid bacteria. Pre-2001 vintage were older at the time of analysis. The significance of this will not be known until it is conclusively determined whether 4-EP can be formed in bottle.

Chatonnet et al. (1992, 1995, 1997) describe as “elevated” and (negatively) affecting wine quality.

Table 1. Mean 4-ethylphenol (4-EP) concentrations of Australian Cabernet Sauvignon and Cabernet Sauvignon/Merlot wines from five regions (Barossa Valley, Coonawarra, Hunter Valley, Margaret River and Yarra Valley) by vintage 1996–2001.

<table>
<thead>
<tr>
<th>Vintage</th>
<th>Number of samples</th>
<th>Mean 4-EP (mg/L)(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996</td>
<td>6</td>
<td>1164</td>
</tr>
<tr>
<td>1997</td>
<td>26</td>
<td>1256</td>
</tr>
<tr>
<td>1998</td>
<td>32</td>
<td>843</td>
</tr>
<tr>
<td>1999</td>
<td>45</td>
<td>845</td>
</tr>
<tr>
<td>2000</td>
<td>62</td>
<td>890</td>
</tr>
<tr>
<td>2001</td>
<td>51</td>
<td>498</td>
</tr>
</tbody>
</table>

Note: 1. The wines of earlier vintage were older at the time of analysis. The significance of this will not be known until it is conclusively determined whether 4-EP can be formed in bottle.

Table 2. Mean 4-ethylphenol (4-EP) concentrations of Australian Cabernet Sauvignon and Cabernet Sauvignon/Merlot wines from five regions (Barossa Valley, Coonawarra, Hunter Valley, Margaret River and Yarra Valley), and the percentage of wines with concentrations of 4-ethylphenol above 425 mg/L and 1000 mg/L, for the 2001 vintage and vintages pre-2001 (1996–2000).

<table>
<thead>
<tr>
<th>Number of samples</th>
<th>Mean 4-EP (µg/L)</th>
<th>% above 425 µg/L</th>
<th>% above 1000 µg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-2001</td>
<td>177</td>
<td>74</td>
<td>40</td>
</tr>
<tr>
<td>2001</td>
<td>51</td>
<td>45</td>
<td>16</td>
</tr>
</tbody>
</table>

Figure 4. Frequency histograms of the ratio 4-ethylphenol : 4-ethylguaiacol (4-EP:4-EG) for a region with a mean January temperature of 22.7°C (region C) and a region with a mean January temperature of 20.4°C (region D).

Chatonnet et al. (1992, 1995, 1997) describe as “elevated” and (negatively) affecting wine quality. Data from the survey also demonstrate an apparent decrease in the mean concentration of volatile acidity (VA) in the wines studied in the survey for the 2001 vintage (data not shown). It should be noted that the interpretation of this data is based on the assumption that the concentration of VA did not increase during bottle storage, as the wines pre-2001 vintage were older at the time of analysis. Dekkera/Brettanomyces yeast are known to increase VA concentrations in wine (Boulton et al. 1996) and winemaking strategies employed to control Dekkera/Brettanomyces growth may also, therefore, lead to a decrease in VA production. In addition, measures used to control Dekkera/Brettanomyces will also result in the control of other microorganisms capable of VA production, such as acetic acid bacteria and lactic acid bacteria.

Sensory perception thresholds\(^1\) and ratios of 4-ethylphenol and 4-ethylguaiacol

Chatonnet et al. (1992) reported that the sensory perception thresholds for 4-ethylphenol and 4-ethylguaiacol alone in a red wine were approximately 605 µg/L and 110 µg/L, respectively. However, when these two compounds are both present in wine, which is invariably the case, the sensory perception threshold of 4-ethylphenol is lower (Chatonnet et al. 1992). It should be noted that Chatonnet et al. (1992) point out that because of the variability in composition of different wines, the perception thresholds quoted are only indicative. Evidence from sensory assessments and analyses conducted at the Institute also suggest that the sensory perception threshold of 4-ethylphenol depends very much on the style and structure of the wine. For example, for a light bodied red wine with little oak influence, the perception threshold of 4-ethylphenol may be as low as approximately 350 µg/L. However, the perception threshold of 4-ethylphenol in a full bodied red wine with intense fruit and considerable oak influence may be as high as approximately 1000 µg/L. That is, the extent to which the sensory properties of a wine may be affected by 4-ethylphenol will depend on the concentration and intensity of other wine components that may mask (e.g. volatile oak compounds), or accentuate (e.g. 4-ethylguaiacol), the aroma of 4-ethylphenol.

The ratio of 4-ethylphenol to 4-ethylguaiacol (4-EP:4-EG) is reported to be typically in the order of 8:1 (Chatonnet et al. 1992, Ribéreau-Gayon et al. 2000). The results from the Institute survey show that 4-EP:4-EG in Australian Cabernet Sauvignon and Cabernet Sauvignon/Merlot wines appears to differ between regions. In addition, there is a large range of ratios in different wines within each region. These points are demonstrated in Figure 4, which presents the range of 4-EP:4-EG for a warm region (MJT\(^2\) of 20.4°C) and a hot region (MJT\(^2\) of 22.7°C).

It is possible to speculate on explanations for the differences in the apparent range of 4-EP:4-EG within and between regions, which may include:

- differences in the concentrations of the precursor compounds for 4-ethylphenol and 4-ethylguaiacol (coumaric and ferulic acids, respectively) between regions;

1. The perception threshold corresponds to the minimum concentration under which 50% of tasters on a panel statistically fail to recognise the difference between a spiked sample and a control (unspiked) sample of wine.
2. MJT denotes ‘mean January temperature’. Note that these temperatures and the classifications of ‘warm’ and ‘hot’ for the regions are those indicated by Dry and Smart (1988).
• other compositional differences between regions, e.g. yeast nutrient concentrations;
• differences in winemaking practices between comparatively warmer and cooler regions, such as the extent of the use of maceration enzymes;
• differences in the ability of different Dekkera/Brettanomyces strains to synthesise 4-ethylphenol or 4-ethylguaiacol;
• differences in the temperature of wines at critical times, e.g. during and after malolactic fermentation; and
• differences in the use of oak.

The Institute’s ongoing investigations will seek to elucidate, over time, the relative importance of these and other factors.

The compositional survey was originally conceived to investigate the incidence and degree of Dekkera/Brettanomyces activity in Australian Cabernet Sauvignon-based wines. The survey has subsequently taken on greater importance as relationships between various compositional measures become apparent, and new methods to quantify various compositional variables are progressively developed. Hence, methods development will remain an important part of the Dekkera/Brettanomyces investigations. Methods for the analysis in wine of the direct precursors to 4-ethylphenol and 4-ethylguaiacol have recently been completed, and analysis of the survey samples for these precursors will be undertaken (using the stored glass ampoules previously described).

Methods for analysis of these compounds, and for the tartaric acid esters of coumaric and fenolic acids in grape homogenates, are currently being investigated. Methods for analysis of 4-vinylphenol and 4-vinylguaiacol, the intermediate precursors of 4-ethylphenol and 4-ethylguaiacol, in wine, have been developed (Elsey 2003). Amino acid profile analysis of the survey samples has begun, which may provide some indication of the nutritional requirements of Dekkera/Brettanomyces yeast.

Control of Dekkera/Brettanomyces during winemaking

There are a number of measures which appear in many cases to be effective in controlling Dekkera/Brettanomyces growth, and in preventing spoilage. It must be stressed that some wine compositional variables are linked, such that a change in one variable, whether intended or not, can have a potentially dramatic flow-on effect leading to the occurrence of an instability, including Dekkera/Brettanomyces spoilage. The main factors associated with the control of Dekkera/Brettanomyces are:

• General cleaning/sanitation;
• Residual nutrients – glucose + fructose and nitrogen;
• Sulfur dioxide;
• pH;
• Turbidity/clarification; and
• Barrel management.

These factors are discussed below, however, the winemaker must bear in mind the interdependence of these factors and address all of them, in order to maximise control of the proliferation of Dekkera/Brettanomyces yeast.

General cleaning/sanitation

Rigorous cleaning and sanitation are important elements in the control of all microbiological infections, including Dekkera/Brettanomyces. Regular cleaning and sanitising of crushers, presses and must lines during vintage, at least daily, is the first step in reducing the populations of unwanted microorganisms. Cleaning processing equipment can prevent the accumulation of organic deposits that may serve as a source of nutrients, and as a haven for microorganisms.

Regular cleaning of tanks and oak barrels (barrel sanitisation is discussed below) should also be an important component of a winery’s quality control program. In addition, winemakers should be aware of the microbial history of any wine entering the winery and any wine that might be used for topping barrels. It is also important for winemakers to know the history and condition of any used barrels brought into the winery.

Residual nutrients

Controlling potential nutrients, including carbon and nitrogen sources, is very important in the control of Dekkera/Brettanomyces, as all yeast need a source of carbon and nitrogen for growth and metabolism.

Residual sugar and nitrogen

Dekkera/Brettanomyces growth rate is enhanced with increasing concentrations of sugar, yet substantial populations of Dekkera/Brettanomyces may develop at sugar levels of less than 2 g/L (Fugelsang 1997). The Institute has observed wines containing a high proportion of budding yeast cells that have concentrations of fermentable sugar below 2 g/L. Dekkera/Brettanomyces yeast are not only capable of growth and metabolism of glucose and fructose (G+F), but can also metabolise other sugars, including galactose and trehalose (Chatonnet et al. 1995, Lodder 1970) and arabinoose (Ribéreau-Gayon et al. 2000). Chatonnet et al. (1995) indicated that the utilisation of 275 mg/L of fermentable sugars by Dekkera/Brettanomyces was sufficient to produce 425 µg/L of 4-ethylphenol, a concentration equal to that which is reported to have a negative impact on the quality of many wines. Chatonnet et al. (1992) also indicated that the assimilation of 300 mg/L of sugars by Dekkera/Brettanomyces was sufficient to produce a population of more than 3000 cells/mL, a population considered by Chatonnet and colleagues to be more than enough to cause a concentration of 4-ethylphenol greater than 600 µg/L in red wine.

During one investigation conducted at the Institute on samples of a wine that were affected by Dekkera/Brettanomyces, the 4-ethylphenol concentration between individual samples was found to vary by up to approximately 2000 µg/L. The concentration of G+F in the samples varied between 100 mg/L and 700 mg/L. These results indicate that Dekkera/Brettanomyces can produce concentrations of 4-ethylphenol that are clearly above the sensory threshold, from growth utilising concentrations of residual sugar in wines that many winemakers would consider ‘dry’.

Winemakers should not automatically assume that alcoholic fermentation is complete in their red wines. The concentration of residual sugar may be below the sensory threshold, and the wines may appear to be dry when tested by hydrometry. However, there may be significant concentrations of G+F in the wines, and therefore the analysis of all red wines for the concentration of residual sugar should be standard practice. An enzymatic assay (or other procedure which gives an accurate result) for the concentration of G+F, rather than a reducing sugar test, is the safest way to be completely sure that primary fermentation is complete.

Analysis of many thousands of G+F results in the Institute’s Analytical Service database revealed that there has apparently been a steady increase in the average sugar content of red wines analysed over the past 18 years (Figure 5a). An attempt was made to remove from the data presented in Figure 5a, wines that had been deliberately made with residual sugar, so that the results in Figure 5a are not skewed. The increase in the concentration of residual sugar corresponds with an increase in the average alcohol concentration in red wines (Figure 5b). There has also been a general increase in the
highest maximum concentrations of alcohol recorded over this period (data not shown). The data for the year 2002 indicate that the mean alcohol concentration in red wines was greater than 14 % v/v, and that 25 % of wines had alcohol concentrations between 14.8 and 16.5 % (data not shown). It is probably reasonable to assume that increased alcohol concentration is a contributing factor to an increasing number of primary fermentations that result in elevated residual sugar concentrations after the completion of fermentation.

It was mentioned above that some wine compositional variables are linked and that changing one winemaking variable can have a potentially dramatic flow-on effect. Such a change, for instance, may be as simple as picking fruit on average one degree Baumé riper than in previous years. The resulting higher alcohol concentration in the wine, all other things being equal, may lead to fermentation problems with the potential for consequent higher concentrations of residual G+F post fermentation. These sugars may then become a substrate for microbial activity, including Dekkera/Brettanomyces. This situation may be exacerbated by excessive nitrogen supplementation of slow fermentations with di-ammonium phosphate (DAP) as a yeast nutrient, in situations where limiting nitrogen is not the reason for the slow fermentation. DAP is often added to fermentations in an attempt to accelerate fermentation rate and to ensure completion of the fermentation. However, if this additional nitrogen is not metabolised during fermentation, the consequently higher concentration of nitrogen in the wine may become a substrate for subsequent microbial activity. Wines containing higher concentrations of alcohol will also tend to take longer to complete malolactic fermentation (MLF), which is considered a crucial time for potential wine spoilage, as at this stage many wines will have a comparatively high pH, with little or no protection from sulfur dioxide (SO₂). It is likely that post MLF, residual sugars coupled with the presence of residual nitrogen will favour the growth of unwanted microflora, including Dekkera/Brettanomyces.

Staff members of the Institute's Industry Services team have liaised with wineries that have had major Dekkera/Brettanomyces problems, but which have overcome them in the past two or three years. One strategy adopted has been to keep fermentations warm during pressing, and for between 12 and 24 hours afterwards. This has the effect of reducing the residual sugar concentrations in these fermentations to less than 0.2 g/L of G+F. Analysis of juices and musts for the concentration of yeast assimilable nitrogen (YAN) will also help winemakers avoid making unnecessary additions of DAP, which may lead to the presence of residual nitrogen after fermentation. A reduction in the concentration of residual nutrients (in the form of G+F and nitrogen) leads to a reduction in the probability and degree of subsequent microbial spoilage.

Figure 6 shows that wines in the survey that contain the highest concentrations of 4-ethylphenol also contain relatively low concentrations of G+F, presumably because Dekkera/Brettanomyces have utilised the available residual sugars.

SO₂ and pH

After many discussions with winemakers in relation to Dekkera/Brettanomyces, it became evident that many problems arose when the use of SO₂ at picking and/or crushing had been reduced or eliminated. In many cases this was the only apparent change in winemaking procedures in particular wineries, during periods where Dekkera/Brettanomyces spoilage became evident for the first time. Once again, this is considered to demonstrate how changing one winemaking practice can have unwanted flow-on effects. The probability of microbiological instability in wine can be substantially reduced by decreasing the population of unwanted...
microorganisms (including Dekkera/Brettanomyces) by the addition of SO₂ during the harvesting process and crushing.

The discussions held between winemakers and Institute staff have also revealed an apparent trend in many wineries to add several small additions of SO₂ during winemaking, rather than larger, less frequent additions. When SO₂ is added to wine, as a guide only approximately 35 to 40% is yielded in the free form (depending on pH and other factors), which is the form that has the important antioxidant and antimicrobial properties. Multiple small additions of SO₂ to a wine rather than one equivalent large addition may result in the concentration of free SO₂ failing to reach a level where it confers a substantive antioxidant or antimicrobial benefit. Larger, less frequent SO₂ additions, yielding greater concentrations of free SO₂, are therefore considered much more effective in achieving the desired antioxidant and antimicrobial effects. After the addition of SO₂ during the harvesting process and/or crushing, another large addition should be made to red wine following the completion of MLF, as it is during MLF when the wine has little or no protection from SO₂, that the population of Dekkera/Brettanomyces and other microorganisms is considered most likely to increase. Typically, it is during aging in barrels after MLF that Dekkera/Brettanomyces spoilage of wine occurs (Chatonnet et al. 1992). Therefore, it is beneficial to make a large addition of SO₂ to red wine after MLF, before aging in barrels, in order to reduce the populations of Dekkera/Brettanomyces and any other potential spoilage microorganisms.

Whilst the appropriate use of SO₂ is one of the means of controlling Dekkera/Brettanomyces, winemakers must bear in mind that these yeast have a relatively high resistance to SO₂, similar to that of Saccharomyces cerevisiae (Romano and Suzzi 1993). Beech et al. (1979) determined that the populations of Dekkera/Brettanomyces (and other wine spoilage microorganisms) in white wines could be reduced by a factor of 10⁴ in 24 hours by maintaining a concentration of 0.8 mg/L of molecular SO₂. Winemakers generally refer to three categories of SO₂: free, bound and total. The free SO₂ is defined as the sum of the unreacted ionic forms, which are the molecular, bisulfite and sulfite forms (Sneyd et al. 1993). Note that the molecular form is the most important in winemaking, as it is this species which is responsible for the antimicrobial activity, and indirectly, the antioxidant properties, of SO₂ in wine (Boulton et al. 1996). It must be understood that the amount of SO₂ in the molecular form, and consequently the effectiveness of the free SO₂, depends on the pH of the wine (Boulton et al. 1996, Margalit 1997, Rankine 1989, Zeecklein et al. 1995). The higher the pH, the less free SO₂ will be in the useful molecular form. Therefore, at higher pH more free SO₂ is required to achieve the desired level of molecular SO₂ and subsequent antioxidant and antimicrobial properties.

In relation to the control of Dekkera/Brettanomyces, Ribéreau-Gayon et al. (2000) indicate that a free SO₂ concentration of 30 mg/L “always results in the total elimination of all viable populations after 30 days”. This statement may not necessarily hold true for all cases in Australian winemaking. As discussed above, the effectiveness of free SO₂ depends on the pH of the wine. For example, for a wine with a pH of 3.4 and a concentration of free SO₂ of 30 mg/L, the desired concentration of 0.8 mg/L of molecular SO₂ will be achieved. However, for a wine with a pH of 3.7 and free SO₂ concentration of 30 mg/L, the desired concentration of 0.8 mg/L of molecular SO₂ will not be achieved, and in fact there will be less than 0.4 mg/L of molecular SO₂ present in such a wine (Margalit 1997). Clearly, 0.4 mg/L of molecular SO₂ will not be as effective in controlling Dekkera/Brettanomyces as 0.8 mg/L of molecular SO₂. In support of this, Industry Services staff members have investigated several wines displaying Dekkera/Brettanomyces problems that have had free SO₂ concentrations in excess of 30 mg/L. Thus, a holistic approach is required to control Dekkera/Brettanomyces, and it is the way that SO₂ is used in combination with other winemaking factors that is considered a key element.

The relationship between the 4-ethylphenol concentration and the pH of the survey wines can be seen in Figure 7. Although there is no obvious relationship, the wines with the highest concentrations of 4-ethylphenol also tend to have higher pH values. It has been reported that lower pH may slow the growth of Dekkera/Brettanomyces (Kalathenos et al. 1995, Van Zyl 1962), however, slower Dekkera/Brettanomyces growth in wines with low pH may simply be a consequence of the increased effectiveness of SO₂ at lower pH. One winery liaising with the Institute had a young red wine in tank with a G+F concentration of 0.8 g/L. The pH of this wine was adjusted to 3.3, yet within a period of three weeks the concentration of 4-ethylphenol in the wine rose from approximately 400 µg/L to 2000 µg/L. This example demonstrates that low pH alone will not prevent spoilage by Dekkera/Brettanomyces yeast and emphasises that a holistic approach is required when attempting to control these yeast.

The analysis of many thousands of pH results in the Institute’s Analytical Service database (data not shown) reveals that there has been little change in the average pH of Australian red wines over the past 18 years. However, the number of wines with what might be considered high pH (3.65 and above) has increased substantially over this period. Further, ‘problem wines’ submitted to the Institute for investigation tend to have substantially higher pH than the general population, perhaps indicating that, all other things being equal, a higher pH will increase the risk of subsequent wine instabilities occurring, including Dekkera/Brettanomyces spoilage. The fact that the average pH of red wines has remained relatively constant during a period when the incidence of Dekkera/Brettanomyces spoilage appears to have increased in many wineries, of itself suggests that any pH effect observed is most likely related to the effectiveness of SO₂. The effect of wine pH on the growth of Dekkera/Brettanomyces will be studied as part of the Institute’s Dekkera/Brettanomyces investigations.
The effect of a delay in the racking of a red wine on the concentration of 4-ethylphenol was investigated by Chatonnet et al. (1995). When racking was delayed by approximately six weeks the 4-ethylphenol concentration in each of several barrels of a particular wine approximately doubled, and the percentage of barrels considered to be tainted with 4-ethylphenol rose from 57% to 100%. Chatonnet et al. (1995) considered that the concentration of free SO₂ remaining in the wine after the delay in racking was too low to sufficiently protect the wine. Thus, if racking is delayed, free SO₂ may be bound, Dekkera/Brettanomyces (and other microorganisms) may proliferate and the concentration of 4-ethylphenol may increase markedly. It is advised that winemakers clarify red wines and add SO₂ as soon as possible after MLF. It is also considered advantageous to identify at the earliest time point possible the completion of MLF, to allow sufficient time for clarification and the addition of SO₂, taking place. As discussed above, the SO₂ added after MLF should be the single largest addition that the wine receives.

Oak barrel management

New versus old barrels Dekkera/Brettanomyces spoilage has occasionally been observed at the Institute in bottle fermented sparkling wines and other still, white table wines. However, Dekkera/Brettanomyces spoilage is generally associated with barrel-aged red wines and in particular, with old barrels (Chatonnet et al. 1992). Microbial colonisation of wood was studied by Swafield and Scott (1995), who were able to show that a variety of microorganisms were capable of establishing populations in the porous structure of wood, which persisted after emptying, cleaning and refilling cycles. It appears that one natural habitat for Dekkera/Brettanomyces is wood, as it has been reported that Brettanomyces has been isolated from tree exudates (Van der Walt 1970). It is therefore considered likely that Dekkera/Brettanomyces are capable of penetrating the porous, cellular structure of oak to establish large populations in used barrels.

Pollnitz et al. (2000) found that wine stored in one set of shaved and refired old oak barrels contained up to 85% less 4-ethylphenol and 4-ethylguaiacol than wine stored in barrels of the same age but which had not been shaved and refired. This effect was attributed to reduced microbiological load on the inner surface of the barrels due to the shaving and refiring process. These results also suggest that old oak barrels may pose a greater risk of spoilage by Dekkera/Brettanomyces than new oak barrels. However, data from the Institute’s commercial Analytical Service database demonstrate a slight positive correlation between the concentrations of oak lactones and vanillin, and the concentration of 4-ethylphenol (data not shown). Higher concentrations of oak lactones and vanillin are found in wine stored in new barrels compared with older barrels. Thus, the fact that higher concentrations of 4-ethylphenol appear to be associated with higher concentrations of oak lactones and vanillin suggests that Dekkera/Brettanomyces spoilage is not just a problem with old oak. Both old and new oak appear to have the potential to promote the growth of Dekkera/Brettanomyces yeast, but perhaps for differing reasons depending on the age of the barrels. It is possible that certain substrates for Dekkera/Brettanomyces growth are more plentiful, and more readily extracted from new oak compared to older oak. It has been reported that both Dekkera and Brettanomyces are capable of utilising the disaccharide cellobiose (Fugelsang et al. 1993). Cellobiose is an α-linked disaccharide of glucose, a fragment of cellulose, which is reported to be an intermediate resulting from the charring or toasting process involved in the production of oak barrels (Boulton et al. 1996, Fugelsang et al. 1993). The presence of cellobiose in oak barrels could also be due to the action of fungal cellobiohydrolases on cellulose. New barrels may, therefore, be better sites for the growth of Dekkera/Brettanomyces than older, used barrels where the cellobiose might have been previously metabolised. However, cellobiose utilisation varies between the strains and species of Dekkera/Brettanomyces (Fugelsang et al. 1993) and the relative importance of this aspect may need to be further studied as part of the Institute’s Dekkera/Brettanomyces spoilage project.

From data collected by a number of wineries, it is also possible to hypothesise that wine stored in new barrels could lose SO₂ more quickly than the same wine stored in older wood. Anecdotal evidence from some winemakers reported to Industry Services staff indicates that this effect may apparently account for as much as 15 mg/L of free SO₂ over six months of storage.

Barrel sanitation

Effective sanitation of barrels is considered crucial if control of Dekkera/Brettanomyces is to be achieved. However, a holistic approach is required, and barrel sanitation alone will not solve a Dekkera/Brettanomyces problem. Whatever sanitation procedure is utilised, it will be wasted if barrels are re-contaminated with wine containing high populations of Dekkera/Brettanomyces. Because of the nature of wooden barrels, there is no certain way to sterilise a barrel once Dekkera/Brettanomyces is established. Between five and six litres of wine can be absorbed into the wood of a barrique (225L barrel) (T. Royal, pers. comm., 2003), creating an ideal environment for Dekkera/Brettanomyces growth. Leaching of 4-ethylphenol into wine from barrels previously contaminated with Dekkera/Brettanomyces may also be an issue.

In a study of different barrel sanitising treatments on the growth of acetic acid bacteria, Wilker and Dharmadhikari (1997) concluded that hot water treatment appeared to be the most effective in reducing populations of these microorganisms. Although there is no proven technique for sterilising barrels, staff of the Institute’s Industry Services believe that heat is the most effective sanitisation method available to many wineries. To sanitise a barrel, it is recommended that the barrel is filled with water at 85°C or above, and is left to stand for at least 15 minutes to allow time
for the heat to penetrate the wood, in order to kill microorganisms. The Institute is aware that some wineries are utilising ozone for barrel sanitisation. However, the Institute has not investigated the use of ozone, and there is a paucity of data from well-designed experiments in the literature. Whilst sanitising barrels may be useful in achieving immediate reduction in Dekkera/Brettanomyces populations, it is considered more effective to reduce the overall risk of infection in the first place by addressing all of the control factors discussed above.

Evidence of control
Some winemakers have apparently been able to reduce the incidence of high 4-ethylphenol concentrations in their wines by implementing the winemaking strategies outlined above, which aim to control the proliferation of Dekkera/Brettanomyces yeast. For example, winery A in Table 3 was one of the first to contact the Institute regarding Dekkera/Brettanomyces control, late in 1998. During 1999 and subsequently, staff from winery A and Institute staff have had many discussions on this issue. Similarly, Winery B in Table 3 first contacted Industry Services regarding the same issue early in 2000. These two wineries are noteworthy for the magnitude of the reduction in 4-ethylphenol concentrations that they have been able to achieve, and it is interesting to note that there appears to be no correlation between the concentrations of 4-ethylphenol and isovaleric acid in the wines referred to in Table 3. It is clear the winemaking staff of each of these wineries have devoted a great deal of time, thought and resources to controlling this problem, and the Institute acknowledges the valuable interaction with staff of these companies, which has greatly contributed to the Institute’s understanding of the issues relating to Dekkera/Brettanomyces spoilage.

Aims of the AWRI’s ongoing investigations into the relationships between Dekkera/Brettanomyces yeast and wine in Australia
Information originating from the AWRI relating to Dekkera/Brettanomyces yeast that is discussed in this paper has been generated by a number of Institute teams under various GWRDC-funded research projects. In July 2003 these activities were combined into a dedicated and ongoing project, which has been alluded to on a number of occasions above. The project has three key objectives.
1. To establish why only a portion of the wines that contain viable Dekkera/Brettanomyces yeast become spoilt.
2. To determine to what extent the propensity of wines to become spoilt is:
   - Environmental: i.e., due to factors such as differences in concentration of precursor compounds, or strains of Dekkera/Brettanomyces yeast, found in different regions.
   - Cultural: i.e., due to differences in vineyard, winemaking and packaging practices.
3. To elucidate strategies to manage Dekkera/Brettanomyces yeast based on the relative importance of various environmental and cultural factors identified.

Some of the key activities that have been identified as necessary to realise these objectives include:

- Continuation of the survey into compositional variables in wine, including those reported to be the major spoilage compounds formed by Dekkera/Brettanomyces yeast.
- Continuation of methods development, including methods for the analysis of coumaric and ferulic acids and the tartaric acid esters of coumaric and ferulic acids, in grapes.
- Continuation of sensory analysis of survey samples, aiming to reliably rate the intensity of reported Dekkera/Brettanomyces spoilage compounds in wine, and to determine the existence of any other compounds that may contribute to Dekkera/Brettanomyces spoilage, or to the overall perception of Dekkera/Brettanomyces spoilage.
- To analyse several hundred isolates of Dekkera/Brettanomyces yeast from geographically diverse Australian wine regions, in order to investigate and quantify the genetic diversity of strains that exist in Australian wineries.
- To identify the most genetically diverse strains, or groups of strains of Dekkera/Brettanomyces, and use these strains in micro-fermentations (in model wine and real wine) to which important precursors and nutrients have been added, in order to ascertain any differences between the strains in their ability to synthesise spoilage compounds, or differences in nutritional requirements or preferences.
- To conduct surveys of the concentrations of precursor compounds of various varieties in different regions.
- To conduct winemaking trials to follow changes in concentrations of reported spoilage compounds and their precursor compounds in fruit throughout ripening and fermentation, and in wine through MLF, barrel storage and a variety of other winemaking processes.
- To conduct a bottling and subsequent storage trial, to investigate any effects of temperature and bottle orientation on the growth of Dekkera/Brettanomyces yeast post-bottling.

Conclusion
The tainting of wine by compounds produced by Dekkera/Brettanomyces yeast is a world-wide problem and presents possible dangers for the Australian wine industry, with an apparent recent increase in the number of wine press articles that refer to this issue. However, the widespread nature and seemingly increasing wine trade and public awareness of this problem in itself presents an opportunity for Australian...
winemakers to gain a competitive advantage if they are able to manage and control it. Data from the ongoing Institute survey suggest that the concentrations of the key Dekkera/Brettanomyces-derived spoilage compounds may in fact be decreasing in Australian Cabernet Sauvignon-based wines. However, such observations must be confirmed by much more extensive data sets to give credence to this hypothesis.

Findings of this project to date confirm reports in the literature that 4-ethylphenol and 4-ethylguaiacol appear to be the main compounds correlated with Dekkera/Brettanomyces-derived off odours in red wines. The results also tend to suggest that the concentration of isovaleric acid is independent of the concentrations of these key spoilage compounds. Therefore, isovaleric acid may not be directly responsible for off odours ascribed to the activity of Dekkera/Brettanomyces. However, isovaleric acid may be involved in additive sensory effects with other Dekkera/Brettanomyces-derived compounds, thereby enhancing the apparent aroma intensity of those other compounds.

Substantial reductions in 4-ethylphenol concentrations in red wines may be achieved by implementing winemaking strategies that aim to reduce the population and proliferation of Dekkera/Brettanomyces yeast throughout the winemaking process. However, it is apparent that a holistic approach is required, with many winemaking aspects being addressed concurrently. In situations where only some of the suggested control strategies are implemented it is likely that reductions in 4-ethylphenol concentrations will be sporadic. It is apparent that wineries that have taken a holistic approach, and which have dedicated significant resources to the issue, have achieved dramatic reductions in 4-ethylphenol concentrations in their wines.

The Institute's ongoing Dekkera/Brettanomyces investigations will seek to establish, over time, the reason why some wines appear not to become spoilt when viable Dekkera/Brettanomyces cells seem to have been present at some stage during their production. Initial investigations will seek to determine to what extent the propensity of wines to become spoilt is due to environmental and/or cultural factors, and flowing from this it is hoped that the project will identify further strategies to manage Dekkera/Brettanomyces during winemaking, for the optimisation of potential wine quality.

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References


Elsey, G. The development of stable isotope dilution assays for the quantification of the important aroma compounds citronellol, 4-vinylguaiacol and 4-vinylphenol in wine: summary of work performed by Katryna van Leeuwen and supervised by Dr Gordon Elsey. Technical Review. (142): 4–5; 2004.


