The Role of Oxygen in the Aging of Bottled Wine

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Abstract.

There are many factors, which are important in the evolution of wines during aging in bottle. Whilst it is accepted that the interaction of limited amounts of oxygen with bottled wine (via closure ingress) will not benefit white wine, it is widely held that limited amounts of oxygen will benefit robust red wines. As consumer acceptance continues of many different forms of wine bottle closures, the need to better understand the role of oxygen during red wine aging in bottle has become paramount, particularly for red wines intended for long term maturation. We have analysed both still and sparkling red wines held in bottle for up to 20 years to determine how the maturation process evolves with varying amounts of oxygen available to the wine. From this, we have been able to demonstrate that red wine will continue to mature and develop both with and without additional oxygen being available to the wine. However, increased availability of oxygen greatly increases the rate at which a red wine will mature, and hence shortens the drinking life of the wine. In an anaerobic environment such as a bottle of red wine sealed with a screw cap or crown seal, some wines may develop reductive characteristics. In contrast, bottled red wine stored in a more aerobic environment such as with a synthetic closure, will prematurely develop oxidised characters.

1. Introduction

All wines will receive some bottle aging before consumption. For the majority of wines, this may only be intended for several months while the bottle progresses through the supply chain to the consumer with the producer's objective to maintain the wine in the condition in which it was packaged. However, for many premium wines, the situation is somewhat more complex, as bottles may be matured for several years prior to release, and may then be expected to cellar for several decades. During cellaring, the wine is expected to progress through three distinct development phases (7); viz a maturing phase followed by a mature or peak phase, and then followed by a declining phase. Whilst a range of factors may influence the rate at which a wine evolves during its bottle aging (e.g. initial wine composition, storage temperature, light exposure), this study focuses on the effect of oxygen (or its practical absence) during bottle maturation.

The uptake of oxygen by wine is slow and prolonged under normal wine conditions (1). Singleton (19) suggested that the consumption of oxygen would continue unabated in wine exposed to air with spoilage oxidation by around 10 saturations (60 mL O_2/L) for white wines and 10 to 30 saturations (60 – 180 mL O_2/L) for red wines. Whilst such high levels of wine oxygenation (and subsequent oxidation) are rarely encountered in practice, it is widely accepted that limited oxygen exposure, particularly early in the life of a red wine is desirable for phenolic development. The mechanisms of oxidative reactions in non-bottled wine have been subject to thorough review (1,6,19) with the oxidation of ethanol to acetaldehyde (via hydrogen peroxide) widely accepted as a major factor (6). This reaction followed by subsequent reactions of the acetaldehyde with tannins and anthocyanins to form ethyl-linked structures is very complex. It is affected by a range of wine parameters including phenolic content, temperature, pH, sulphur dioxide concentration (bisulphite) and light penetration (5, 6,9,11,19,23). In addition to the oxygen mediated reactions, other reactions involving phenolics but not oxygen, also take place in bottled wine (18). These reactions include polymerisation and condensation of tannins, breakdown and condensation of anthocyanins, and precipitation of larger polymers. In addition to reactions that affect colour and tannin structure, the presence or absence of oxygen also influences wine aroma development. Ribereau-Gayon *et al* (18) suggest that the bouquet of a majority of great wines develops as a result of reduction, whereas in contrast flatness (particularly attributable to aldehydes) is an oxidative phenomenon.

The requirement to better understand the practical role of oxygen in determining wine style during bottle aging has been brought to the fore with the increasing use of alternative wine bottle closures by the Australian wine industry (15). Previous research on bottled wine has generally been limited to white wine, ranging from 25 years ago (17) to the more recent closure trial conducted by The Australian Wine Research Institute (AWRI) (10). These two studies focussed on white wine, and in particular aromatic wine styles. Rankine *et al*. (17) compared wines bottled with screw cap and cork closures, and concluded that white wines matured better under screw cap than under cork but did not elaborate any further than this. The AWRI study used screw cap, synthetic and cork closures, and also found that screw cap preserved the wine better than both synthetic and cork based closures. However, they found the wines stored under screw cap showed a reduced character, probably due to the limited amount of oxygen in the wine's environment (10). Further studies at the AWRI by Kwiatkowski *et al* (13) have found that red wine bottled with ullage heights of 4 and 64 mL respectively showed no significant differences for reduced characters after 18 months of storage.

A recent article by Stelzer (22) has shown that winemakers have many and varied opinions on whether oxygen is necessary for red wine maturation in bottle. It is now very evident that the wine industry requires additional information, particularly in the area of full-bodied red wine (15). This study investigates:

- The effects of oxygen ingress via natural cork, screw cap (roll on tamper evident, ROTE), and synthetic closures on a super premium still red wine, evaluated over 7 years post-bottling.
- The effects of oxygen ingress via crown seal closures on sparkling red wines over a 20-year period post-bottling.

The results reported will contribute to the understanding of the 3 phases of development and maturation of red wines in both slightly aerobic and virtual anaerobic conditions, in a practical and commercially relevant format.

2. Methods

Bin 389 - A Penfolds super premium (Bin 389) still red wine from the 1996 vintage was subjected to standard commercial winemaking and storage conditions prior to bottling. The wine was a blend of Shiraz and Cabernet Sauvignon, sourced from various vineyards within South Australia and matured in new and used American oak casks for 14 months prior to bottling (July 1996 to September 1997). Copper fining was used to remove any sulphide residues in the wine after fermentation (May 1996). A selection of closures were used in commercial bottling equipment, consisting of ROTE, natural cork (44 x 24 mm reference 2) and 2 commercially available synthetic closures. Chemical analysis of the wine immediately post-bottling (December 1997) is summarised in Table 1. All chemical analysis was conducted in accordance with methods outlined by Iland *et. al*. (12) at Southcorp Wines' Nuriootpa laboratory. Once bottled, all wines were stored on site at Nuriootpa in an underground cellar prior to assessment. All bottles were stored on their side, except ROTE, which was stored upright.

Table 1 Chemical Analysis of Bin 389 at point of bottling (11/12/1997).

Anolyte	Concentration	Anolyte	Concentration
Residual Sugar (g/L)	1.7	Fe (mg/L)	2.1
Alcohol (%)	14.2	Ca (mg/L)	55.5
Free SO2 (mg/L)	30	Na (mg/L)	98
Total SO2 (mg/L)	87	K (mg/L)	1217
pH	3.54	Acetaldehyde (mg/L)	20.5
Titratable Acidity (g/L)	6.5	Ethyl Acetate (mg/L)	79.5
Volatile Acidity (g/L)	0.73	Methanol (mg/L)	135.5
Malic acid (g/L)	0.09	Turbidity (NTU)	0.2
Cu (mg/L)	0.05		

Chemical and Spectral Analysis (20) of the wine was conducted at 2.5, 4.5 and 6.5 years following bottling. Sensory analysis was also conducted at the same time using panels of between 8 – 12 Southcorp Winemakers. Each replicate used for tasting was a blend of equal proportions from 3 bottles of that replicate and a randomised block design with ranking (16). Panellists were instructed to rank wines from 1 ("most developed") to 4 ("least developed"). Data was analysed using a nonparametric Friedman-type statistic, and a nonparametric analogue to Fisher's least significant difference (16).

An additional tasting using descriptive analysis was conducted at 7 years following bottling (December 2004) at the AWRI using a trained panel, consisting of 7 members of the AWRI and 3 Southcorp winemakers. The panellists were both male and female, all had extensive experience in wine tasting and in some cases descriptive analysis. Prior to formal assessment, a session for term and attribute selection, as well as judge training was held. The list of agreed attributes necessary to describe the wines was fruit intensity, spicy, caramel, chocolate, developed, reduced, oxidised and tri-chloro-anisole taint (TCA). Each panellist was then presented with 4 randomised single glass samples (30 mL aliquot) each representing a wine from a closure type. Panellists were isolated in single booths with sodium lighting and were asked to score each attribute on a scale from 0 to 9 for each tasting sample. Results were registered and analysed using analysis of variance (ANOVA) and XL.Statistics (2).

Sparkling Red Wine - All fruit for the sparkling red (SR) wines was sourced from the Seppelt Great Western vineyard over a period of 20 years. Base wines from the 1984, 1987, 1994 and 1999 vintages were processed and fermented with standard winemaking practices. Base wine was held in large oak vats for approximately 16 months prior to secondary fermentation in crown sealed bottles. All wines were stored in the underground cellars at Seppelts, Great Western in constant temperature (15°C) until the point of evaluation. Wines were disgorged 4 days prior to sensory evaluation to remove yeast sediment. All SR wines analysed had been stored in a virtual anaerobic environment through the sealing capacity of a crown seal. Chemical analysis for all base wines was conducted at Southcorp Wines Seppelts laboratory in line with current industry practices at the time of analysis. These wines were also held under constant pressure (approximately 6 bar) for the duration of their storage period, which in some cases spans 15 - 18 years. A summary of the base wine analysis is shown in table 2.

Table 2. Summary analysis of SR Shiraz prior to secondary fermentation.

Vintage	**Alcohol %**	**pH**	**Titratable Acidity**	**Volatile Acidity**	**Residual Sugar**
1984	10.8	3.01	8.3	0.22	1.7
1987	12.0	3.67	5.1	0.6	2.1
1994	12.1	3.57	6.2	0.45	2.0
1999	12.9	3.61	6.0	0.62	1.8

Sensory analysis of SR wines was conducted through descriptive analysis using the same 10 trained members from AWRI panel and Southcorp Wines in December 2004. Tasting protocol, attribute selection and analysis of results were conducted using the same methods as outlined for the Bin 389 trial but with some modification for attributes. The list of attributes scored for the SR wines were as follows; fruit intensity, spicy, chocolate, developed, leesy, dimethyl-sulphide (DMS), reduced, aldehyde and bitterness.

3. Results and Discussion

The amount of any additional oxygen seen by a wine during bottle aging is determined by the effectiveness of the oxygen barrier created by the bottle's closure. For natural cork, indirect measurements of oxygen permeability have been determined to be several tenths of a mL of O_2 in the first few weeks after bottling, several hundredths of a mL of O_2 in the next four months; but as high as several mL of O_2 per year for bottles stored upright (Ribereau-Gayon cited in Casey, (3). An internal study by Southcorp Wines (8) found that the permeability of natural cork could be highly variable; the (log-normal) distribution observed for 35 randomly selected natural corks is shown here in Figure 1.

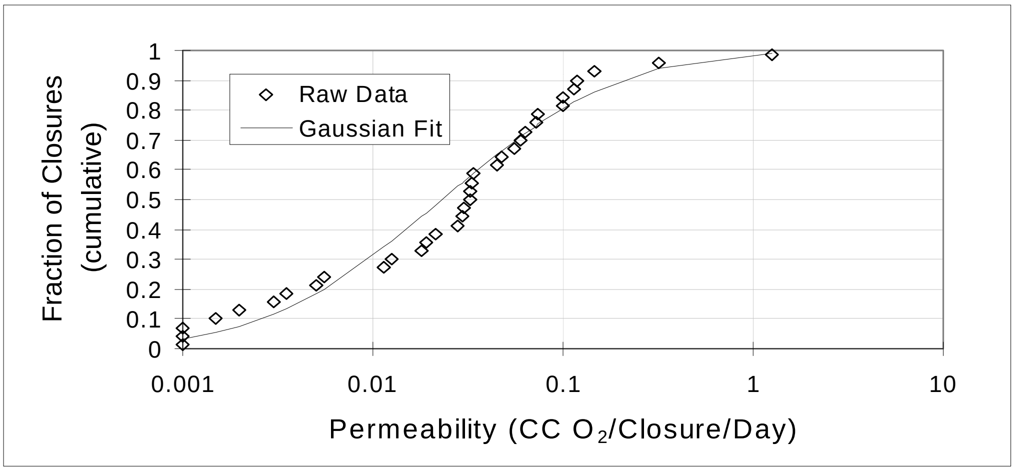

Figure 1: Distribution of closure permeability (measured in situ in cut-off bottle necks after insertion with a production corker) for 35 randomly selected 44 x 24 mm reference 2 natural cork closures.

Whilst the degree of variability seen in this population (from < 0.001 to > 1.0 CC O_2/day) should not be considered typical, it is certainly not unusual for natural cork. Other alternate closures examined by Southcorp in this study found that ROTE was an effective oxygen barrier (< 0.001 CC O_2/closure/day) while the synthetic closures available at the time of the study showed measurable amounts of oxygen permeability (typically 0.010 CC O_2/closure/day) but without the variability seen with natural cork (8).

Bin 389 Chemical Analysis

Chemical analysis results obtained at each assessment are summarised in Figures 2,3,4 and 5 below. The results for free SO_2 (Figure 2) show a greater rate of loss of free SO_2 for synthetic closures than for natural cork and ROTE at the first assessment (2000). This difference continued to be observed at the following two assessments, with the synthetic closures having lost all free SO_2 by the 2002 assessment.

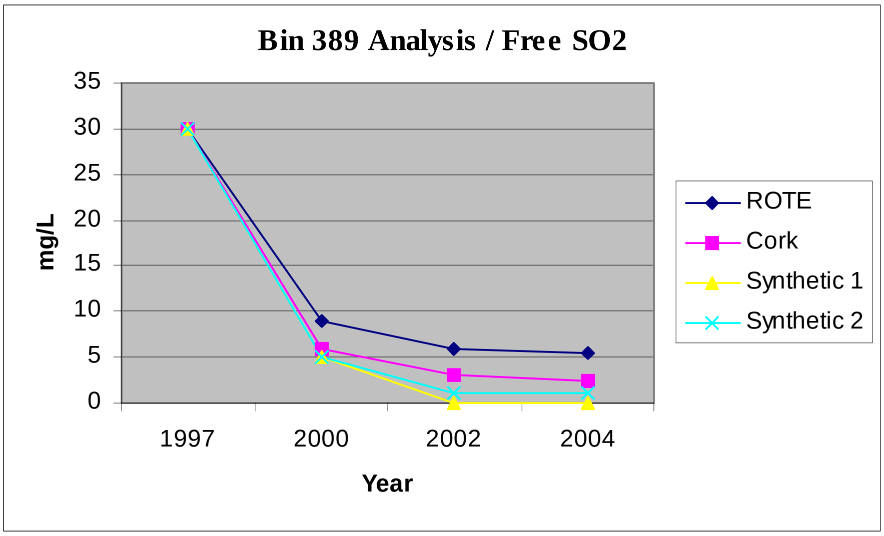

Figure 2: Changes in free SO_2 concentration over time for Bin 389 (average of 3 bottles).

The results for total SO_2 shown in Figure 3 show the same trends as free SO_2 (Fig.2) with the synthetic closures having retained the least total SO_2, ROTE the most, and natural cork with intermediate levels. For all closure types, more SO_2 was consumed over time during the first 3 years after bottling. This may be due to a combination of early SO_2 loss reactions (e.g. dissolved oxygen and other oxidative species in the wine at bottling) and also reduced SO_2 reaction rates (oxidative and non-oxidative) due to the reduced concentration of SO_2 limiting the rate of reaction. The continual loss of free and total SO_2 occurring in the ROTE bottles may be due to small but finite levels of oxygen ingress, or through other non-oxygen related SO_2 reactions.

Whilst the concentrations of total SO_2 in the wine were relatively consistent between bottles for the synthetic and ROTE closures at all tastings, the natural cork had highly variable total SO_2 results (eg individual bottles had levels of 22, 38, 41 mg/L in 2000; 12, 21, 27 mg/L in 2002; 13, 13, 18 mg/L in 2004), with the highest total SO_2 results comparable to the ROTE total SO_2, and the lowest total SO_2 results comparable to the synthetics total SO_2. This variability in SO_2 levels observed in individual bottles under cork is of particular interest as Casey (4) has argued that “random oxidation” is more about the level of oxidants present in a wine and incorporated at the point of bottling rather than the closure type used. The evidence presented here confirms that there is the potential for considerable variability within cork populations to oxygen ingress and that this variability is a most likely contributor to “random oxidation”. Moreover, it was noted by Godden *et al* (10) that natural cork closures were more variable in physical properties than either synthetic or ROTE closures.

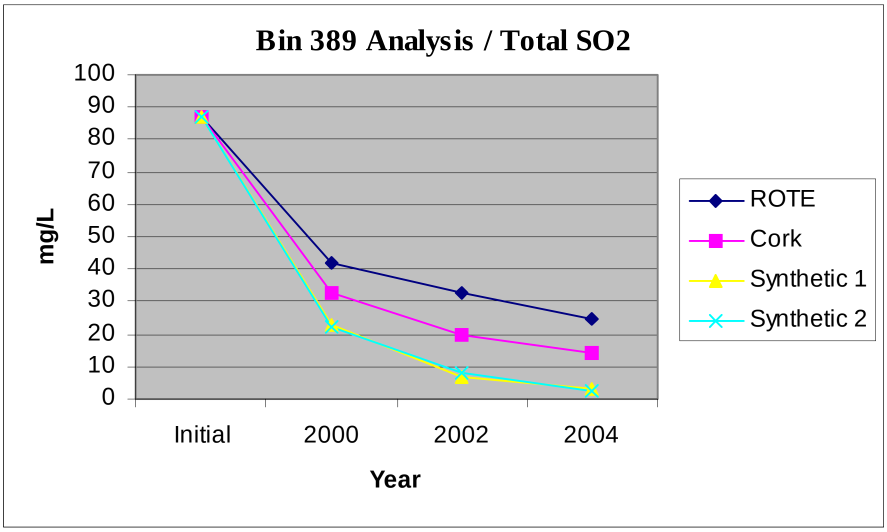

Figure 3: Changes in total SO_2 concentration over time for Bin 389 (average of 3 bottles).

The results for wine colour density in Figure 4 and wine hue in Figure 5 show that by 2000, the synthetic closures had the greatest expression of wine colour due to decreases in SO_2 bleaching and increased brown ($A_{420\ nm}$) colour. However, this colour declined rapidly between 2000 and 2004, with the increased brown colour and decreased red colour ($A_{520\ nm}$) resulting in an increased hue or browning of the wine ($A_{420\ nm}/A_{520\ nm}$). In comparison, the ROTE samples showed a small loss of wine colour at each assessment (due primarily to loss of red colour) and a minimal increase in hue. The natural cork samples had an intermediate colour result between that of the ROTE and synthetic closures. By 2004, the wine colour density of all samples was relatively similar with trends suggesting that the wines bottled under the ROTE closure will continue to have a lower hue in subsequent assessments, and with their more stable colour density, will soon have the highest colour density of the four wines.

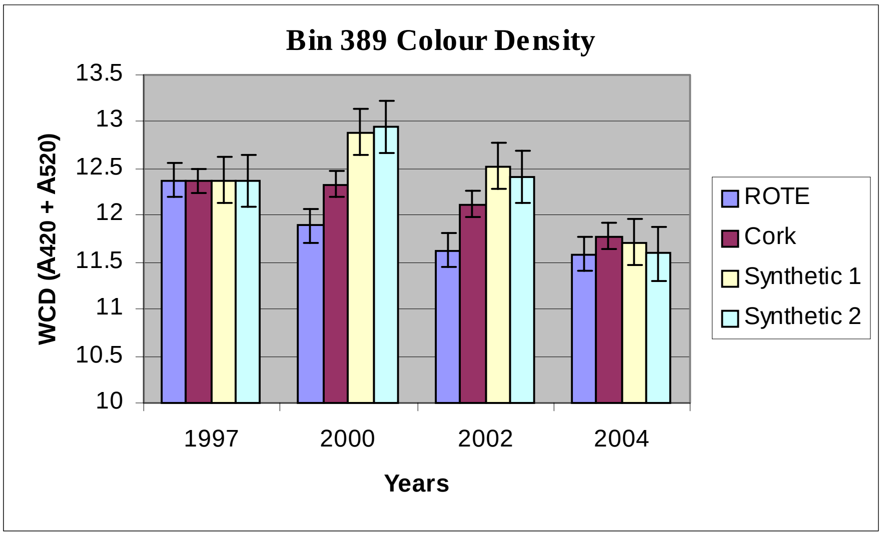

Figure 4: Changes in wine colour density over time for Bin 389 (average of 3 bottles) $P < 0.05$.

These results show that for wine bottled with ROTE closures, the phenolic reactions that influence wine colour are still progressing even in the practical absence of ongoing oxygen for reaction. This allows the wines colour and tannin structure to continue to evolve with time.

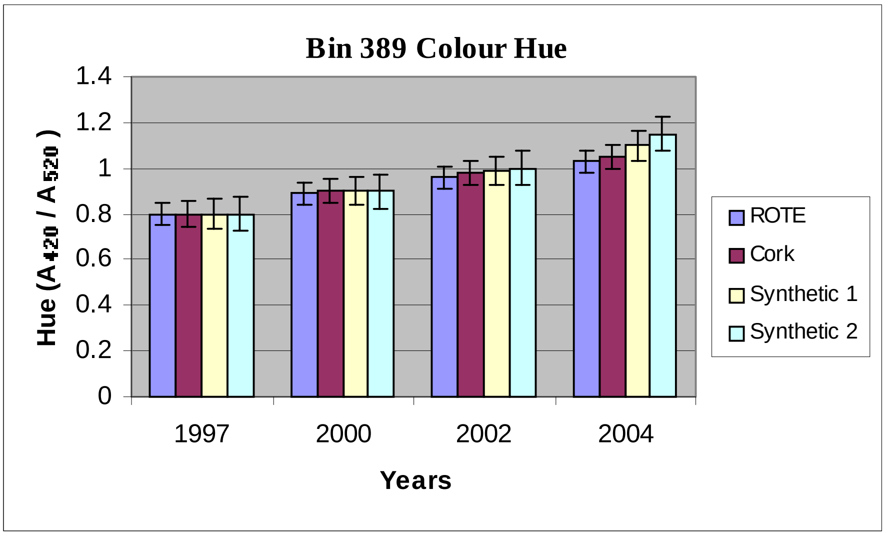

Figure 5: Changes in wine colour hue over time for Bin 389 (average of 3 bottles) $P < 0.05$.

Bin 389 Development Ranking

Sensory evaluation rankings for the Bin 389 trial wines are summarised in Table 3. Interestingly, at the first assessment in 2000, there was no significant difference in the “development” ranking for the 4 closure types. Development was defined as the stage a wine was at in relation to the 3 phases of a wines maturation cycle (7). Differences in the wines were due to more subtle changes, e.g. slight reductive character with ROTE, and flatness with the synthetic closures (possibly oxidation). However, by 2002, the wines bottled under synthetic closures were clearly ranked as the most developed, ROTE the least developed, and natural cork with an intermediate level. This would indicate that the synthetic closure wines had probably reached their peak by 2002 – showing rich, chocolate palate structures, however, they lacked the primary fruit characters observed in the ROTE and natural cork wines. By 2004, the two synthetic wines were again clearly ranked as most developed. However, the synthetic wines were probably well into their decline phase, with definite oxidation problems, whereas the ROTE and natural cork wines had very similar average ranks

Table 3: Development ranking for each closure type (1= "most developed", 4 = "least developed").

	ROTE	Natural Cork	Synthetic 1	Synthetic 2
Average Rank August 2000	2.2[a]	2.8[a]	2.4[a]	2.6[a]
Average Rank June 2002	4.0[a]	3.0[b]	1.5[c]	1.5[c]
Average Rank July 2004	3.6[a]	3.4[a]	1.4[b]	1.4[b]

* Average ranks between the 4 different closure type wines with the same superscript not significantly different to each other ($P < 0.05$).

Clearly, this similarity in rankings between ROTE and natural cork could not have occurred if the ROTE closures had not allowed development of the wine, comparable to that achieved with natural cork. These two samples had retained much more fruit character, but still clearly showed evolution of colour, nose, and structure during aging. In short, the wines evolved irrespective of oxygen availability to the wine.

Descriptive Analysis

Bin 389 – A summary of the descriptive analysis for the 1996 Bin 389 is shown below in Figure 6.

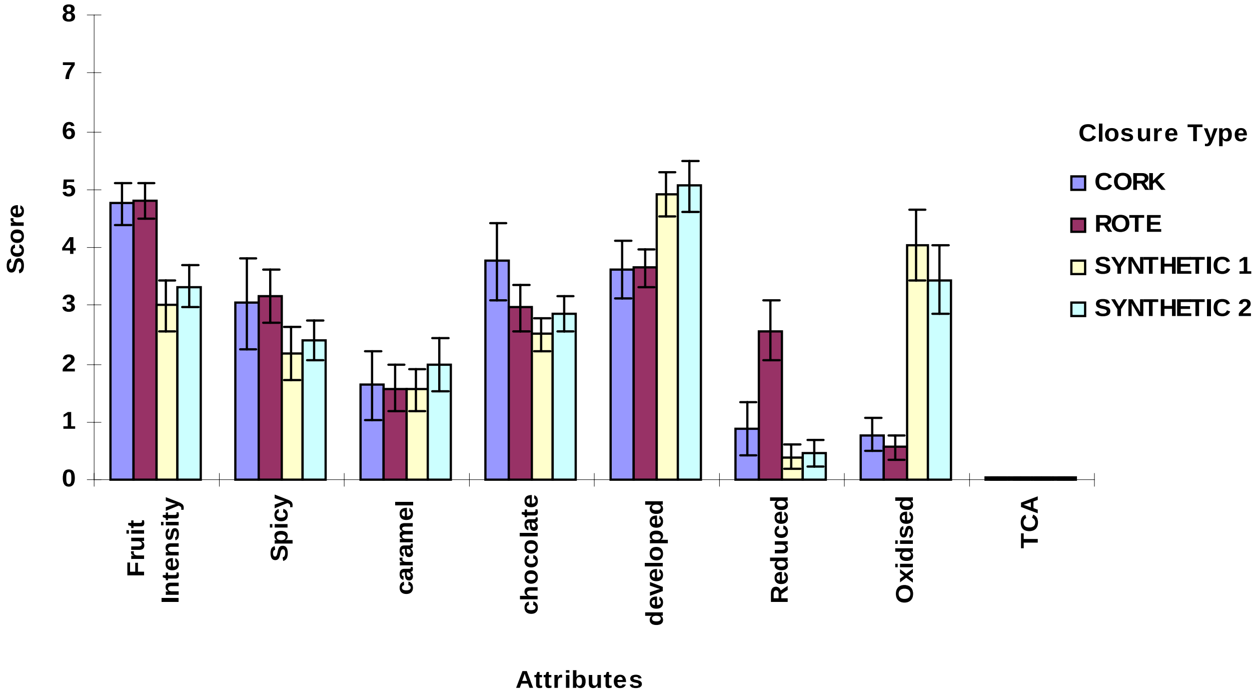

Figure 6: Attribute scores for Bin 389 trial wines.

This data excludes one of the bottles sealed with a cork which was TCA affected and shown to skew the data.
The major points observed from the Bin 389 descriptive analysis are as follows;

- Wines utilising cork and ROTE closures were rated as having retained significantly higher levels of fruit intensity than either wines under synthetic closures.
- The wine with a ROTE closure was rated slightly higher for Spicy than the wine under synthetic 1, but was not significantly different to wines with cork or the synthetic 2 closure
- There was no significant difference observed for the Caramel attribute between all wines under all closures.
- The wine with a cork closure was rated slightly higher in Chocolate than the wine under synthetic 1, but was not significantly different to wines with ROTE or the synthetic 2 closure.
- Wines sealed with both synthetic 1 and 2 closures were rated as significantly more developed than wines sealed with either ROTE or cork closures.
- The wines sealed with ROTE were rated as significantly higher for reduced characters than any other wines. (This could be attributed to winemaking conditions prior to bottling with either, sulphite and sulphate being a source of hydrogen sulfide formation during vinification (21). The levels recorded were not considered to be unacceptable for commercial wines.
- Wines utilising synthetic closures were rated as significantly more oxidised than wines using either cork or ROTE closures.

When conducting ANOVA on the data obtained from the descriptive analysis session, the program did not allow for separation of the TCA affected cork data and it was therefore excluded from the following summary. The observations are as follows;

Bin 389 ANOVA results (excluding all cork data)

- Wines sealed with ROTE had significantly more fruit aroma than either wines using synthetic 1 or 2 closure type **
- Wines sealed with ROTE had significantly more spicy character than either wines using synthetic 1 or 2 closure type *
- There was no significant difference in wines sealed with all closures for caramel or chocolate attributes.
- Wines under both synthetic closures were rated as being significantly more developed than the wines sealed with the ROTE closure **
- Wines sealed with ROTE were rated as having significantly more reduced characters than wines under synthetic type closures ***
- Wines under both synthetic closures were rated as being significantly more oxidised than the wines sealed with a ROTE closure ***

*$P<0.01$, ** $P<0.05$, *** $P<0.001$.

These results concur with the findings from the development ranking and show that oxygen availability to the wines through ingress via a closure will simply accelerate the maturation process and lead to premature oxidation of the wines.

Sparkling Red

SR Descriptive Analysis – A summary of the descriptive analysis for the SR wines is shown below in Figure 7. This data includes all bottles, however, one of the 1999 Sparkling Shiraz had considerable aldehyde levels in comparison to the other.

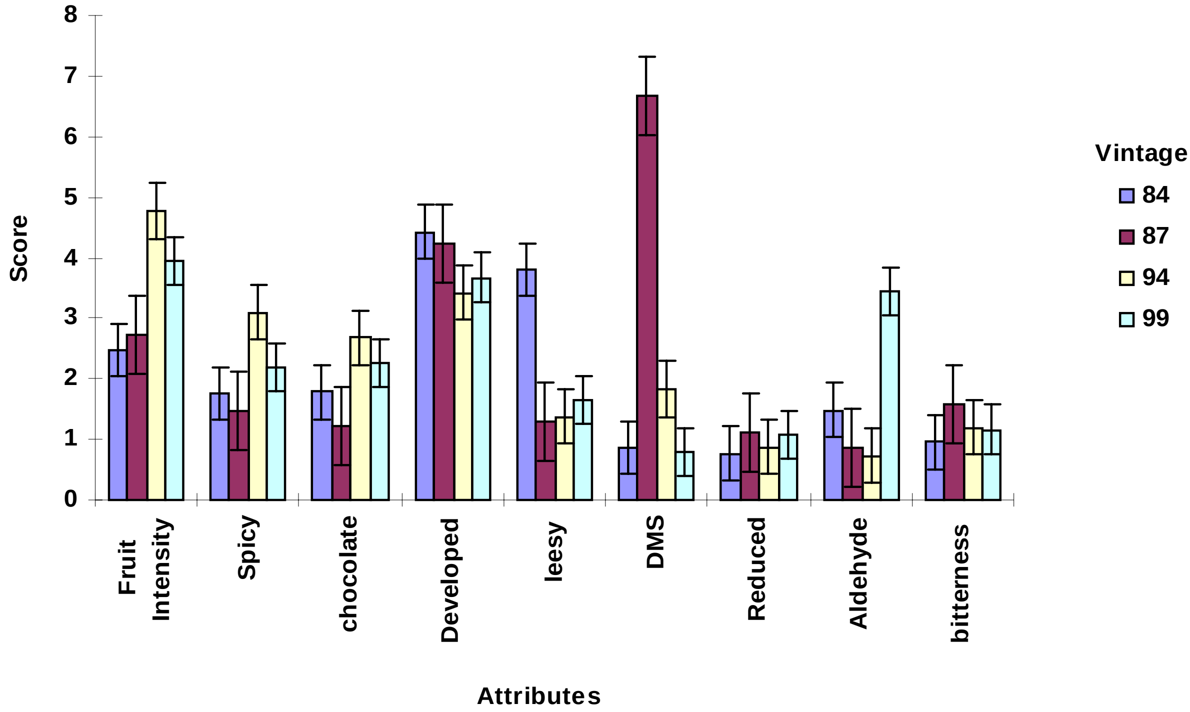

Figure 7: Attribute scores for SR Shiraz

The aldehydes should be viewed in context as part of the maturation process. The major points observed from the SR descriptive analysis are as follows;

- Wines from both 1994 and 1999 were rated significantly higher in fruit intensity than 1984 and 1987.
- The 1994 vintage wine was rated as having significantly higher spice character than the other 3 wines.
- The 1994 wine was rated significantly higher for chocolate character than 1984 and 1987, but not for 1999.
- Wines from 1984 were rated as being more developed than 1994, but not 1987 and 1999. This changes if you exclude the sample rated high in aldehydes from 1999 (data not shown here). In this instance there is a gradient from most (1984), to least (1999) developed in line with expected trends.
- The 1984 wine was rated as significantly higher in leesy character than the other 3 vintage wines.
- Wines from 1987 were very high for DMS character and it should be noted that vintage and climatic variations are most likely the major contributors for this character.
- One of the bottles from the 1999 vintage allowed a significantly higher aldehyde character to be recorded in comparison to the other 3 vintage wines.

The levels of aldehyde present in the youngest wine (1999) with limited oxygen and sulphur dioxide present would suggest that these compounds were a by-product of secondary fermentation in bottle. It has been noted previously that yeast strain, pH, nutrient availability, SO_2 levels and temperature are among the many variables that can influence the production of acetaldehyde during fermentation (1). Also of note for the SR wines was the absence of any high levels of reductive characteristics. It was noted by some panellists that in some of the wines there were very low levels of reductive characters, but in general there was a lack of strong attributes in this area. This was a little surprising given previous comments and reported findings (10,22). This may be due to the preparation of the base wines following primary fermentation and a yeast with low nitrogen requirements utilised for secondary fermentation. Alternatively, absorption by dead yeast cells and subsequent riddling and disgorging may also have contributed to a lack of reductive characters along the lines suggested by Lavigne-Cruge and Dubourdieu (14).

SR Spectral Analysis (20) of the wines was shown to have similar trends with results attained by descriptive analysis. Results of wine colour density and hue in Figure 8 show that the older vintages have the highest hue but lowest density due to increased brown and decreased red colours. This agrees with expected trends for a series of vintaged wines ranging from very old to very young. These results are also interesting in that some SR wines were matured over a long period of time in an environment free from any significant levels of SO_2 or oxygen, but are very similar to the Bin 389 colour analysis trends. This implies that bottled red wines with limited SO_2 and oxygen available during the maturation process will continue with influential phenolic reactions for wine colour in a similar way to wines bottled with commercial levels of SO_2 available to the wine. However, it must be noted that the possibility of different levels of colour at the finish of initial vinification for the SR wines must be taken into consideration and may have influenced the results presented in Figure 8.

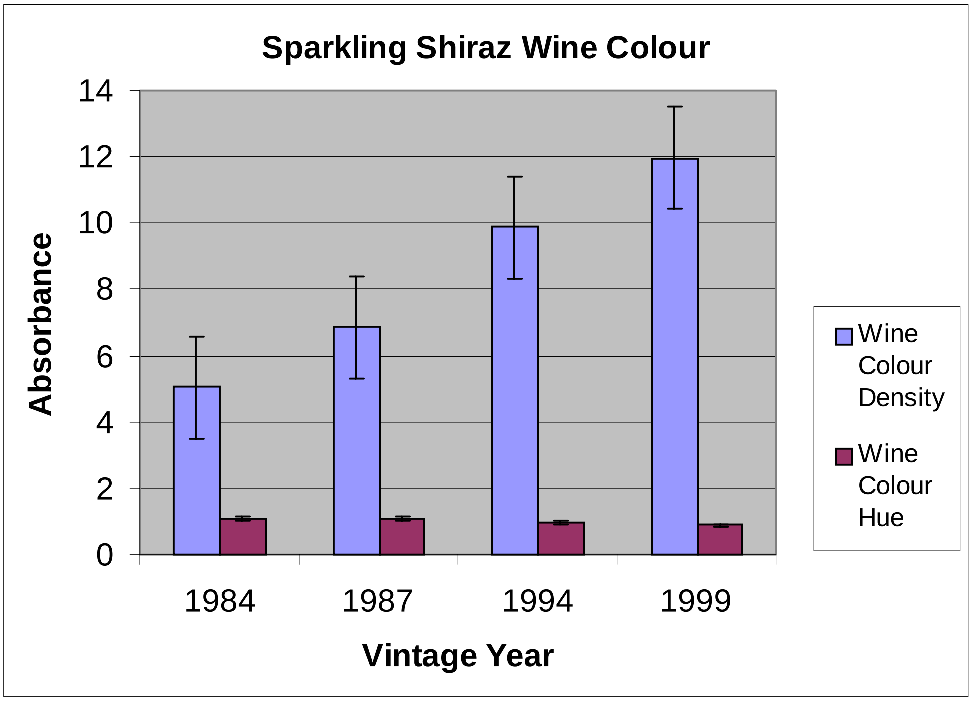

Figure 8 Wine colour density and wine colour hue of SR Shiraz ($P<0.05$)

Although it is almost certain that each wine will have had an initial colour difference, the trend displayed between wines in Fig. 8 is quite clear. The wine from 1984 has the highest hue and therefore brown colour, while the 1999 wine has the highest wine colour density, lowest hue and therefore, red colour. If the role of oxygen was critical in the reactions necessary for wine colour evolution over prolonged periods then the observed results in Fig. 8 would be unlikely. A summary of the recorded levels of wine colour density and hue from the different vintages suggests;

- Wines from 1984 and 1987 were not significantly different to each other in either wine colour density or hue, but were different to 1994 and 1999 wines.
- Wines from 1994 and 1999 were not significantly different to each other in either wine colour density or hue.
- Although vintage variation should be considered, the trends observed in the analysis would suggest that the wines were maturing in accordance with expectations even in the absence of oxygen in the wines environment.

Conclusion

From this study it is apparent that oxygen was not a vital component for the ongoing evolution and maturation of these red wines after bottling. It is clear that access to small but measurable amounts of oxygen (eg synthetic closures, approximately 4 CC O_2/bottle/year) will accelerate the evolution and maturation of red wine through oxidative reactions. However, red wine will continue to evolve without measurable oxygen ingress via a closure, primarily through what is assumed to be anaerobic reactions. Some bottled wine may express “reductive” characters in such an anaerobic environment, in contrast to the wine developing oxidised characters in a more aerobic environment. Given the low ranking scores for reductive characteristics observed in the Bin 389 trial wines, the low levels recorded in this instance are not considered to be commercially unacceptable.

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