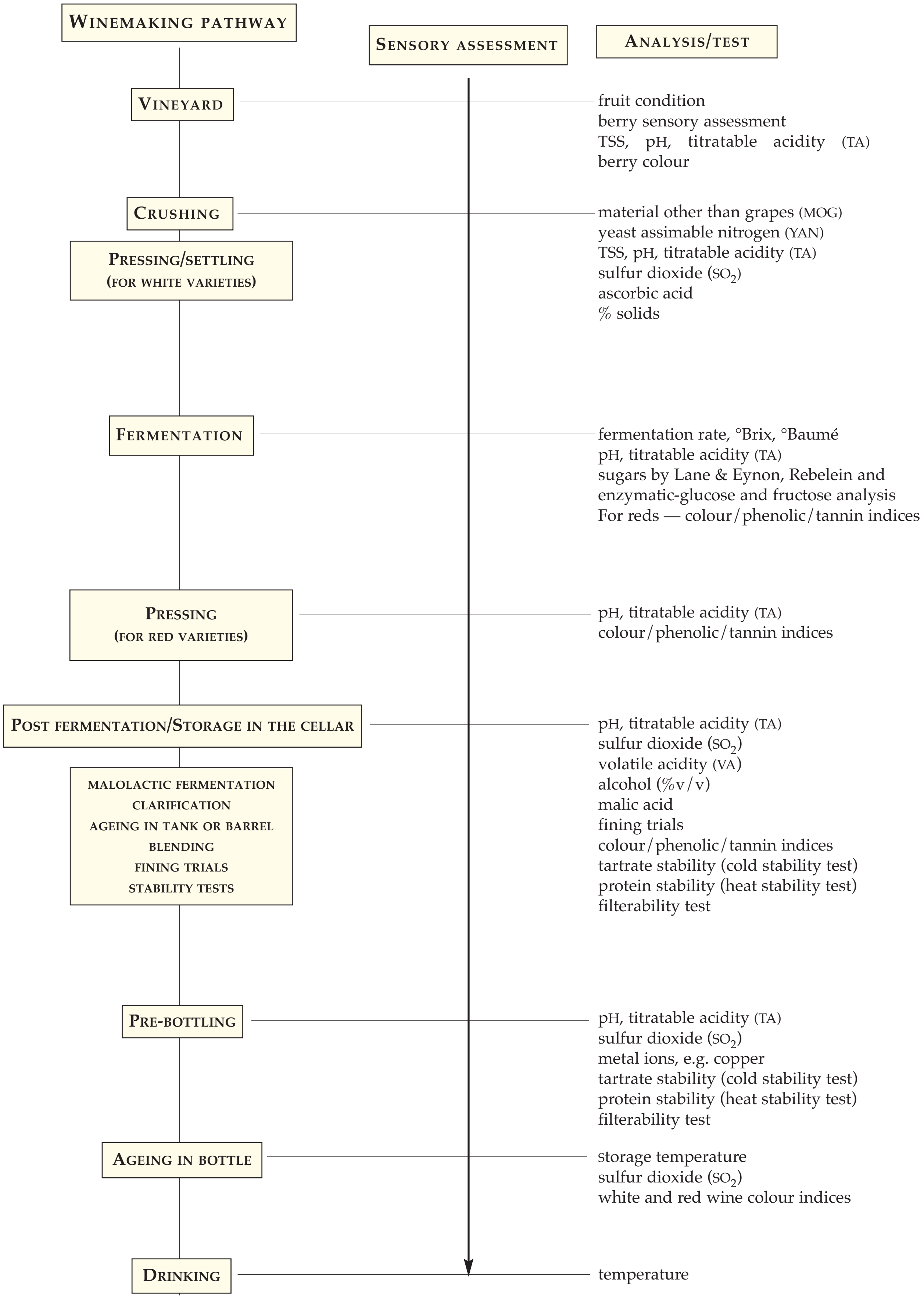

Figure 1.1 Analyses, tests and observations and their timing during the winemaking pathway

5. Sulfur Dioxide

5.1 TYPES AND STRUCTURES

Sulfur dioxide (SO_2) is a colourless gas at room temperature. It has an irritating odour.

Sulfur dioxide in wine originates from that produced by yeast and that added throughout the winemaking process. Final levels depend on several reactions/events that take place throughout the life of the wine.

5.2 THEORY AND CONCEPTS

5.2.1 SPECIES OF SO_2

In water or in aqueous environments such as juice, must or wine SO_2 exists as several species in equilibrium (Figure 5.1).

H_2SO_3

SO_2: H_2O

$$\underset{\text{molecular } SO_2}{SO_2 + H_2O} \rightleftharpoons \underset{\text{bisulfite}}{H^+ + HSO_3^-} \rightleftharpoons \underset{\text{sulfite}}{H^+ + SO_3^{2-}}$$

Figure 5.1 The species of SO_2 present in juice or wine

The weak acid species (H_2SO_3) has not been isolated and therefore the left hand side of the equilibrium is written as SO_2 hydrated/associated with H_2O (SO_2:H_2O) or as $SO_2 + H_2O$ (Figure 5.1); the hydrated/associated species acts as a weak acid.

The equilibrium between the species is dependent on the pH, and to a lesser extent ionic strength, temperature and alcohol concentration of the medium. The relative distribution of the SO_2 species with pH is determined by their pK_a values.

Reported values for the pK_{a_1} and pK_{a_2} for SO_2 in aqueous solution vary, e.g. pK_{a_1} 1.78 to 2.0 and pK_{a_2} 7.0 to 7.21 (Waterhouse et al. 2016). A distribution diagram for SO_2 in aqueous solution using values for pK_{a_1} of 1.81 and pK_{a_2} of 7.2 is illustrated in Figure 5.2.

In wine the pK_{a_1} and pK_{a_2} values are different from those in aqueous solution because of the effect of ethanol, temperature and ionic strength of the wine on the dissociation equilibrium. The higher the ethanol concentration and the temperature of the wine, the higher the pK_a (higher ethanol concentration and temperature suppress the dissociation); the higher the ionic strength of the wine, the lower the pK (the presence of other ions increases the dissociation) (Waterhouse et al. 2016).

Because of the above influences on pK_a values, empirically derived correction factors are applied to nominate the pK_a values in wine (Waterhouse et al. 2016). This difference in pK_a values for aqueous solution, juices and wines is important in constructing the pH dependent distribution diagram for SO_2. It means that the distribution diagram for SO_2 (shown in Figure 5.2) could vary slightly for each juice and wine.

The differences in the distribution pattern from the aqueous representation in Figure 5.2 is likely to be greater for wines than juices.

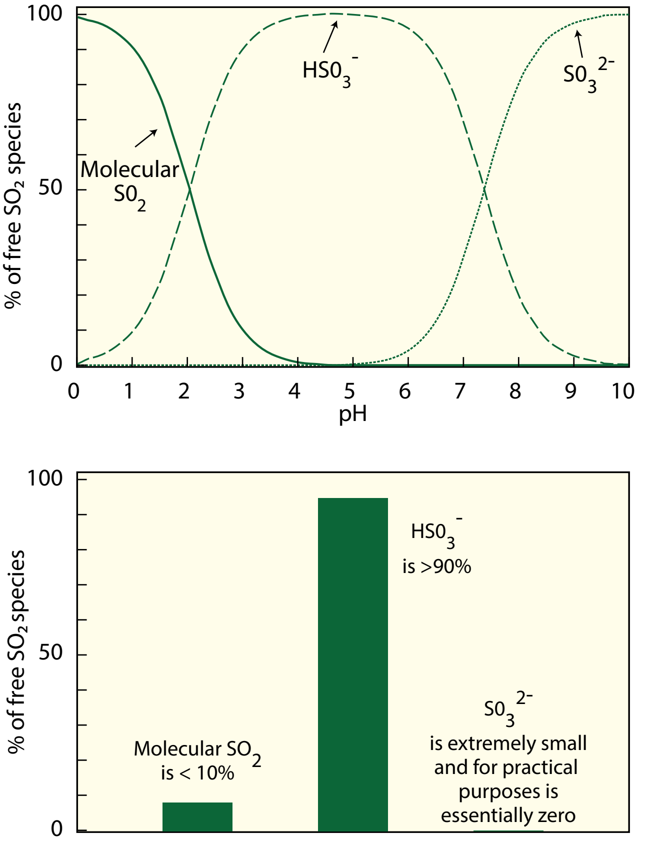

Figure 5.2 The distribution diagram for SO_2 in aqueous solution. Note, for juice the graph will be similar and for wine the lines move slightly to the right.

5.2.2 FORMS OF SO_2

FREE FORMS

In winemaking, SO_2 is described as being either in 'free' or 'bound' forms or states. We use the term 'form' as this is what is commonly used in practice.

The free form of SO_2 consists of molecular SO_2, the bisulfite (HSO_3^-) and the sulfite (SO_3^{2-}) species.

Because there are extremely low levels of SO_3^{2-} present in juice or wine, free SO_2 is essentially the combination of molecular SO_2 and HSO_3^-, existing in a pH dependent equilibrium.

BOUND FORMS

In juice or wine, SO_2 (as the HSO_3^- species) can combine with compounds present in the juice or wine and when this takes place SO_2 is referred to as being in the 'bound' form.

The bound form of SO_2 consists of SO_2 bound either weakly or strongly to compounds present in the wine.

TOTAL SO_2

Total SO_2 is the sum of free and bound forms

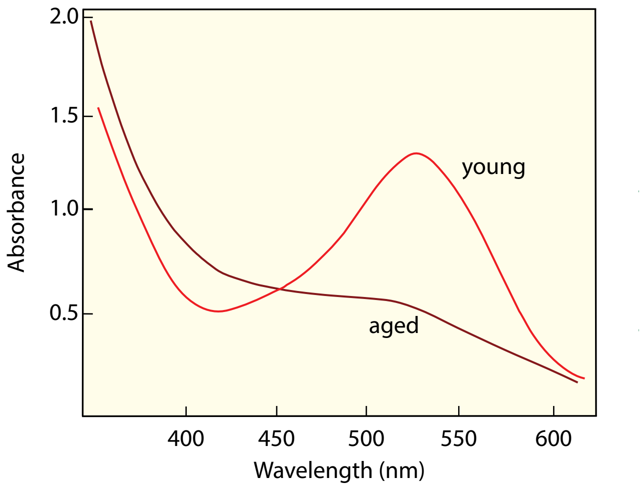

Figure 6.8 Examples of a spectral scan of a young wine and an older wine. Note as the wine ages A_{520} decreases and A_{420} increases (modelled on a figure in Somers 1998).

Table 6.9 An example of the effect of pH change on wine colour density

Wine	Wine colour density (A_{520} + A_{420})		
	pH 3.7	pH 3.4	% change from pH 3.7 to 3.4
wine 1	4.8	5.6	+17
wine 2	7.7	9.6	+25
wine 3	13.4	15.7	+18

Table 6.10 An example of the effect of sulfur dioxide addition to a red wine on wine colour density

	Free sulfur dioxide concentration (by aspiration)	Wine colour density (a.u.)	% decrease relative to base wine
base wine	2.6	11.5	-
+ addition of sulfur dioxide	8.6	10.7	7
	18.7	9.6	17
	27.2	8.8	24
	36.0	8.0	30
	51.0	7.7	33
	81.0	6.9	40

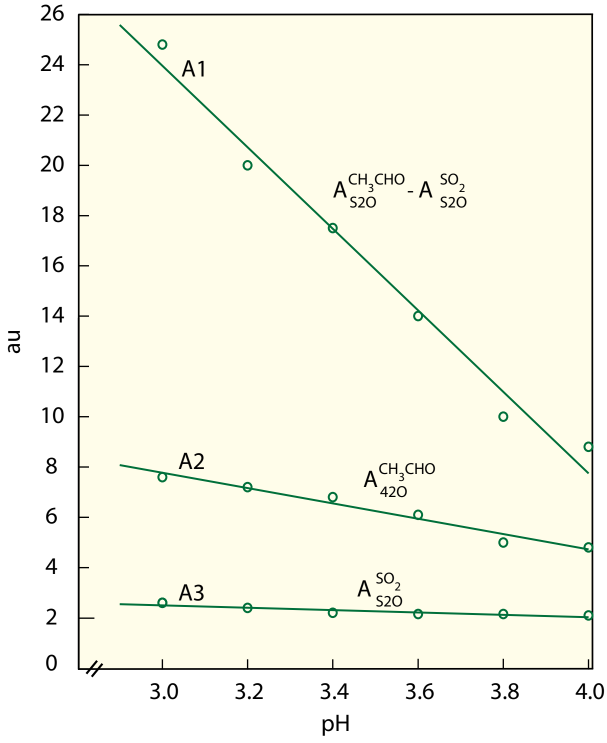

Figure 6.9a The response in A_1 ($A_{520}^{CH_3CHO}$ - $A_{520}^{SO_2}$), A_2 ($A_{420}^{CH_3CHO}$) and A_3 ($A_{520}^{SO_2}$) as a result of a change in pH for a wine with high total anthocyanins concentration (data from Richter 1987).

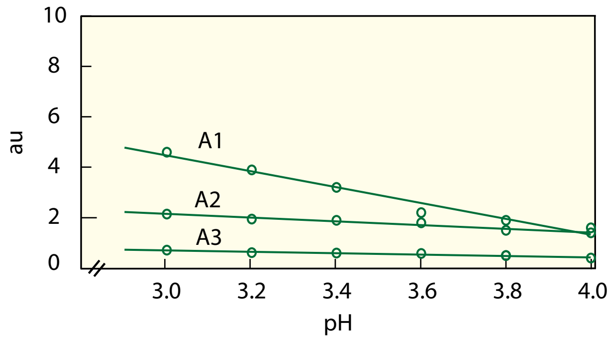

Figure 6.9b The response in A_1 ($A_{520}^{CH_3CHO}$ - $A_{520}^{SO_2}$), A_2 ($A_{420}^{CH_3CHO}$) and A_3 ($A_{520}^{SO_2}$) as a result of a change in pH for a wine with low total anthocyanins concentration. Codes to A1, A2 and A3 are shown on the top graph (data from Richter 1987).

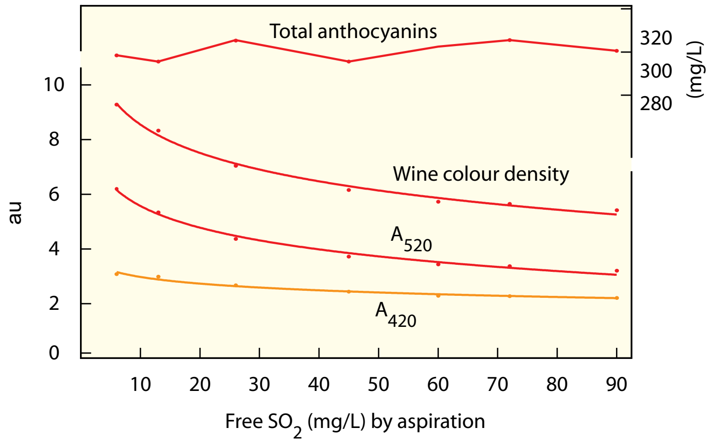

Figure 6.10 An example of the response of total anthocyanins, wine colour density, A_{520} and A_{420} as a result of a change in sulfur dioxide (SO_2) concentration

9. Metal ions and complexes

WHITE WINE STUDIES: Below we summarise the possible species/forms of Cu in white wine and how they can be categorised through the colorimetric assay developed by Clark and co-researchers. We use the terms of Clark et al. (2020):

(a) 'non-sulfhydryl bound-Cu' — this is comprised largely of complexes of Cu(II) bound to organic acids, most likely tartrate, and is termed 'Cu-organic acid complexes'. Note, this complex may contain traces of aquated Cu(II) ions in equilibrium with the organic-acid bound Cu(II).

(b) 'sulfhydryl-bound Cu' — this is comprised largely of complexes where Cu(I) is bound to either H_2S to form Cu_2S complexes (termed Cu-sulfide complexes) or to thiols to form Cu-thiol complexes. The complexes may not be pure. For example, Cu_2S may incorporate other wine components into its structure.

Cu in its various forms can be measured by advanced instrumentation or by a newly developed colorimetric assay. The research group at The National Wine and Grape Industry Centre, Wagga Wagga, Australia, led by Andrew Clark has developed a colorimetric assay that allows measurement of total Cu, 'non-sulfhydryl bound-Cu' (i.e. Cu-organic acid complexes) and 'sulfhydryl-bound Cu' (i.e. Cu-sulfide complexes and Cu-thiol complexes), (Clark et al 2018, 2020, Kontoudakis et al. 2020).

The absorbance measurement can be performed on a UV-vis spectrophotometer using a 4 cm pathlength cell.

The method involves reacting a colourimetric reagent 2,2'-bicinchoninic acid dipotassium salt (BCA) with Cu(I) to form a purple coloured complex. The purple coloured complex absorbs at 563 nm and can be measured spectrophotometrically. The addition of ascorbic acid aids in converting Cu(II) to Cu(I) and the addition of silver(I) nitrate induces dissociation of suspended Cu(I) sulfide to form non-sulfide bound Cu. The addition of silver nitrate to the analysis sample and modification to the timing of the absorbance reading allows the different species/forms of Cu to be determined.

A scheme, based on the studies of Clark and co-researchers, categorising the different possible forms of Cu in white wine is described below (Figure 9.1).

Possible species /forms of copper in white wine

- Total copper (Cu)
  - Non- sulfhydryl-bound Cu
    - Cu-organic acid complexes
  - Sulfhydryl-bound Cu
    - Cu-thiol complexes
    - Cu-sulfide complexes

Measurement of different species/forms of Cu in white wine

Total Cu — measured by a) Flame atomic absorption spectroscopy, b) Inductively coupled plasma-optical emission spectroscopy ICP-OES or other advanced instrumentation or c) colorimetric assay (with Ag(I)).

Cu-organic acid complexes — measured by electrochemical techniques, and often termed 'Electrochemically detected Cu'. Also measurable by the colorimetric assay as Cu immediately chelated by BCA and detected colorimetrically (without Ag(I)).

Cu-thiol complexes — measured by the colourimetric assay as Cu chelated by BCA after 1 minute but before 30 minutes and detected colorimetrically (without Ag(I)).

Cu-sulfide complexes — Cu not detected colorimetrically using BCA and 30 minutes incubation (no Ag(I) added); there may be a minor contribution from thiol-bound Cu.

Cu-sulfhydryl complexes - determined as the difference between 'Total Cu' and 'Electrochemically detected Cu'. This may also be determined colorimetrically (without Ag(I)) as the Cu that is not chelated by BCA within 1 minute.

Figure 9.1 Possible species /forms of copper in white wine and measurement of different species/forms of Cu in white wine

RED WINE STUDIES: The species/forms are similar to those shown for white wines.

Total Cu can be measured by techniques shown above for white wine.

Cu-organic acid complexes can be measured by electrochemical techniques.

Cu-sulfhydryl complexes can be determined as the difference between 'Total Cu' and 'Electrochemically detected Cu'.

The colorimetric assay cannot be used for red wines as anthocyanins and red-pigmented polymers interfere in the absorbance measure at 563 nm.

Clark and colleagues have developed a filtration method — diatomaceous earth depth filtration followed by Cu measurement in the filtrate (Clark et al. 2020); this approach allows for the determination of Cu-sulfide complexes from other forms (e.g. Cu-organic acid and Cu-thiol complexes in red wines. A colorimetric method is being investigated.

'Over time in wine, at low oxygen conditions, the Cu-organic acid complexes are gradually converted to sulfhydryl-bound forms. Therefore, in bottle aged white and red wine, copper predominantly exists in the sulfhydryl-bound copper form. (Kreitman et al. 2018, Kontoudakis et al. 2019b, Clark et al. 2020).

10.12 KEY POINTS

- Gases used in the winemaking process include air, carbon dioxide, nitrogen, argon and various mixtures of carbon dioxide/nitrogen and carbon dioxide/argon. They are used to prevent/limit oxygen (air) ingress into the juice/wine.

- Oxygen (air) can enter the wine at various stages in the winemaking pathway, e.g. crushing, pressing, pump overs, transfers and through bottle closures.

- In a wine bottle, oxygen is present in the wine matrix as dissolved oxygen (DO), as a gas in the headspace of the bottle (HSO) and for bottles sealed with a cork, the oxygen encapsulated in the cork. Normally only DO and HSO are measured to obtain a value of 'Total packaging oxygen' (TPO). Levels of DO pre-bottling and TPO post-bottling should be as low as possible.

Oxidation is part of the wine ageing process. Gradual oxygen ingress can lead to desirable ageing reactions. Uncontrolled oxygen ingress leads to erratic and unpredictable ageing processes.

- Measurement of oxygen concentration can provide a guide to the potential risk of oxidation.

- The decrease in SO_2 concentration over time provides a good indication of the extent of oxidation.

- CO_2 concentration can affect the taste and mouthfeel characteristics of not just sparkling wine but also white and red still wines.

If unpleasant and unwanted volatile sulfur compounds
are not desired as part of the wine style.

A strategy could be —

a) aim to achieve the lowest possible concentration of unwanted ('reductive') volatile sulfur compounds and their precursors at all times in the life of the wine.

Although the concentration of H_2S, thiols, thioacetates and other volatile sulfur compounds can be determined by advanced instrumentation, these compounds are normally measured only in research studies. In practice sensory perception is an important tool in detecting these characters.

b) It seems that it may be necessary to have an adequate Cu concentration in the wine to bind any unwanted volatile sulfur compounds present, but not too high to initiate excessive oxidation/reduction reactions. Further research will clarify this issue.

In modern day winemaking, with the use of yeast that produce very low/low levels of unwanted volatile sulfur compounds and a greater understanding of viticulture and winemaking practices that limit formation of volatile sulfur compounds, the natural levels (and any pick-up during winemaking) of Cu in the wine may be adequate to achieve the desired/targeted Cu level. In many cases Cu addition may not be required or desired. The mean concentration of Cu in Australian wine is about 0.3 mg/L. Higher concentrations (especially >0.5 mg/L?) are most likely associated with contamination and Cu fining operations.

Determination of the concentration of Cu (and its various forms) post-fermentation and pre-bottling would assist decisions relating to targeted Cu concentrations (and any Cu additions, if required) and the likelihood of 'reductive' character development in the wine during storage and in bottle.

The risk of unwanted volatile sulfur compounds accumulating in a wine post-bottling is higher under low oxygen condition (i.e. strong reductive conditions). The choice of a closure with sufficient OTR is an important part of this strategy. Pre-bottling choose a closure that allows some small and gradual oxygen ingress and that suits the desired flavour development in bottle over time.

The 'reductive' character may not appear until months/years post-bottling.

If additions of Cu are required using a Cu fining operation, additions of Cu should be determined via sensory trials and should be at the lowest rates possible. Post-fermentation, after a Cu fining operation most of the Cu remains in the wine as Cu-sulfide complexes.

Aim for lower SO_2 and pH conditions rather than higher, while still ensuring that SO_2 levels are within targeted guidelines (Chapter 5) and that acid balance is maintained.

The development of relatively simple methods, e.g. colorimetric, to determine the concentration of the different species/forms of Cu in wine would help advance knowledge of desirable Cu concentrations in different wine styles.

It appears that post-fermentation and, particularly pre-bottling,
Cu concentrations above 0.5 mg/L offer a higher risk
for the development of 'reduced' aromas
than Cu concentrations below 0.5 mg/L Cu.
The risk is higher under low oxygen conditions.

Figure 13.3 The flavour of red wines

THE FLAVOUR OF RED WINES

COLOUR

light red
crimson
mid red
plum-red
burgundy
brick-red
red
purple black
inky-black
magenta
mahogany
tawny
orange
brown

PRIMARY AROMA — examples of descriptors for primary fruit characters of red wines

anise
beetroot
berry
boysenberry
blackcurrant (cassis)
blackberry
boiled lolly
capsicum
cherry (red/black)
chocolate
cinnamon
clove
confectionery
cranberry
dusty
eucalypt
fragrant
fruit cake
fruity
herbaceous
herbs
inky
jammy
juniper berries
kirsch
leafy
licorice
menthol
minty
mulberry
olives (green/black)
pepper (white/black)
perfumed
plum
plum pudding
prune
pumpkin
raspberry
rhubarb
sappy
sarsaparilla
spearmint
stewed plum
strawberry
spice
tobacco
tomato leaf
vegetative
violet

TASTE

SWEET SENSATIONS:
cloying, sweet, unctuous

SOUR/ACIDITY SENSATIONS:
acidic, boney, bracing, brisk, chalky, crisp, edgy, firm, flinty, fresh, lively, metallic, minerally, pebbly, penetrating, refreshing, savoury, sharp, steely, tangy, tart, tingling, vibrant, vivid, zesty, salivating

BITTERNESS:
burning sensation over the tongue and back of the mouth, grippy, hard, sharp

SALTY:
salt, salty

UMAMI:
broth, chicken, dried fish, meat extracts, mushroom, savoury foods, soy sauce, truffle

AGED AROMA — examples of descriptors for aged fruit characters of red wines

bacon
bacon fat
balsamic
barnyard
brambly
briary
cedar
chocolate
cigar-box
cinnamon
coffee
cowyard
earthy
farmyard
feral
forest floor
gamey
humus
leather
meaty
mocha
mushroom
paneforte
porty
prune
raisin
salami
savoury
smoked meats
smoky
sweet fruit
tobacco
toffee
truffle
walnut

STYLE/QUALITY CHARACTERS

attack — evolution — length and finish

balanced
complex
elegant
generous
lingering
long
rich
seemless
short
tight
persistent
powerful

MOUTHFEEL/TEXTURE

light-
medium-
or
full-bodied

abrasive
astringent
chalky
close-knit
coarse
dense
dusty
drying
fat
firm
furry
grainy
grippy
hard
heavy
hot
juicy
lacy
lean
linear
minerally
oval
powdery
puckering
round
salivating
sandy
satiny
silky
smooth
soft
supple
talc
thick
thin
tight
velvety
viscous
warm
watery