REPORT OF TASMANIAN PINOT NOIR FORUM
PINOT NOIR COLOUR STABILITY TRIAL

Background

This GWRDC – RITA funded project was established to study the concept of colour stability in Pinot Noir wines. Members of the Tasmanian Pinot Noir Forum initiated and participated in the study. The project included presentations and discussion of aspects of grape and wine colour at the 2006 and 2007 Tasmanian Pinot Noir Forums and an examination of the change in colour parameters of wines produced by some members of the Forum.

Wines examined in the project

Each participant was asked to provide a set of wines that had been produced from either different vineyards (or sections of the one vineyard) or by different winemaking practices.

A summary of the set of wines examined in the 2005 vintage is detailed below—

- Winery 1: Three wines, (A, B and C) were produced from fruit from the same vineyard but 3 different yeasts were used to conduct three separate fermentations, via the normal fermentation practice of that winery
- Winery 2: Two wines, (A and B) where wine A was produced with a 9 day pre-ferment soak with no addition of SO_2 pre-soak followed by the normal fermentation practice of that winery and wine B was produced with a 12 day pre-ferment soak and a 150 mg/L SO_2 addition pre-soak followed by the normal fermentation practice of that winery.
- Winery 3: Two wines (A and B) where wine A was produced with a 3 day pre-ferment soak with a 50 mg/L SO_2 addition pre-soak followed by the normal fermentation practice of that winery and wine B with a 10 day pre-ferment soak and a 200 mg/L SO_2 addition pre-soak followed by the normal fermentation practice of that winery.

A summary of the set of wines examined in the 2006 vintage is detailed below—

- Winery 4: Two wines (A and B) produced from fruit from two separate sections of the one vineyard. The fruit for wine A was from vines judged as not stressed during the ripening period and the fruit for wine B was from vines judged as stressed during the ripening period. The wines were produced in a similar manner via the normal fermentation practice for that winery.
- Winery 5: Two wines (A and B) where wine A was produced via the normal fermentation practice of that winery and wine B was produced with a 14 day pre-ferment soak and a 90 mg/L SO_2 addition pre-soak followed by the normal fermentation practice of that winery.
- Winery 6: Two wines (A and B) where wine A was produced via the normal fermentation practice of that winery and wine B was produced via the normal fermentation practice but with a post-fermentation maceration period, where the cap was kept in contact with the wine for 10 days (at the end of this period the cap had completely sunk).

- Winery 7: Two wines (A and B) produced from fruit from two separate sections of the one vineyard. The fruit for wine A was from vines trained to a split-canopy training system and the fruit for wine B was from vines trained to a vertical shoot positioned training system. The wines were produced in a similar manner via the normal fermentation practice for that winery, but in this case included a pre-ferment soak period and an extended post-ferment maceration period of approximately 3 weeks.

In 2005 wine colour measures were determined on wines at the completion of fermentation, after malolactic fermentation and after approximately 8 months post the completion of fermentation. Berry colour measures were determined on samples of berries used in the fermentation of the wines for wineries 1 and 2.

In 2006 wine colour measures were determined on wines at approximately 4 months after the completion of fermentation (some wines had completed MLF at this stage) and approximately 8 months post the completion of fermentation. Berry colour measures were determined on samples of berries used in the fermentation of the wines at all participating wineries.

Results and discussion

Measurement of berry colour

Berry colour is determined by extracting a sample of berries with a 50% v/v solution of ethanol, diluting a portion of the extraction mixture with 1M HCl and measuring the absorbance of this solution at 520 nm on a spectrophotometer. The berry colour measure can be expressed as content (mg anthocyanins per berry) or as concentration (mg anthocyanins per gram berry weight). The concentration measure is used when relating berry colour measures to wine colour measures.

Relationship between berry colour and wine colour measures

Previous studies have shown that –

- Berry colour for the variety Pinot Noir varies from approximately 0.4 to 1.5 mg anthocyanins per gram berry weight.
- Low values of berry colour, eg 0.4 to approximately 0.6 mg anthocyanins per gram berry weight are usually associated with light coloured wines, which often lack flavour intensity.
- Comparatively, berries with higher berry colour concentration are generally associated with wines of higher wine colour, but at values of berry colour above approximately 0.8 mg anthocyanins per gram berry weight the colour of the associated wines is generally regarded as being acceptable for Pinot Noir wines.
- High values of berry colour, eg above 1.3 mg anthocyanins per gram berry weight can be associated with wines which often are regarded as having too intense colour for the variety Pinot Noir. These wines often are described as being dry-red like. They can have stewed plum/jammy/raisin characters, particularly if the high concentration of berry colour has resulted from berry shrivel.

Relevance of berry colour measures to this project

Values, as mg anthocyanins per gram berry weight for berry colour of berries associated with wines in this project were —
winery 1 (0.70), winery 2 (0.88), winery 4 (A = 1.28, B = 1.35), winery 5 (0.93), winery 6 (1.18), winery 7 (A = 1.11, B = 1.04).

The values for berry colour indicates that in most cases the berries are suitable to produce wines with acceptable colour. When there were differences in the source of the fruit, ie where A and B values are shown, there was only a small difference between the A and B samples.

Changes in wine colour measures during fermentation and storage
During fermentation anthocyanins are extracted from the skins and tannins are extracted from the skins and the seeds. The anthocyanins can remain free or combine with monomeric compounds (eg catechins, extracted mainly from the seeds) and tannins extracted from the skins and seeds to form red coloured polymeric pigments.

Monomeric compounds (eg catechins, mainly extracted from the seeds) can combine with each other or with tannins extracted from the skins and seeds. Tannins can also combine with each other to form larger tannins. These combinations of monomers and tannins result in the formation of yellow/brown coloured polymeric pigments. Anthocyanins can also be oxidised to compounds that are coloured yellow-brown.

Free anthocyanins and red and yellow-brown polymeric pigments, as well as being involved in the reactions described above, can be absorbed onto yeast lees or precipitate from the wine during storage. When the concentration of free anthocyanins reaches low levels, most of the red colour of a wine is due to the presence of red coloured polymeric pigments. These red coloured polymeric pigments are often referred to as stable red colour, as once formed they are present for most of the life of the wine. Even though red coloured polymeric pigments are present in aged wines, brown coloured pigments often dominate the colour of an aged wine and thus the wine appears orange-brown, tawny or brown.

Wine colour measures
Wine colour measures are normally determined by the method of Somers and Evans (1977). These measures include —
Wine colour density ($Abs_{520} + Abs_{420}$)
Wine colour hue (Abs_{420}/Abs_{520})
Total anthocyanins (mg/L)
Total phenolics (au)
Estimate of red coloured polymeric pigments as given by $Abs_{520}^{SO_2}$
Abs_{520} is a measure of the red colour of the wine and relates to the concentration of free anthocyanins in the red coloured form and red coloured polymeric pigments.
Abs_{420} is a measure of the yellow-brown colour of the wine and relates to the concentration of yellow/brown pigments, including monomer and polymeric compounds.
$Abs_{520}^{SO_2}$ is the absorbance measure after the addition of sulfur dioxide (SO_2) to the wine sample. The addition of SO_2 bleaches the colour of a portion of the free anthocyanins, but

has only a small effect on the colour of the red coloured polymeric pigments. Compared to free anthocyanins, the red coloured polymeric pigments are much less susceptible to bleaching by SO_2, to colour change with changes in pH and to oxidation. Therefore these red coloured polymeric pigments are regarded as being associated with stable red colour and thus this measure is considered to provide an estimate of colour stability.

Wine colour density measures, particularly the Abs_{520} measure, are affected by wine pH and SO_2 concentration. In wine the free anthocyanins exist in a pH dependant equilibrium — with increasing pH the concentration of red coloured forms of the anthocyanins decreases. When SO_2 is added to the wine (or produced in the fermentation) a portion of the SO_2 can bind to free anthocyanins and bleach their red colour. Therefore, in any one wine where changes in pH and SO_2 concentration occur, eg as a result of acid or SO_2 addition, the higher the pH and the higher the SO_2 concentration the lower the concentration of red coloured free anthocyanins and thus the lower the measures of Abs_{520} and wine colour density.

Because wine pH and SO_2 concentration affect the measure of wine colour density, in this study the expression of wine colour density was adjusted so that it was expressed for each wine at the same pH (3.6). As well, according to the method of Somers (1977) an addition of acetaldehyde (CH_3CHO) was made to the sample of wine prior to measuring Abs_{520} and Abs_{420} to allow for any bleaching effects of sulfur dioxide present in the wine on the wine colour density measure. Sulfur dioxide (SO_2) binds more strongly with the added acetaldehyde molecules than it does with the anthocyanins present in the wine, thus the anthocyanins previously bound to SO_2 are released and there is a resultant increase in red colour and in Abs_{520}. The addition of acetaldehyde also results in the release of red coloured compounds from polymeric pigments but the extent of this effect is much smaller than the effect on anthocyanins. Therefore overall, $Abs_{520}^{CH_3CHO}$ gives the absorbance due to red coloured pigments (anthocyanins and red polymeric pigments) in the wine without the bleaching effects of any SO_2 present in the wine. The addition of acetaldehyde also results in the release of yellow/brown coloured compounds from yellow/brown polymeric pigments, but again the extent of this effect is much smaller than the effect on anthocyanins. Overall, $Abs_{420}^{CH_3CHO}$ gives the absorbance due to yellow/brown coloured pigments without the bleaching effects of any SO_2 present in the wine. This approach, where pH and SO_2 effects are allowed for before the absorbance measures are taken, provides data that leads to a valid comparison and interpretation of any differences in wine colour measures in wines produced from fruit from different vineyards or by different winemaking practices.

The measures used to follow changes in wine colour in this project were:
Modified Wine Colour Density ($Abs_{520}^{CH_3CHO} + Abs_{420}^{CH_3CHO}$) at pH 3.6,
Estimate of red coloured polymeric pigments as given by $Abs_{520}^{SO_2}$
Total anthocyanins (mg/L),
Total phenolics (au).

Relevance of wine colour measures to this project

- Results of winery 1: The use of different yeasts to conduct the fermentation can result in differences in modified wine colour density and in the measurement of the estimate of red coloured polymeric pigments (a measure of stable colour). Two yeasts (those associated with ferments B and C) resulted in fermentation conditions which lead to higher measures of modified wine colour density and of stable colour than the yeasts that conducted ferment A (Figures 1a and 1b).
- It is interesting to observe that most of the compounds contributing to stable red colour were formed by the end of fermentation (Figure 1b). Other studies with other varieties have reported a similar trend.
- While the data of Figures 1a and 1b indicate that a higher modified wine colour density and measure of stable red colour at the end of fermentation is associated with higher values of these parameters at the later stages of wine maturation, data from the trials of wineries 2, 3 and 5 indicate a different trend. The difference between the treatments investigated in the trials of wineries 2, 3 and 5 related to a cold soak period with high SO_2 addition. This treatment resulted in a delayed formation of stable red colour compounds (compare values of the estimate of red coloured polymeric pigments in Tables 2, 3 and 5 at the first and final measurements). For example, for winery 2 (Table 2) the measures of stable red colour were post-fermentation A = 0.7 and B = 1.0, but at 8 months post-fermentation A = 0.8 and B = 1.6, representing a larger difference at the later stage; and for winery 5 (Table 5), the measures for stable red colour were at 4 months post-fermentation A = 1.2 and B = 0.8, but at 8 months post-fermentation A = 1.3 and B = 1.5. In these B treatments it is likely that the anthocyanins previously bound to SO_2 are gradually released to form stable red pigments. Therefore, when interpreting a comparison of a pre-soak and high SO_2 treatment with another treatment it is best to compare modified wine colour density and stable red colour measures at a time later than at the end of fermentation. An appropriate time to compare wine colour values would be after major winemaking processes, eg fermentation and MLF are complete and prior to the wine being stored in barrel or tank.
- Results of winery 4: The wine produced from fruit of vines judged as being stressed had higher values for the measures of modified wine colour density and stable red colour than that from fruit of vines judged as less stressed.
- Results of winery 6: The wine produced with an extended post-fermentation maceration period had lower values of the measures of modified wine colour density and stable red colour. It is likely that colour compounds are lost through precipitation during the period of extended maceration.
- Results of winery 7: The wine produced from fruit from vines trained to VSP canopy system had lower values of the measures of modified wine colour density and stable red colour than that of the wine produced from fruit from vines trained to a split-canopy system. The decrease in the measures of total anthocyanins concentration in wines from this winery was greater than the decreases observed in other wines in the trial (data in Table 7 compared with data in the other Tables). The reason for this is unclear, but it maybe associated with the extended post-fermentation maceration period. When there is an extended post-fermentation maceration period there is opportunity for the anthocyanins to be

absorbed on yeast lees. Other wines in the trial either had no or a shorter post-fermentation maceration periods compared with the wines of Winery 7, therefore the effects of absorption of anthocyanins on yeast lees may have been greater for the wines of Winery 7 compared to that of the other wines in the trial.

Sensory aspects of the wines of the different treatments

The wines were tasted by participants at the Tasmanian Pinot Noir Forum. At the time of tasting the wines were approximately 9 months old. Wines were presented without the participants knowing the identity of the wine and in random order. In 2005 a preference between the wines of each trial was recorded. In 2006 the wines were scored for overall quality using a scale of 1 to 5 where a score of 5 represented an excellent quality Pinot Noir wine.

2005 wines

In 2005 there were 40 tasters.

- Winery 1: all tasters preferred wines B and C to wine A; wines B and C were equally preferred
- Winery 2: equal preference for wine A and B
- Winery 2: equal preference for wine A and B

2006 wines

In 2006 there were 30 tasters.

- Winery 4: 18% of tasters preferred wine A, 27% had equal preference and 55% preferred wine B
- Winery 5: 32% of tasters preferred wine A, 28% had equal preference and 40% preferred wine B
- Winery 6: 33% of tasters preferred wine A, 15% had equal preference and 52% preferred wine B
- Winery 7: 42% of tasters preferred wine A, 33% had equal preference and 25% preferred wine B

There was no obvious relationship between the wine colour measures and wine score. The wine with the higher wine colour measures did not necessarily achieve the higher score. This indicates that provided the wine has sufficient colour intensity other sensory aspects contribute to the evaluation of wine style and quality.

In 2006 the concentration of wine tannins was determined by the AWRI Tannin method (Sarneckis et al 2006). The values of the wine tannin concentration (g/L) were winery 4 (A = 1.0, B = 1.3), winery 5 (A = 0.6, B = 0.5), winery 6 (A = 1.1, B = 1.0) and winery 7 (A = 2.3, B = 2.4). The score out of 5 for the set of 2006 wines was plotted against tannin concentration (Figure 2). There appears to be a trend towards a lower wine score with either low or higher wine tannin concentration. At the tasting, a number of participants commented on the excessive tannin sensations perceived in the wines of winery 7. These wines had an extended period of post-fermentation maceration and it is possible that the

higher wine tannin concentration was due to extraction of tannins from seeds during this time.

Conclusion

There was no field/fermentation replication of the treatments as the aim of this RITA project was to obtain data that could contribute to discussion of colour stability aspects. Therefore, it should be appreciated that the points covered in the discussion and conclusion are based on observations of the trends in the data and not a statistical assessment.

- The modified wine colour measure ($Abs_{520}^{CH_3CHO} + Abs_{420}^{CH_3CHO}$) at pH 3.6 and the estimate of red coloured polymeric pigments ($Abs_{520}^{SO_2}$) can be used to follow the change in colour of wine without the effects of pH and SO_2 on the wine colour measurements.
- The measure of the estimate of red coloured polymeric pigments ($Abs_{520}^{SO_2}$) provides an estimate of stable red colour.
- When a pre-soak with high SO_2 addition is employed as part of the winemaking process, the formation of stable red colour compounds may be delayed until sometime after the completion of fermentation. Comparisons between this type of treatment and other treatments using this measure should be determined post-MLF.
- Winemakers need to consider fermentation conditions that enhance the formation of stable red colour compounds.

Some guidelines:

a) achieve high (or sufficient) concentration of colour in berries.
b) achieve efficient extraction of colour and tannin from skins during fermentation on skins — higher rather than lower temperatures; gentle but thorough mixing to maximise skin extraction but limit seed extraction
c) the use of a pre-soak period with SO_2 addition appears to be beneficial in that higher concentrations of stable red pigments are formed in wines treated this way in comparison to wines where no SO_2 addition was made pre-soak. However, it needs to be realised that the formation of the stable red coloured compounds is delayed until some time after the end of fermentation.

- The observed relationship between wine score and wine tannin concentration requires further investigation.

References

Sarneckis, C.J., Dambergs, R.G., Jones, P., Mercurio, M., Herderich, M.J. and Smith, P.A. (2006) Quantification of condensed tannins by precipitation with methyl cellulose: development and validation of an optimised tool for grape and wine analysis. Australian Journal of Grape and Wine Research 12, 39-49.

Somers, T.C. and Evans, M.E. (1977) Spectral evaluation of young red wines: anthocyanin equilibria, total phenolics, free and molecular SO_2, chemical age. Journal of Science, Food and Agriculture 28, 279-287.

Table 1. Wine colour measures for winery 1 samples for the 2005 vintage at three stages of development. Each value represents the mean of 3 measures

Wine colour measures	Post-fermentation			Post-malolactic			8 mths post-fermentation		
	A	B	C	A	B	C	A	B	C
Modified wine colour density (au)	8.1	9.0	9.3	7.7	8.3	8.8	6.6	7.1	7.6
Estimate of red coloured polymeric pigments (au)	2.0	2.6	2.3	2.1	2.3	2.3	2.1	2.2	2.4
Total anthocyanins (mg/L)	193	187	234	146	140	164	143	131	146
Total phenolics (au)	60	59	67	51	49	57	58	55	60

A, B, C = different yeasts; post-malolactic measurement was approximately 4 months post-fermentation

Table 2. Wine colour measures for winery 2 samples for the 2005 vintage at three stages of development. Each value represents the mean of 3 measures

Wine colour measures	Post-fermentation		Post-malolactic		8 mths post-fermentation	
	A	B	A	B	A	B
Modified wine colour density (au)	4.8	6.0	6.1	7.0	5.0	6.6
Estimate of red coloured polymeric pigments (au)	0.7	1.0	1.0	1.5	0.8	1.6
Total anthocyanins (mg/L)	310	311	208	218	232	217
Total phenolics (au)	36	37	36	44	39	49

A = no SO_2; B = 150 mg/L SO_2 pre-ferment soak; post-malolactic measurement was approximately 6 months post-fermentation

Table 3. Wine colour measures for winery 3 samples for the 2005 vintage at two stages of development. Each value represents the mean of 3 measures

Wine colour measures	Post-fermentation		Post-malolactic		8 mths post-fermentation	
	A	B	A	B	A	B
Modified wine colour density (au)	9.0	5.7	-	-	5.5	7.7
Estimate of red coloured polymeric pigments (au)	1.2	0.9	-	-	1.1	1.7
Total anthocyanins (mg/L)	442	285	-	-	165	220
Total phenolics (au)	44	36	-	-	32	40

A = 50 mg/L SO_2; B = 200 mg/L SO_2 pre-ferment soak; post-malolactic measurement was approximately 6 months post-fermentation; no post-malolactic sample was taken as the wine was still undergoing MLF at the stage when the samples were taken for the other wines; therefore, post-malolactic values are not reported in this table.

Table 4. Wine colour measures for winery 4 samples for the 2006 vintage at two stages of development. Each value represents the mean of 2 measures

Wine colour measures	4 months post-fermentation		8 months post-fermentation	
	A	B	A	B
Modified wine colour density (au)	7.8	9.6	6.7	8.0
Estimate of red coloured polymeric pigments (au)	1.3	1.6	1.7	1.9
Total anthocyanins (mg/L)	198	265	192	222
Total phenolics (au)	33	41	35	41

A = wine made from vines assessed as being stressed;B = wine made from vines assessed as not being stressed

Table 5. Wine colour measures for winery 5 samples for the 2006 vintage at two stages of development. Each value represents the mean of 2 measures

Wine colour measures	4 months post-fermentation		8 months post-fermentation	
	A	B	A	B
Modified wine colour density (au)	8.2	6.7	6.2	6.1
Estimate of red coloured polymeric pigments (au)	1.2	0.8	1.3	1.5
Total anthocyanins (mg/L)	191	108	169	159
Total phenolics (au)	36	33	36	35

A = no SO_2; B = 90 mg/L SO_2 pre-ferment soak

Table 6. Wine colour measures for winery 6 samples for the 2006 vintage at two stages of development. Each value represents the mean of 2 measures

Wine colour measures	4 months post-fermentation		8 months post-fermentation	
	A	B	A	B
Modified wine colour density (au)	10.1	10.2	9.6	8.2
Estimate of red coloured polymeric pigments (au)	1.4	1.6	2.3	2.0
Total anthocyanins (mg/L)	203	239	247	246
Total phenolics (au)	43	49	44	41

A = normal ferment; B = normal ferment plus post-fermentation maceration period

Table 7. Wine colour measures for winery 7 samples for the 2006 vintage at two stages of development. Each value represents the mean of 2 measures

Wine colour measures	4 months post-fermentation		8 months post-fermentation	
	A	B	A	B
Modified wine colour density (au)	10.1	9.4	8.8	8.0
Estimate of red coloured polymeric pigments (au)	1.9	1.9	3.3	2.8
Total anthocyanins (mg/L)	328	324	111	107
Total phenolics (au)	66	76	65	68

A = vines trained to a split-canopy; B = vines trained to a vertical shoot positioned canopy

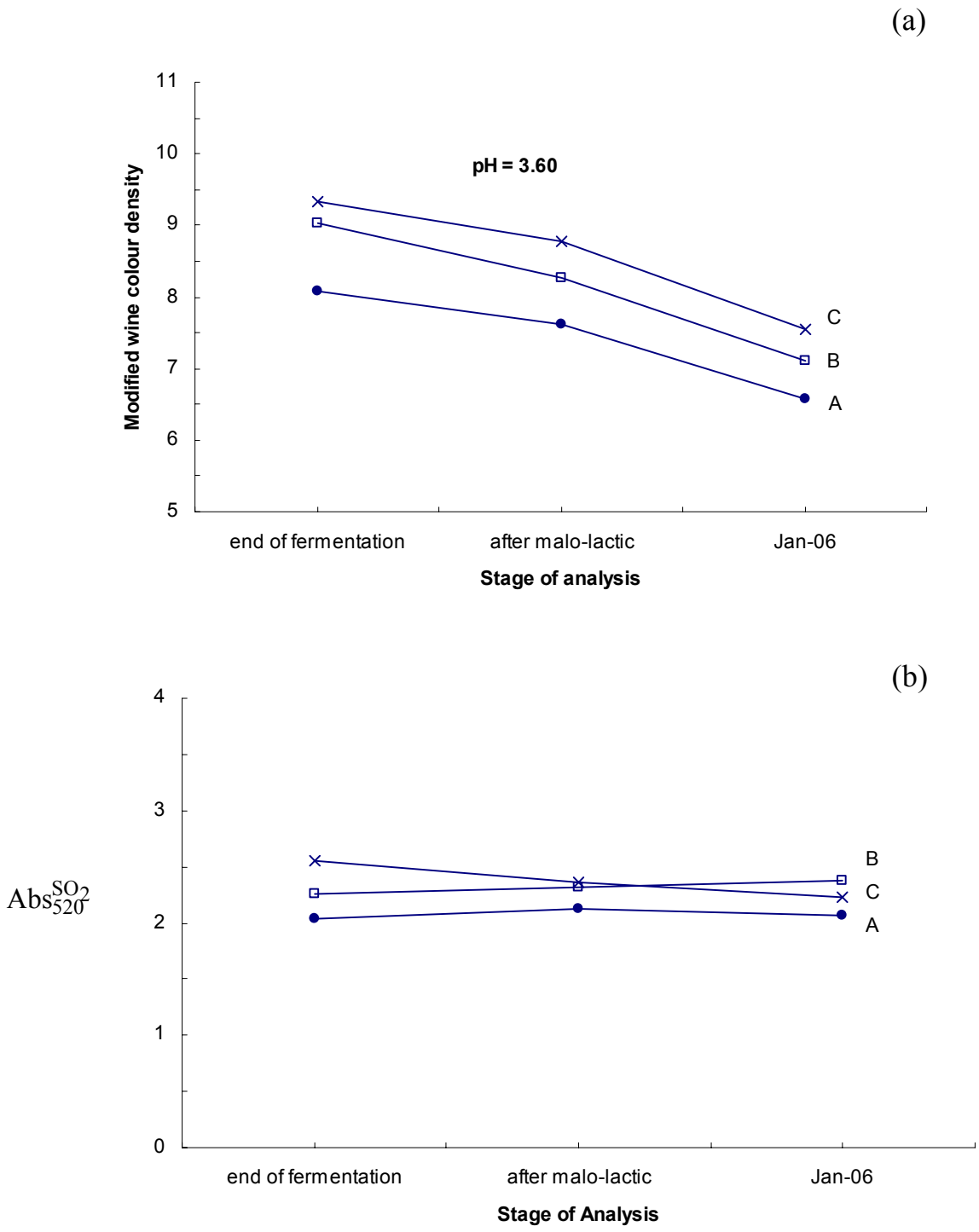


Figure 1: (a) Change in modified wine colour density and (b) the estimate of red coloured polymeric pigments (measured as Abs_{520}^{SO2}) over time for wines from winery 1. "After malolactic' stage was 4 months post-fermentation and 'Jan 06' was 8 months post-fermentation

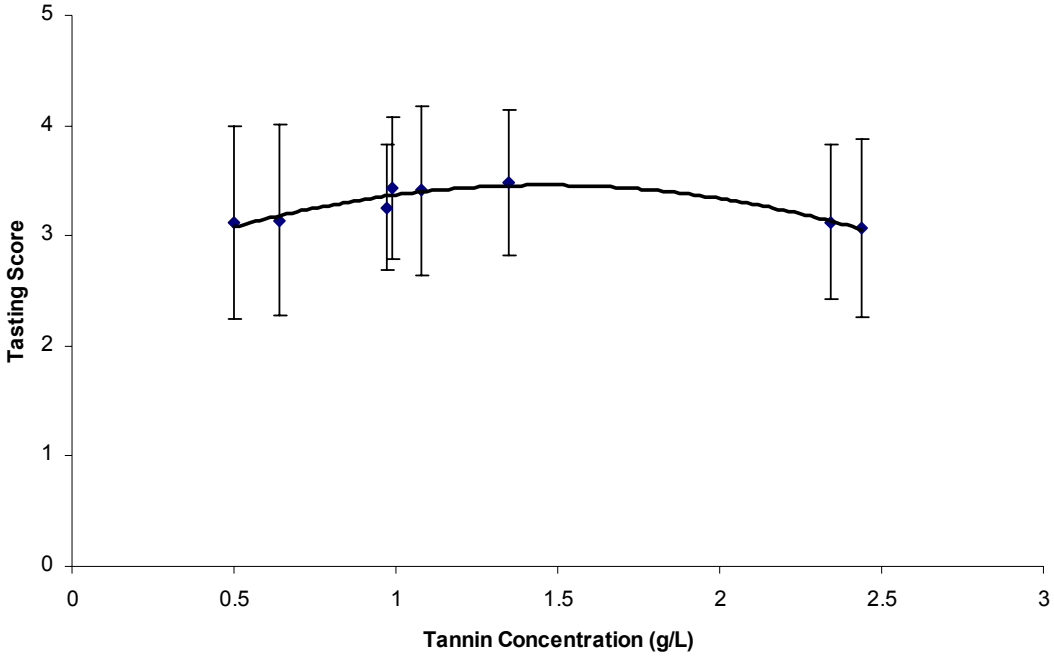


Figure 2: The relationship between wine tannin concentration and tasting score