

MICRO-OXYGENATION IN CONTEMPORARY WINEMAKING

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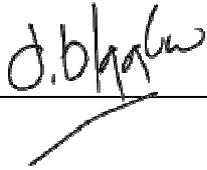
Dissertation submitted in partial fulfillment for the Cape Wine Master Diploma

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DECLARATION

“I, Duane Alexander Blaauw, declare that this report is my own, unaided work. It is submitted in partial fulfilment of the requirement for the diploma of Cape Wine Master to the Cape Wine Academy. It has not been submitted before for qualification of examination in this or any other educational organisation.”

Signed

A handwritten signature in black ink, appearing to read 'd. blaauw', is written over a horizontal line. The signature is cursive and somewhat stylized.

Date

30 January 2009

ABSTRACT

Micro-oxygenation is a new, innovative wine-making technique which involves the controlled introduction of low concentrations of oxygen during early wine maturation. It is claimed that micro-oxygenation can reproduce the benefits of barrel-aging but in a much shorter time and at a fraction of the cost. Micro-oxygenation is supposed to result in wines with soft, accessible tannins as well as greater colour stability which is obviously appealing to winemakers vying for a share of the increasingly competitive global market. The technique was originally developed in France but is now used throughout the world including South Africa.

This report provides details on the practical application of micro-oxygenation. It also summarises the available literature on the basic science underlying the technique and reviews the scientific studies which have attempted to evaluate the impact of micro-oxygenation on wine quality, structure and flavour.

Our current understanding of the basic science indicates that oxygen certainly does play a role in the phenolic reactions that relate to colour stabilisation and maturation. However, that in itself does not prove that the micro-oxygenation technique is consistently able to enhance the desired reactions. Scientific evaluations of current applications of micro-oxygenation have provided evidence that micro-oxygenation can increase colour and improve colour stability. Although micro-oxygenation does produce changes in tannin structure, the actual impact on astringency and mouthfeel are less certain. At present there is little support for the claim that micro-oxygenation enhances fruitiness or reduces green herbaceous flavours in wine. However, the potential risk of increases microbial spoilage associated with micro-oxygenation has also not been clearly proven. Wine anti-oxidant activity is of topical interest because of its importance in the potential health benefits of wine. Although it is possible that micro-oxygenation may decrease wine anti-oxidant capacity this aspect also requires further study.

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ACRONYMS AND ABBREVIATIONS

[x]	: Concentration of x
A	: Anthocyanin
A-T	: Anthocyanin bound to Tannin
AF	: Alcoholic Fermentation
CI	: Colour Intensity
°C	: Degrees Celsius
DO	: Dissolved Oxygen
H ₂ O	: Water
H ₂ O ₂	: Hydrogen Peroxide
HCl	: Hydrochloric acid
MDP	: Mean Degree of Polymerisation
MLF	: Malo-Lactic Fermentation
mg/L	: Milligrammes per Litre
ml/L	: Millilitres per Litre
MOX	: Micro-oxygenation
O ₂	: Oxygen
OD	: Optical Density
OD ₅₂₀	: Optical Density at 520nm
nm	: Nanometres
PPO	: Polyphenol Oxidase
ROS	: Reactive Oxygen Species
SO ₂	: Sulphur Dioxide
T	: Tannin
TAC	: Total Anti-oxidant Capacity
VA	: Volatile acidity

PART I: BACKGROUND

CHAPTER 1: INTRODUCTION

“Oxygenation is unavoidable in winemaking, so we better know what’s happening.”
(Singleton, 1987)

“While exposing wine to limited amounts of oxygen is not a recent innovation, the traditional methods of execution have not, in many cases, given the winemaker adequate regulation of either the rate or the quantum of exposure. The novelty of micro-oxygenation is in the ability of the practitioner to have substantial control over the total amount of oxygen and the rate at which it is delivered to the wine”
(Pour-Nikfardjam and Dykes, 2003)

“Micro-oxygenation is one of the most controversial and misunderstood areas of modern winemaking. Its proponents claim that it can bring about seemingly miraculous changes in wine while its detractors claim it does not work, or worse, that it destroys wine.”
(Paul, 2002)

A large part of the science, and the art, of winemaking has to do with managing exposure to oxygen. The detrimental effects of excessive oxygen - in the form of browning, development of aldehyde aromas, and the loss of fruit flavour - have been recognised since Pasteur (Halliday and Johnson, 2003). Preventing oxygen contact is the primary concern of modern reductive methods for making wine. This is especially true of most contemporary white winemaking in the New World. On the other hand, winemakers deliberately encourage oxygen exposure at certain steps in the production process. For example, it is well known that some aeration of the must augments alcoholic fermentation and well-established winemaking practices such as racking promote oxygenation. Many of the benefits of wine maturation in oak barrels are due to the slow permeation of oxygen through the barrel walls but long-term bottle ageing then takes place in a predominantly oxygen-free environment. Once opened, however, a short period of air contact, in the form of decanting or ‘breathing’, is thought to improve wine quality before consumption. Clearly, winemaking is a complex balance between protection from oxygen and controlled aeration so that any oxygen exposure during winemaking requires careful consideration with regard to dose and timing.

Micro-oxygenation (MOX) is a relatively new winemaking technique which involves the controlled addition of small amounts of oxygen at various stages of the winemaking process (Moutounet *et al.*, 1998; Silva *et al.*, 1999; Rowe and Kingsbury, 1999; Parish *et al.*, 2000). The development of micro-oxygenation has partly been driven by the need to find ways of reproducing the benefits of barrel-ageing in shorter time and at lower cost in an increasingly competitive market (Blackburn, 2004). But there has also been support for the argument that traditional oxygenating processes, such as racking or topping-up, are inherently variable and difficult to regulate, whereas the micro-oxygenation method

provides more direct control and predictability (Nel, 2001; Jones *et al.*, 2004). Interestingly, this technological approach to manipulating oxygen exposure has found favour among both Old World and New World winemakers so that micro-oxygenation has become an essential part of the contemporary winemaker's toolkit in most winemaking countries (Zoecklein *et al.*, 2002).

The proponents of micro-oxygenation claim that the technique results in improved colour, structure and flavour in treated wines. It is the phenolic compounds in wine that are responsible for wine's colour and structure so the claimed improvements due to micro-oxygenation must be produced through biochemical reactions involving phenols and oxygen. There is now a fairly large body of basic scientific research on phenolic reactions in wine and their role in the fundamental oenological processes of oxidation and ageing. This literature is clearly relevant to understanding the potential impact of micro-oxygenation. On the other hand, there are only a few rigorous scientific investigations which have directly evaluated the impact of micro-oxygenation on wine colour, structure or flavour, although the number of studies is growing.

1.1 SCOPE OF THE REPORT

The main objective of this report is to review the current state of knowledge with regard to micro-oxygenation. It will describe the historical development of the technique and its present-day practical application. It will summarise the basic principles and claimed benefits of micro-oxygenation but also attempt to evaluate those claims against the available scientific evidence from both the broader literature on phenolic reactions in wine as well as the more specific, and more recent, scientific investigations of the impact of micro-oxygenation.

The primary focus of this report is on the winemaking technique known as micro-oxygenation. Although the role of oxygen in winemaking more broadly will be an important theme throughout the text, other related oenological practices that promote oxygenation - such as pumping over, topping up, racking and decanting - will not be considered in any detail (see Chapter 2). Given the typical applications of micro-oxygenation, the emphasis will definitely be more on red winemaking than on white winemaking. Indeed, the clear benefits of reductive winemaking under certain circumstances are accepted without debate - our concern here is more to identify when and how a little oxygen may be beneficial. Wine styles other than natural dry wines will not be discussed at all even though oxygen obviously plays an important role in the production of specific oxidative styles of wine such

as sherry and port. Lastly, there is a rapidly growing literature on the anti-oxidant role of phenolic compounds and their potential health benefits, but again this will be considered only very briefly in relation to the potential impact of micro-oxygenation on wine anti-oxidant capacity.

1.2 CONTRIBUTION OF THE REPORT

The purpose of this review is to provide an up-to-date description and synopsis of micro-oxygenation. There are a number of excellent and relatively recent reviews on wine phenolic chemistry (Monagas *et al.*, 2005; Fulcrand *et al.*, 2006; Ribéreau-Gayon *et al.*, 2006c; Parker *et al.*, 2007) and the role of oxygen in winemaking (du Toit *et al.*, 2006b; Danilewicz, 2007; Li *et al.*, 2008), but the intended contribution of this report is to present this somewhat technical material for a practitioner, rather than an academic audience.

Another key objective of the report is to summarise the latest available scientific evidence on the benefits and risks of micro-oxygenation. Although there are a number of general reviews on micro-oxygenation (Parish *et al.*, 2000; Zoecklein *et al.*, 2002; Blackburn, 2004; Pour-Nikfardjam and Creasy, 2004; Goode, 2005), none of these are very recent and few probe beyond the broad assertions of the micro-oxygenation industry. Although a number of more rigorous studies of micro-oxygenation have been published in the last few years (del Carmen-Llaudy *et al.*, 2006; Cano-López *et al.*, 2006; du Toit *et al.*, 2006a; Pérez-Magariño *et al.*, 2007), a systematic review of this evidence has not yet been produced.

1.3 OUTLINE OF THE REPORT

This report is broadly divided into four main parts. Part I (Chapters 1 – 3) outlines the themes to be explored in this report and introduces the micro-oxygenation technique by providing a definition and brief history. Part II (Chapters 4 – 5) describes the commercial claims and practical application of micro-oxygenation. Part III of the report (Chapters 6 – 7) reviews the available scientific evidence concerning the relevant chemical reactions as well as the proven advantages and disadvantages of micro-oxygenation. Finally, Part IV provides some general conclusions and recommendations.

CHAPTER 2: DEFINING MICRO-OXYGENATION

The focus of this report is on the technique known as *micro-oxygenation*. However, there are a number of terminological and definitional issues that need to be clarified because different writers and disciplines use different terminology and definitions. That is the purpose of this short introductory chapter.

In this report *micro-oxygenation* will be simply defined as “the intentional and controlled addition of small measured amounts of oxygen to wine” (Paul, 2002). *Oxygenation* usually refers to the deliberate exposure of wine to oxygen and implies a positive impact on wine quality. In contrast, the term *oxidation* (or *oxidised*) is more frequently associated with the negative consequences of excessive oxygen exposure (Blackburn, 2004). Chemists, however, use the words oxidation and reduction simply to describe the exchange of electrons in redox reactions.

Micro-oxygénation was the phrase coined when the technique was developed in France and *micro-oxygenation* is still the most common usage. *Aeration* and *micro-aeration* are sometimes used as synonyms for oxygenation and micro-oxygenation, though strictly aeration means exposure to air whereas oxygenation implies treatment with pure oxygen. In practice, however, both terms are used for both air and pure oxygen exposure. *Micro-bullage* is another synonym which invokes the characteristic bubbling of oxygen but this term is not in widespread use and, in fact, more modern equipment strives to make the bubbles as small and unobtrusive as possible.

Micro-oxygenation is generally accepted as being the deliberate introduction of oxygen and involving the use of specialised equipment to regulate the doses of oxygen that are administered. So the term does not usually include the passive oxygen exposure that occurs during barrel ageing nor the range of winemaking practices – such as pumping over, topping up and racking – where oxygen exposure may be intentional but is not well measured (Rieger, 2000).

However, there is less consensus on how low the dose needs to be to qualify as *micro-oxygenation* (Paul, 2002), whether or not the oxygen treatment has to be continuous (Jones *et al.*, 2004), and if micro-oxygenation can only be used if exposure happens over a long period of time (Waterhouse, 2004). For example, a number of authors use *macro-oxygenation* to refer to the higher dose exposures (in the order of 2-4 mg/L/day) of fairly

short duration (usually one or two weeks) which are sometimes used in the initial stages of oxygen treatment (typically before malolactic fermentation). These commentators reserve the term *micro-oxygenation* for very low dose treatment (more like 2-4 mg/L/month) that is applied over the period of a number of months after malolactic fermentation (Paul, 2002; Loch, 2002; du Toit *et al.*, 2006a). Although there is clearly merit in this distinction, it is not used consistently in the literature or the industry and will not be strictly adhered to in this report. Similarly, although micro-oxygenation is usually administered continuously and over longer periods of time, some practitioners recommend interrupted doses or short bursts of treatment and these applications will still be considered part of the broader micro-oxygenation methodology.

There have been some developments and extensions of the original equipment and techniques and these variants are sometimes distinguished by specific terms or brand names. For example, *cliquage* is the term coined by OenoDev to refer to their application of micro-oxygenation in barrel and O₂mate is the brand name of a method which utilises oxygen diffusion rather than bubbling. Some of these terms are discussed in Chapter 5 on the practical applications of micro-oxygenation.

Lastly, *hyper-oxidation* (or sometimes *hyper-oxygenation*) is a specific technique which has been used in white winemaking where the must is deliberately oxidised prior to fermentation by adding high doses of oxygen (Bird, 2005). The phenolic compounds precipitate out and are removed during clarification but it is argued that the wines produced by this process are lighter and more stable (Cheynier *et al.*, 1991; Schneider, 1998). The oxidation of white must is a fundamentally different process to the oxidation of red wine so this method is not usually considered as part of the topic of micro-oxygenation and is not included in this report. Schneider (1998) provides a detailed review of hyper-oxygenation for the interested reader.

CHAPTER 3: THE HISTORY OF MICRO-OXYGENATION

Micro-oxygenation is a relatively new addition to the oenological toolkit. This chapter briefly outlines the early development of micro-oxygenation and its subsequent uptake around the world. It also identifies some of the key scientific discoveries in phenolic and oxidation chemistry that underlie the claims of the technique.

Micro-oxygenation was developed in the early 1990s by Patrick Ducournau, a winemaker in the Madiran region of South West France (Dempsey, 2001). The Madiran appellation produces red wines made from a blend of Tannat, Cabernet Sauvignon and Cabernet Franc although the wines from the top estates are predominantly Tannat (Robinson, 2006). Given the predominance of Tannat, it is not surprising that Madiran wines are well known for their tannic and astringent character and that they require very long bottle-ageing before they are ready for consumption. Ducournau was motivated to develop micro-oxygenation in order to soften the tannic structure of the Tannat and produce wines that could be consumed earlier.

After three years of initial research Ducournau and Laplace registered a patent for their micro-oxygenation method in 1993 (Lemaire *et al.*, 2002). They set up a company called OenoDev to provide micro-oxygenation equipment and expertise to the broader winemaking market (Goode, 2005). Further research and development in these early years was undertaken at a number of French Universities, and in particular through the work of Michel Moutounet at Montpellier University (Rieger, 2000; Moutounet *et al.*, 2001). Micro-oxygenation was finally authorised for use in Europe by the European Commission in 1996 (Robinson, 2006).

In 1998, OenoDev enlisted an American company, Vinovation, to expand their activities in the United States (Vinnovation, 2001b). Together these two companies were responsible for much of the early expansion of micro-oxygenation around the world and remain important players in the industry. However, the market is now much more diverse and there are a number of new companies selling micro-oxygenation equipment and providing micro-oxygenation consultancy services. Some of the more influential companies include Parsec in Italy (Parsec, 2008), Stavin in the United States (Bowyer and McCord, 2007; Stavin, 2008), and Wine Network in Australia (Parish *et al.*, 2000; Paul, 2002; Kelly and Wollan, 2003).

Accurate recent figures on worldwide sales of micro-oxygenation equipment are not available. France remains the largest user of micro-oxygenation with over 2000 units in operation by 2006, mainly in Bordeaux (Robinson, 2006). A similar number of units have been sold worldwide. Uptake has been significant in Italy (Dempsey, 2001), Chile (Goode, 2005; Robinson, 2006) and the United States (Zoecklein *et al.*, 2002). For example, in a 2007 survey of American wineries, 16% of small wineries, 50% of medium-sized wineries, and 83% of large wineries admitted to using micro-oxygenation on some of their wines (Goldfarb, 2007). Internationally, some of the most high profile producers publicly promoting micro-oxygenation include Randall Graham of Bonny Doon (Graham, 2003), E&J Gallo (Rieger, 2000), Domaine Chandon (Dempsey, 2001), and Vasse Felix (Otto, 2003). The market in South African is still relatively small but the technique has been used by a number of producers and local sales of the equipment continue to increase (Euroberry, 2008a).

Phenolic chemistry is a very important and rich area of wine research. The fundamental reactions which seem to be implicated in micro-oxygenation were well known by the time the technique was actually developed (Rieger, 2000).

Vernon Singleton and his associates at UC Davis in California elucidated the key mechanisms involved in the oxidation of phenolic compounds in musts and wines in the 1970s and 80s (Singleton, 1987). The fact that colour stability in wine was due to anthocyanins reacting with other compounds to produce large polymeric pigments was first identified by Pascal Ribéreau-Gayon and Emile Peynaud in 1962 [cited in (Thorngate and Singleton, 1994)]. The work detailing these reactions between anthocyanins and tannin molecules was mainly completed by Somers in the remaining years of that decade (Somers, 1971). Another very important part of the puzzle was research in the early 1970s that demonstrated that acetaldehyde (produced from the oxidation of ethanol) was the link in certain condensation reactions between anthocyanin and tannins (Wildenradt and Singleton, 1974; Timberlake and Bridle, 1976).

Of the more recent discoveries which may be of some relevance to the chemistry of micro-oxygenation are the discovery of the category of compounds known as pyranoanthocyanins (Fulcrand *et al.*, 1996; Monagas *et al.*, 2005; Fulcrand *et al.*, 2006), and research on the central role of iron and copper in the oxidation reactions of phenols in wine (Danilewicz, 2003; Danilewicz, 2007).

PART II: PRACTICAL EXPERIENCE OF MICRO- OXYGENATION

CHAPTER 4: INDUSTRY CLAIMS ABOUT MICRO-OXYGENATION

The industry now has over 15 years experience on the use of micro-oxygenation. The practical insights derived through that experience may be useful even if all the claims have not yet been properly evaluated or scientifically proven. Part II of this report is concerned with presenting the accumulated practical experience on the application of micro-oxygenation. This Chapter summarises the typical industry claims about the benefits of micro-oxygenation but also lists the acknowledged risks. Chapter 5 will then describe the usual methods and techniques involved in micro-oxygenation. The data presented in Part II will be contrasted with Part III which reviews the available scientific evidence on the subject of micro-oxygenation.

4.1 BENEFITS OF MICRO-OXYGENATION

The benefits of micro-oxygenation as outlined in the Vinovation promotional material are shown in Table 1. This characterisation has proven particularly influential since it is often cited verbatim as the list of benefits of micro-oxygenation by practitioners and academics alike (Rieger, 2000; Nel, 2001). Vinovation provides no experimental data to justify these claimed benefits but the Vinovation notes are essentially a translation into English from the original OenoDev documentation, so presumably the list has its origins in the early testing and development of the technique in France.

Table 1: Vinovation/OenoDev - Goals of Micro-oxygenation

Tannin and mouthfeel restructuring	Increased body. Softer, richer tannins. Rounder and more supple mouthfeel.
Colour stability	Early polymerisation results in more intense, oxidation-resistant pigmentation.
Aroma integration	Enhanced fruit forwardness and integration of oak aromas and vegetal aspects.
Combat sulphides and reductive aromas	Feed quantified oxygen tailored to the wines individual need. Achieve optimum balance and overcome wine's tendency to produce sulphides.
Longevity potential	Treatment is not beneficial in promoting early release. Does not prematurely age wine.

From (Vinnovation, 2001a)

These then are the essential benefits of micro-oxygenation as claimed by the industry: treating your red wine with a course of micro-oxygenation will result in a softer, more accessible wine with improved colour and better flavour (Parish *et al.*, 2000; Paul, 2002).

The advantage of micro-oxygenation emphasised most prominently by supporters is the improvement in wine structure. Indeed that was why the technique was originally developed – to make harsh tannic wines more accessible and ready to drink earlier. In fact the Vinovation promotional material prefers the term ‘integrated tannin management’ to micro-oxygenation (Vinnovation, 2001b). It is proposed that treatment with low doses of oxygen will speed up tannin polymerisation resulting in softer tannins, decreased astringency, and an overall improvement in mouthfeel.

The added oxygen will also promote reactions between anthocyanins and tannins to produce larger molecules known as polymeric pigments. Polymeric pigments are more deeply coloured and are more resistant to degradation, effects of pH changes, and bleaching by sulphur dioxide (SO₂). So that the overall impact of micro-oxygenation should be both enhanced red wine colour and more stable colour over time (Paul, 2002).

These two benefits, improved palatability and enhanced colour, are more traditionally achieved by ageing red wine in oak barrels over a period of months. Although maturation in oak barrels will also impart advantageous oak flavours to wine, it was established in the 1970s and 80s that many of the benefits of barrel-ageing are due to the slow diffusion of oxygen through barrel walls and that the resulting low doses of oxygen promote beneficial oxidation reactions which modify wine structure and colour (Atanasova *et al.*, 2002; Cheynier, 2002). Indeed, it was those discoveries that suggested to the inventors of micro-oxygenation that low dose oxygen treatment would be an alternative way to achieve the same outcomes. Therefore, micro-oxygenation is frequently presented as a substitute for barrel maturation, with the possible advantages of lower cost, faster maturation, and more scientific control (Jones *et al.*, 2004; Paul and Gore, 2006; Dykes and Kilmartin, 2007). With the addition of oak substitutes, such as oak staves or oak chips, it may then be possible to replicate completely the effects of oak barrel-ageing in stainless steel tanks, which is an attractive option for winemakers, particularly for medium-range wines (Zoecklein *et al.*, 2002; Goode, 2005).

Interestingly, although these changes induced by micro-oxygenation are interpreted by many as speeding up the ageing of wine (Zoecklein *et al.*, 2003), Vinovation takes considerable effort in their documentation to present it differently. As shown in Table 1,

they promote the modifications in mouthfeel and colour stability but argue that these changes are not about hastening ageing or allowing earlier release of the wine. Presumably, they are concerned with the negative connotations of premature ageing and implications for long-term longevity, but the semantic distinctions are not completely clear or convincing, and it is somewhat of a stretch to present micro-oxygenation as actually increasing longevity.

The other stated benefits of micro-oxygenation have to do with improvements in aroma and flavour. It is frequently claimed that micro-oxygenation is effective in decreasing vegetative and herbaceous flavours of wine (Parish *et al.*, 2000; Paul, 2002; Blackburn, 2004). The origins of this claim are unclear since there is no obvious association between herbaceousness and reductive or oxidative conditions, but it may be linked to the notion that micro-oxygenation is successful in mitigating the effects of harsh green tannins.

The contention that micro-oxygenation is able to decrease the sulphide aromas associated with reduction seems more logical and is somewhat easier to understand. However, the simplistic argument that problems caused by reduction can be easily corrected by oxidation or oxygenation should not be accepted uncritically because many of the malodorous reductive sulphur compounds are extremely resistant to all corrective efforts and treatments (Ribéreau-Gayon *et al.*, 2006b).

A number of other possible benefits of micro-oxygenation are mentioned less frequently in the industry. These include:

- Micro-oxygenation could increase long-term oxidative stability, providing some protection against later, more harmful, oxidation (Parish *et al.*, 2000);
- A related idea is that the use of micro-oxygenation could lower SO₂ requirements in winemaking (Robinson, 2006; Theron, 2007);
- Micro-oxygenation produces more complex wines than stainless steel tank production alone (Bird, 2005);
- Micro-oxygenation used before alcoholic fermentation could provide additional oxygen for yeast metabolism and decrease the incidence of sluggish or stuck fermentations (Paul, 2002; Zoecklein *et al.*, 2003); and
- Micro-oxygenation could be used to prolong white wine maturation on lees producing wines with increased body and freshness.

4.2 RISKS OF MICRO-OXYGENATION

“In some ways, I feel like a dynamite salesman. Everybody just wants a stick, but you need support and training too, because if you fry your wine and micro-oxygenation doesn’t work, you’re poisoning the process for everyone. You’ve got to buy the education with the stick of micro-oxygenation dynamite”

Clark Smith, Vinovation. Quoted in (Dempsey, 2001)

The above quote by the current (2008) President of Vinovation indicates that the industry does recognise that there are risks associated with the use of micro-oxygenation. The most important identified risks of micro-oxygenation are summarised in Table 2.

Table 2: Identified Risks of Micro-oxygenation

- | |
|---|
| <ol style="list-style-type: none">1. Palate dryness2. Phenolic precipitation resulting in colour loss3. Development of aldehyde / oxidised aromas and flavours4. Increased volatile acidity (VA)5. Microbial spoilage |
|---|

Summarised from (Paul, 2002; Blackburn, 2004)

The most important concern is clearly over-treatment resulting in excessive polymerisation, tannic dryness, colour loss, decreased freshness, and the development of aldehyde and oxidised aromas. This is an important risk because there are no easy markers of the transition from complete treatment to over-treatment. The industry is quick to point out that there is a steep learning curve with this technology and that treatment with micro-oxygenation requires experience, significant attention and careful monitoring. Therefore, as implied in the above quote, many suppliers of micro-oxygenation equipment include a more or less compulsory package of consultancy and support to go with the new machinery.

There is also the possibility that oxygenation will promote the growth of aerobic organisms. Acetic acid bacteria (AAB) and *Brettanomyces* are the most important aerobic organisms of importance in winemaking (Paul, 2002; du Toit *et al.*, 2006a). Acetic acid bacteria could cause increased volatile acidity (VA) as well as complete spoilage of the wine, whereas *Brettanomyces* contamination is associated with the increased production of malodorous volatile phenols (Ribéreau-Gayon *et al.*, 2006b).

A number of other possible disadvantages of micro-oxygenation are mentioned in the literature but have not yet been confirmed. For example, there are unanswered questions about:

- The impact of micro-oxygenation on wine anti-oxidant capacity (and therefore the health benefits of red wine) (de Beer *et al.*, 2008);
- The long-term ageing potential of treated wine;
- Whether or not micro-oxygenation increases the production of sulphide dimers; and
- The effects of micro-oxygenation on other important aroma compounds such as pyrazines (Dempsey, 2001).

CHAPTER 5: MICRO-OXYGENATION METHODS AND TECHNIQUES

Like many other viticultural practices, there is unfortunately no consistent approach to the application of micro-oxygenation. What is common is the use of specialised equipment to deliver measured amounts of oxygen to wine. Different suppliers have developed slightly different machinery but the basic principles of the different technologies remain similar.

However, there is significant variation in the timing, dose and duration of oxygen treatment recommended by different suppliers and practitioners. Indeed, the industry seems completely opposed to the idea of a standardised recipe for micro-oxygenation and has strongly promoted the idea that treatment needs to be tailored to the needs of each wine (Rieger, 2000; Vinovation, 2001b). This approach is clearly appropriate but, of course, it does make things somewhat complicated for the novice micro-oxygenator.

This Chapter provides a brief overview of the practical methods of micro-oxygenation. The underlying principles and common applications of micro-oxygenation are outlined first. That is then followed by a description of the basic equipment in current use before exploring some of the practical issues in actually managing micro-oxygenation.

5.1 BASIC PRINCIPLES OF MICRO-OXYGENATION TREATMENT

The key challenge of micro-oxygenation treatment is to promote beneficial oxidation reactions whilst avoiding excessive oxidation which would spoil the wine.

Therefore, one of the most important fundamental principles of micro-oxygenation treatment is that the rate of oxygen addition to wine should be lower than the rate at which the oxygen is consumed through chemical reactions (Lemaire *et al.*, 2002; Pour-Nikfardjam and Dykes, 2003; Jones *et al.*, 2004).

The ability of a wine to take up oxygen, its oxygen absorptive capacity, is mainly determined by its total phenolic content because it is the phenolic compounds in wine that react with oxygen. So a light white wine could take up about 60 ml O₂ /L whereas the figure for red wine is more in the order of 600 ml/L (Singleton, 1987). However, it is somewhat more complicated than that. For one thing, different categories of phenolic compounds react differently with oxygen so that it is the types of phenolics present, rather than the total phenolic concentration, that is relevant. More importantly, the total absorptive capacity of a

wine depends on the rate of oxygen addition. Rapid exposure to high doses of oxygen quickly overwhelms the wine's capacity resulting in oxidation and browning. However, the same wine could tolerate fairly large amounts of oxygen administered in small doses because the oxygen is slowly consumed through reactions with phenols. It is these slow oxygen-induced polymerisation reactions that are implicated in the positive changes associated with wine ageing (Fulcrand *et al.*, 2006; Ribéreau-Gayon *et al.*, 2006a), and it is these types of reactions that micro-oxygenation seeks to emulate (Lemaire *et al.*, 2002; Bautista-Ortín *et al.*, 2007).

In addition to phenolic composition, a wine's ability to consume oxygen will also be influenced by other factors such as the availability of alternative substrates, anti-oxidants and oxidation catalysts. The principal anti-oxidants in wine include SO₂ and ascorbic acid while metal ions in wine act as important catalysts of oxidation reactions (du Toit *et al.*, 2006b; Danilewicz, 2007; de Basquiat, 2008). Yeast lees have a very high oxygen absorptive capacity so the presence of lees will also significantly increase a wine's ability to consume oxygen (Fornairon-Bonnefond *et al.*, 2003; Salmon, 2006; Mazauric and Salmon, 2006).

If the rate of oxygen addition is lower than the rate of oxygen consumption in phenolic reactions then oxygen will not accumulate in the wine. So the optimal oxygen dose in micro-oxygenation should not produce an increase in the concentration of dissolved oxygen (Paul, 2002). This principle is used in the monitoring of micro-oxygenation (Pour-Nikfardjam and Dykes, 2003) but it should also be noted that the dissolved oxygen concentration depends on the solubility of oxygen in wine which is very temperature dependent – wine at 25°C and completely saturated with oxygen would contain 7 mg O₂/L whereas at 5°C the figure would increase to 10 mg/L.

5.2 APPLICATIONS OF MICRO-OXYGENATION

Micro-oxygenation is not appropriate for all wines (Parish *et al.*, 2000). Micro-oxygenation is simply another technique available to winemakers and if used at all, its use must be tailored to specific circumstances and particular wines.

Following the potential benefits of micro-oxygenation listed in Section 4.1, some of the potential applications of micro-oxygenation and the key choices that need to be made are summarised in Table 3. The vast majority of micro-oxygenation applications have been in

red winemaking though there may be some specific indications where it could be useful as part of white winemaking (Rieger, 2000).

Table 3: Potential Applications of Micro-oxygenation (MOX)

Objectives	Suitable Wines / Applications	Important Choices to be Made
1. Improved colour and mouthfeel	Green wines High tannic wines Low colour varieties Medium-quality wines not worth barrel ageing	<u>Timing</u> Pre-MLF Post-MLF Pre- + Post-MLF <u>Dose and duration</u> Higher dose, short duration Lower dose, long duration <u>Additions</u> Oak alternatives MOX + barrel ageing
2. Decrease herbaceous flavours	Green wines	Timing Dose and duration
3. Removal of reductive sulphur flavours	Wines with reductive faults	Timing Dose and duration
4. Prolonged ageing on lees	White wines benefiting from prolonged lees contact	Post-MLF Dose and duration
5. Improved alcoholic fermentation	To prevent sluggish or stuck fermentations	During alcoholic fermentation Very high dose, short duration

Although the industry promotional material usually presents more lofty aspirations, in current practice there appear to be two main motivations for the application of micro-oxygenation (Goode, 2005):

1. As a cost-saving technique: to replicate some of the advantages of wood ageing without the cost of expensive barrels; and
2. As a remedial technique: to correct faulty wines with harsh green tannins or with herbaceous or reductive sulphur aromas.

The most common goal or application of micro-oxygenation, therefore, would be to improve wine structure and colour (Table 3). Information on remedial applications to remove off-odours is extremely limited and there is even less real experience on its use to improve lees ageing of white wines (Zoecklein *et al.*, 2003; Tibbits, 2003; Dykes and Kilmartin, 2007). Micro-oxygenation equipment could also be used to introduce relatively high doses of oxygen during alcoholic fermentation which has been shown to be beneficial for yeast metabolism and preventing stuck fermentations (Paul, 2002; Jones *et al.*, 2004; Salmon,

2006). Whether these newer applications of oxygenation in winemaking can still be characterised as micro-oxygenation raises difficult definitional issues (see Chapter 2).

Micro-oxygenation was originally developed to improve very tannic varieties such as Tannat or Cabernet Sauvignon, and the technique is most widely used in regions and appellations where those varieties predominate. Certainly, full-bodied tannic wines are better candidates for micro-oxygenation. Thinner, medium- or light-bodied, red wines are much more risky and may become harsh and dried out after micro-oxygenation (Paul, 2002). For example, experience with Pinot Noir has not been encouraging and the technique is not used in Burgundy despite its enthusiastic uptake in other regions of France (Rieger, 2000). Therefore, micro-oxygenation is not really promoted as a way of improving poor colour alone. Vinovation (2001b) warns that low colour risks dryness and recommend a tannin to anthocyanin ratio of at least 4:1. However, it has also been suggested that micro-oxygenation would be useful for certain varieties, such as Sangiovese, that are relatively low in colour but high in tannins (Robinson, 2006).

However, very tannic wines are confined to specific appellations such as Madiran or are the result of faulty viticulture (picking grapes too early or from virus-infected vines). In practice, the most widespread use of micro-oxygenation is as a cheaper alternative to barrel-ageing for medium-quality wines intended for short to medium-term consumption (Paul and Gore, 2006; Goldfarb, 2007). In using micro-oxygenation for this purpose there are a number of choices to be made (Table 3). Micro-oxygenation can be applied before malolactic fermentation (MLF), after MLF or both. This will also obviously influence the dose and duration of micro-oxygenation treatment. Unfortunately there are no clear rules to help the micro-oxygenation beginner but these decisions are discussed in more detail in Section 5.4.3. If the objective is to mimic barrel ageing in stainless steel tanks then alternative oak products such as segments, staves, or chips may be added concurrently with micro-oxygenation (McCord, 2003; Gore, 2007; Bowyer and McCord, 2007).

Although the proponents of micro-oxygenation claim that the technique should not be relegated to medium-quality wines (Vinnovation, 2001b), many producers currently use it exclusively for this end of the market, and it is very unlikely that micro-oxygenation will ever replace traditional long-term maturation in new oak barrels for premium quality wines (Paul, 2002). However, the future may see combination therapy for quality wines. Newer micro-oxygenation products allow micro-oxygenation in barrel (Memstar, 2008) and there have been some trials on using micro-oxygenation before normal barrel ageing (Sartini *et al.*, 2007; Ortega-Herás *et al.*, 2008).

5.3 EQUIPMENT FOR MICRO-OXYGENATION

5.3.1 Basic Equipment

Oxygen is widely available, either in the form of air or pure oxygen, and it would not be very complicated to bubble oxygen into a wine tank. However, as discussed above, micro-oxygenation treatment involves the controlled addition of small measured amounts of oxygen to wine, and the main objective is to ensure that the rate of oxygen addition is lower than the rate of oxygen consumption by the wine.

These requirements presents two main technical challenges for any micro-oxygenation equipment:

1. To be able regulate the flow of oxygen to the very low rates required for micro-oxygenation (in the order of 2-5 ml/L/month); and
2. To ensure that the oxygen diffuses into the wine where it is needed for beneficial oxidation reactions, rather than just bubbling through the wine and accumulating in the tank headspace where it could cause harmful oxidation and the growth of acetic acid bacteria.

Different equipment suppliers have solved these problems in different ways, but an illustrative setup, based on the original OenoDev invention, is shown in Figure 1 overleaf.

There are three main components in a micro-oxygenation device:

1. Oxygen supply.
2. Metering device.
3. Delivery device.

Oxygen **supply** is usually from a standard pressurised oxygen cylinder. Oxygen rather than air is used because it is better for regulating the exact dose of oxygen supplied. Medical or food grade quality oxygen is usually used. The OenoDev solution to the **metering** problem is to use two chambers connected by regulator valves (Figure 1). The first chamber is of known volume and filled at a high fixed pressure. Predetermined doses of oxygen are injected at periodic intervals into the second low-pressure chamber. From the second chamber the oxygen is then **delivered** slowly and at low pressure through a small polyamide tube into the wine tank. At the end of the tube is a ceramic or stainless steel diffuser/sinter which introduces very fine bubbles of oxygen into the wine. The diffuser should be lowered to close to the bottom of the tank, but above the lees. Even so, the wine maturation tank needs to be at least 2.2 m tall in order to provide sufficient path length for the oxygen to permeate through the wine and avoid headspace accumulation (Rieger,

2000; du Toit, 2007a). The volume of the tank is less critical than the height, and the technique has been used on a wide range of tank sizes. The diffuser is prone to fouling so it needs to be thoroughly cleaned between uses.

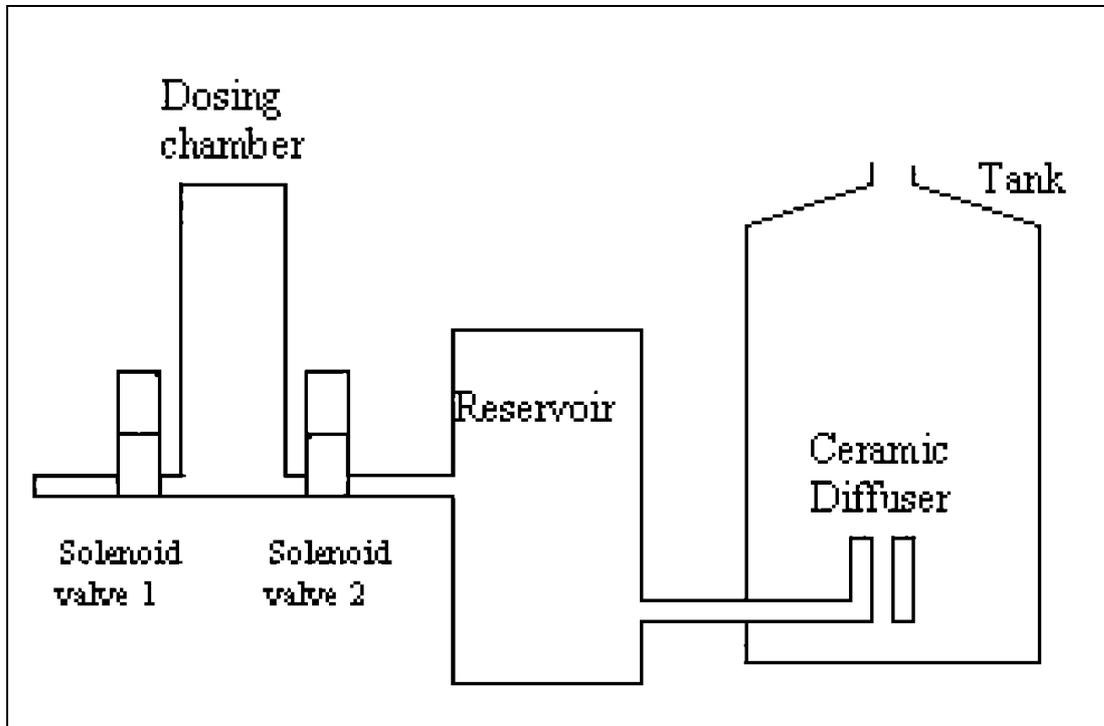


Figure 1: Basic Components of a Micro-oxygenation Setup

Source: (Parish *et al.*, 2000)

Internationally, the main suppliers of basic micro-oxygenation equipment include OenoDev (Vinnovation, 2001b), Parsec (Parsec, 2008) and Stavin/Westec Tanks (Stavin, 2008). The main distributor of micro-oxygenation units in South Africa is Reines Trading/Euroberry (Euroberry, 2008b) who are the local agents for Parsec. By mid 2008, Euroberry claimed to have 23 micro-oxygenation clients in South Africa, including a wide range of producers - from bulk-producing co-operatives to premium quality estates (Euroberry, 2008a). The equipment provided by the three different manufacturers differs mainly in the dosing control. The OenoDev and Parsec machines are based on similar principles though OenoDev's controller is electrochemical and measures the dose required in ml O₂/L whereas Parsec's units are microprocessor controlled and measure in mg/L (Table 4). The Stavin *Ox Box* is a more simple device that regulates oxygen flow which makes dosing a little more complicated (Loch, 2002). A number of different models are available from each supplier according to the cellar's needs - from small, manual devices that can be moved from tank to tank, to large built-in automated units that regulate micro-oxygenation in multiple tanks at once (Table 4).

Table 4: Micro-oxygenation Equipment from Major Suppliers

Supplier	Metering Device	Units of Measure	Models	Details
Vinnovation / OenoDev	Electrochemical Dual chamber	O ₂ dose ml/L/month	Compact	Portable, Single diffuser
			Eco2	Fixed 1-4 diffusers
			Visio6	Fixed 6-60 diffusers
Parsec	Micro-processor control Dual chamber	O ₂ dose mg/L/month	OxyGenius Plus	Portable Single diffuser Micro + Macro-oxygenation
			SAEn 4000	Fixed 3-15 Diffusers
			SAEn 5000	Fully expandable system
Stavin / Westec Tanks	Gas chromatograph flow controller	O ₂ flow	Ox Box	Portable Single diffuser

From (Vinnovation, 2001b; Zoecklein *et al.*, 2002; Stavin, 2008; Parsec, 2008; McCord, 2008)



Figure 2: Commercial Micro-oxygenation Equipment

5.3.2 Newer Innovations

Although the basic technology described in Section 5.3.1 has been around for more than 15 years now, it remains the standard approach to micro-oxygenation around the world. Some newer methodologies that may become more important, and more widespread, in future include:

1. Diffusion methods: *O₂mate* and *(barrel)mate*
2. Oxygen-permeable tanks: Flextank
3. Electro-chemical micro-oxygenation.

Diffusion methods are based on the diffusion of oxygen across a permeable membrane instead of the usual bubbling method (Kelly and Wollan, 2003; Paul and Kelly, 2005). The technique was invented by Mark Kelly of Wine Network Australia and is distributed by Memstar under the brand name of *O₂mate* (Memstar, 2008). The technique depends on specialised polydimethylsiloxane tubing which is permeable to oxygen and will not clog up. The tubing is placed inside the maturation tank and connected to an un-pressurised system supplying atmospheric oxygen at the required dose.

It is argued that the microscopic bubbles produced by diffusion are a much better way of delivering oxygen into wine than the large bubbles produced by traditional methods, and that the system mimics more closely the diffusion of oxygen that occurs during normal barrel ageing (Paul and Kelly, 2005). Because headspace accumulation of oxygen is not a problem with diffusion technology, one advantage of the system is that it doesn't require a path length of 2.2m and can therefore be used in smaller tanks. Indeed, Wine Network have also developed a micro-oxygenation solution for use in barrels called *(barrel)mate*. Kelly and Wollan (2003) calculate that the maximum amount of oxygen diffusion that can occur with normal barrel maturation is only 2.5 ml/L/month whereas some wine requires up to 8 ml/L/month for optimal polymerisation reactions. The *(barrel)mate* system is able to provide the supplementation required. The diffusion technology is the subject of an international patent and is currently undergoing large scale commercial trials.

Even more intriguing are the **oxygen-permeable tanks** developed by Flextank in 2004 (Flecknoe-Brown, 2004; Flecknoe-Brown, 2005; Flecknoe-Brown, 2006). These wine-maturation vessels are made from high density polyethylene which is permeable to oxygen so that the vessels actually allow oxygen through the walls in a similar way to normal oak barrels. The polyethylene tanks have been specially designed to limit the degradation of wine aroma compounds and to inhibit bacterial spoilage – problems typical of other plastic tanks. The amount of oxygen delivered depends on the size of the tank but the oxygen

dose from the standard 1000 L tank is equivalent to micro-oxygenation in stainless steel tanks (Flecknoe-Brown, 2004). Not only are Flex tanks cheaper than barrels but they have a much longer lifespan and require less labour. Trials with this technology are also ongoing (Flecknoe-Brown, 2006).

Lastly, **electrochemical micro-oxygenation** utilises the industrial process of electrolysis to produce oxygen and oxidation reactions in wine (Fell *et al.*, 2007). The technique involves passing an electric current through a glass carbon electrode. One advantage of this approach is that it oxidises phenols and oxidises ethanol to acetaldehyde without the production of hydrogen peroxide (H₂O₂) or other reactive oxygen species. An initial trial produced similar effects to traditional micro-oxygenation methods (Fell *et al.*, 2007) but the technique is not yet commercially available.

5.4 MANAGING MICRO-OXYGENATION

This section briefly summarises some of the practical issues in managing micro-oxygenation. The key decisions that need to be made include:

- Is micro-oxygenation indicated or likely to be helpful?
- When should the treatment be started?
- What dose of oxygen is required?
- What monitoring should be done?
- When should the treatment be stopped?

5.4.1 Phases of Micro-oxygenation

The OenoDev/Vinovation documentation presents a theory of micro-oxygenation which describes three distinct phases of tannin and aroma development during micro-oxygenation, which they label structuration, harmonisation, and over-oxygenation respectively (Vinovation, 2001b). This theoretical framework is illustrated in Figure 3.

The model was presumably based on their early research in France (Lemaire, 1995) but it has not subsequently been verified by other researchers (du Toit *et al.*, 2006b), and it is somewhat difficult to reconcile with what is now known about the oxidation reactions that occur during ageing (See Chapter 6). Nevertheless, this theory of micro-oxygenation has also been extremely influential in the industry and in the literature, and so is briefly described here.

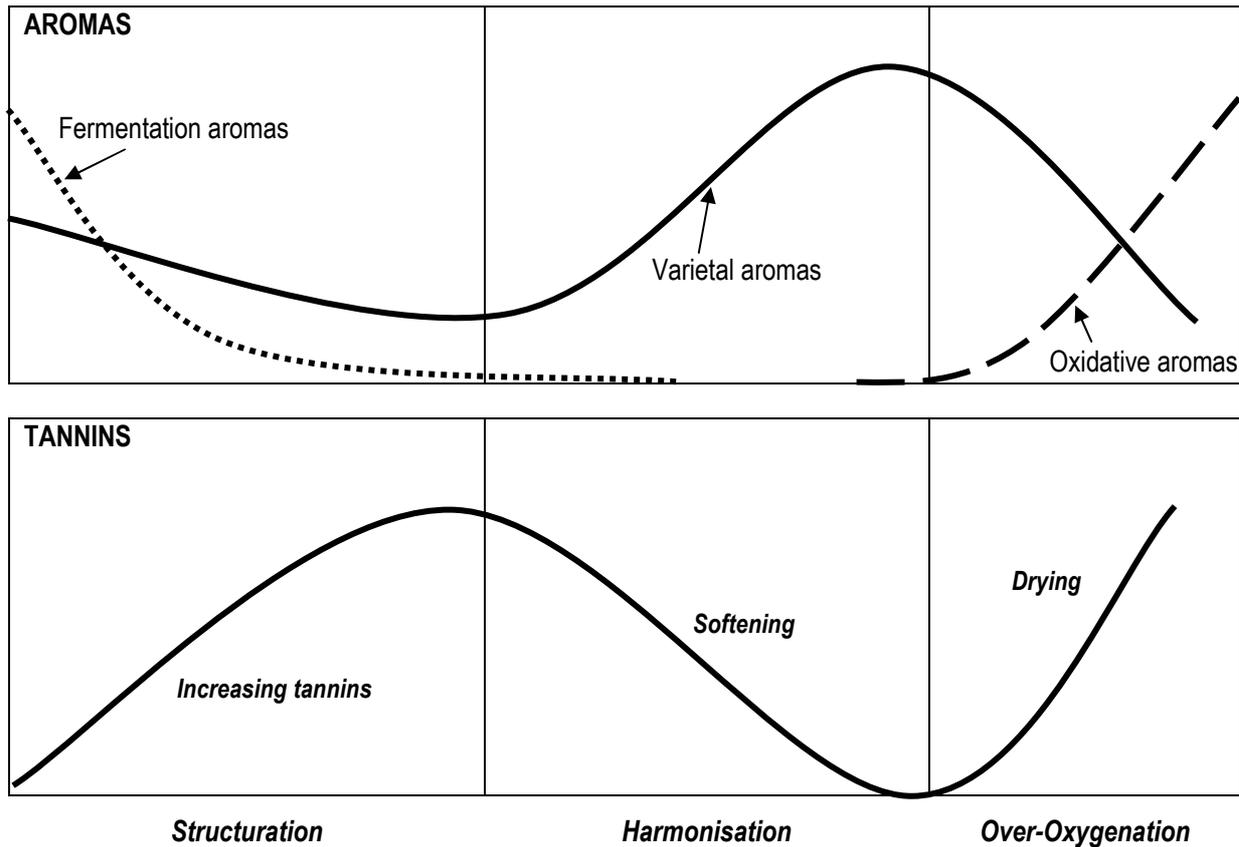


Figure 3: Vinovation / OenoDev Phases of Micro-oxygenation

Drawn from (Parish *et al.*, 2000)

5.4.1.1 Structuration (Structuring) Phase

In the OenoDev/Vinovation model, micro-oxygenation actually makes the structure of the wine worse before it gets better. It is proposed that during the early stages of oxygen addition tannins get more aggressive, astringency increases, and there is even a decrease in aromatic intensity and complexity (Figure 3). According to the promotional material this phase can take from 1-6 months depending on the wine and micro-oxygenation conditions (Vinovation, 2001b).

5.4.1.2 Harmonisation Phase

However, according to this theory, if the winemaker persists with micro-oxygenation treatment they will eventually be rewarded by an exact reversal of the phenomena observed during *Structuration*: tannins will soften, mouthfeel will improve, and fruitiness will return. It is proposed that optimal *Harmonisation* usually takes twice as long as the structuration phase.

5.4.1.3 Over-oxygenation Phase

Unfortunately, if micro-oxygenation continues too long, the wine may deteriorate again as it enters this third phase of development. Over-treatment will result in dryness, thinner body, a decrease in fruit flavours, and eventually the development of oxidation aromas.

If the OenoDev/Vinovation model is correct it makes micro-oxygenation an extremely complex endeavour. There is a very small 'sweet spot' that needs to be found – the window in Figure 3 where the impact of treatment is optimal or, indeed, where treatment has actually resulted in an improvement over starting conditions, is extremely small; and there are the clear dangers associated with under- or over-treatment on either side. Also, despite the claims that micro-oxygenation allows a more scientific application of oxygen than traditional oxygenation strategies, the monitoring parameters and end-points here are far from clear-cut. Lastly, it is evident that there is no standard recipe that can be followed because each wine is different and will require its own customised treatment regimen.

It is not surprising, therefore, that the industry promotional literature also places a lot of emphasis on the technical support required to use micro-oxygenation, the long learning curve involved, and the importance of monitoring in managing micro-oxygenation.

5.4.2 Micro-oxygenation Conditions

As has already been suggested, each wine and each micro-oxygenation treatment will be different. Some of the factors known to affect the impact of micro-oxygenation include the following:

5.4.2.1 Initial Phenolic Structure of the Wine

The impact of micro-oxygenation on wine colour and structure will obviously be influenced by the initial phenolic structure (particularly tannin and anthocyanin concentrations) of the wine. As discussed in Section 4.1, micro-oxygenation is best for full-bodied tannic red wines whereas over-treatment can happen fairly quickly with thinner wines so micro-oxygenation should be used with caution in low colour wines (du Toit, 2007b).

As will be discussed in the next Chapter there are a number of different condensation reactions occurring at the same time during ageing and the competition between them is influenced by the relative concentrations of tannins and anthocyanins. On the basis of their practical experience with the technique, Vinovation (2001b) recommends a tannin to anthocyanin (T:A) ratio of at least 4:1 and provides the following table as guidance:

Table 5: Micro-oxygenation Treatment according to T:A Ratio

[Tannin]	[Anthocyanin]	Impact on Micro-oxygenation Treatment
<i>High</i>	<i>High</i>	Rapid T-A condensation reactions May require high O ₂ doses
<i>High</i>	<i>Low</i>	T-A reactions easy to produce Risk of dryness
<i>Low</i>	<i>High</i>	T-A reactions more difficult to produce But no risk of dryness
<i>Low</i>	<i>Low</i>	T-A reactions difficult to produce Not optimal candidate for micro-oxygenation

Drawn from: (Vinnovation, 2001b)

This also means that any other winemaking processes which modify wine phenolic content will influence micro-oxygenation. So, for example, fining decreases total phenols while the addition of oenological tannins, using oak alternatives, and blending in press wine will increase the phenolic substrates for micro-oxygenation.

5.4.2.2 Wine Turbidity

Micro-oxygenation is less effective on very turbid wines so it is usually only initiated after pressing and settling (Paul, 2002).

5.4.2.3 The Temperature

Temperature has an important effect on oxygen solubility and the rate of oxidation reactions (de Basquiat, 2008). Lower temperatures significantly increase oxygen solubility but will decrease the speed of beneficial polymerisation reactions (Nel, 2001). Therefore, it is usually recommended that the temperature be controlled at about 15°C for micro-oxygenation.

5.4.2.4 The SO₂ Concentration

Sulphur dioxide (SO₂) is an anti-oxidant and interferes with many of the key oxidation reactions of interest: for example, it reacts with hydrogen peroxide (H₂O₂) and acetaldehyde which are required for condensation reactions, and also binds directly with anthocyanins inhibiting the production of polymeric pigments. Therefore, micro-oxygenation requires relatively low concentrations of SO₂ to be effective.

On the other hand, micro-oxygenation may increase the growth of aerobic organisms so some minimal SO₂ is required to protect against microbial spoilage.

5.4.2.5 Timing of Micro-oxygenation

As will be discussed in more detail in Section 5.4.3, micro-oxygenation appears to be more effective before malolactic fermentation than afterwards (Paul, 2002).

5.4.2.6 The Rate of Oxygen Addition

More rapid and dramatic results are produced with higher oxygen flow rates but if the rate of addition is higher than the consumption capacity of the wine (see Section 5.1), the dissolved oxygen concentration will increase and encourage harmful oxidation and spoilage of the wine.

5.4.2.7 Other Aeration Practices

Other winemaking practices, such as racking, that add oxygen to the wine obviously need to be taken into account when planning and managing micro-oxygenation.

5.4.3 Timing and Sequencing of Micro-oxygenation

As discussed in Section 5.2, there are three main stages during the winemaking process when micro-oxygenation could be applied (Zoecklein *et al.*, 2002):

1. Early alcoholic fermentation (AF),
2. Post-fermentation, before malolactic fermentation (pre MLF),
3. Post-fermentation, after malolactic fermentation (post MLF).

Each of these applications has its own proponents, objectives and protocols. However, most practitioners recommend that micro-oxygenation be started immediately after the completion of alcoholic fermentation (AF) and before malolactic fermentation (pre-MLF MOX) (Paul, 2002; Lemaire *et al.*, 2002; Zoecklein *et al.*, 2003). It is argued that micro-oxygenation is much more effective in improving wine structure before malolactic fermentation when tannins and anthocyanins are still mostly in simple monomeric form. Tannin and anthocyanin reactions occur independently of micro-oxygenation so if the treatment is only started post-MLF it has to act on substrates that have already undergone some polymerisation and condensation (Nel, 2001). Practical experience suggests that this is less effective and may produce very large molecular weight molecules that could precipitate out of solution resulting in the loss of colour and structure (Rieger, 2000).

A second argument for pre-MLF micro-oxygenation is that the effectiveness of micro-oxygenation depends significantly on SO₂ levels (Section 5.4.2) and SO₂ concentrations are usually lower at this stage in order to promote MLF. Most winemakers sulphite their wine after MLF is completed to increase protection against microbial spoilage during

maturation. Micro-oxygenation in the face of high SO₂ levels is more difficult and will take much longer to produce the same effect. However, it has also been argued that this slower micro-oxygenation in the presence of higher SO₂ is more analogous to conditions that prevail during normal barrel ageing (Paul, 2002).

Lastly, malolactic bacteria consume acetaldehyde during MLF. Therefore, any excess acetaldehyde produced during pre-MLF MOX will be eliminated by the bacteria producing a better organoleptic product (Vinnovation, 2001b). This useful side-effect does obviously not occur in post-MLF micro-oxygenation (post-MLF MOX).

However, most winemakers want their red wines to undergo MLF, and there is usually insufficient time between alcoholic fermentation and MLF for complete micro-oxygenation treatment. Therefore, pre-MLF MOX usually involves fairly high doses of oxygen for only a few weeks. It has been proposed that winemakers could delay MLF, using Lysozyme or other means (Nel, 2001; Paul, 2002), in order to prolong pre-MLF MOX, but not all winemakers are enthusiastic about such an approach (Tibbits, 2003).

Therefore, a common strategy at present is to do both pre-MLF and post-MLF MOX. Micro-oxygenation treatment starts with a short course of higher dose pre-MLF MOX, stops when MLF occurs spontaneously, and then restarts with post-MLF MOX at much lower doses and for a much longer period (Theron, 2007; du Toit, 2007b).

5.4.4 Dosage and Duration of Micro-oxygenation

As has been mentioned before there is no standardised recipe for micro-oxygenation and current practice advocates that the appropriate dose of micro-oxygenation be determined for each case depending on the initial structure of the wine, the objectives of treatment, and the resources available.

The broad guidelines for micro-oxygenation doses come from earlier measurements on typical oxygen exposures during traditional winemaking. For example, Singleton (1987) determined many years ago that when red wine was repeatedly saturated with oxygen, it seemed to improve with up to 10 saturations of oxygen (approximately 60 ml O₂ /L) and only showed definite deterioration after 40 saturations (~180 ml O₂ /L). Similarly, it has been calculated that typical vinification activities added 40-78 mg O₂ /L to wine whereas ageing in new barrels would expose wine to a further 54-121 mg/L in total (Vivas and Glories, 1995; Ribéreau-Gayon *et al.*, 2006a).

Oxygen exposure during normal barrel ageing is in the order of 2.5 ml/L/month (Nel, 2001; Kelly and Wollan, 2003). Typical doses during micro-oxygenation are somewhat higher as shown in Table 6.

Table 6: Typical Micro-oxygenation Doses

Timing	Typical Dose ‡		Typical Duration
	ml/L/month	mg/L/month	
<i>Pre-MLF MOX</i>	10-50	15-65	2-4 weeks
<i>Post-MLF MOX</i>	0.1-5.0	0.15-6.5	1-6 months

‡O₂ exposure can be measured in both ml/L or mg/L which causes some confusion in the literature.
At 15°C 1.0 ml/L = 1.35 mg/L.

Sources: (Rieger, 2000; Zoecklein *et al.*, 2002; Bird, 2005)

The dose of oxygen administered will obviously depend on the overall treatment objective - the elimination of reductive defects requires lower O₂ doses than accelerated maturation, for example (de Basquiat, 2008).

Further, the dose of oxygen required needs to be fine-tuned to the wine's phenolic structure and ripeness (Paul, 2002; Zoecklein *et al.*, 2003), and is likely to vary for different varieties, vineyards and vintages (Rieger, 2000).

Most equipment suppliers provide additional guidance for determining the appropriate dose of micro-oxygenation required in the form of various tables, normograms, and computer programmes (Parsec, 2008). For example, Vinovation's (2001b) more detailed specifications for micro-oxygenation dosage are shown in Table 7 and Table 8.

Table 7: Vinovation: Classification of Wines

		Greenness				
		Very green	Green	Ripe	Very Ripe	Over Ripe
Concentration	Most Concentrated	A	B	C	D	Not Recommended
	Concentrated	B	C	D	E	Not Recommended
	Dilute	C	D	E	F	Not Recommended
	Most Dilute	D	E	F	G	Not Recommended

Table 8: Vinovation: Oxygen Dose for Different Wine Types

Wine Type †	Pre-MLF Dose (ml/L/month)	Pre-MLF Duration	Post MLF Dose (ml/L/month)	Post-MLF Duration
A	60	2-4 weeks	2-10	6 months
B	60	2-4 weeks	2-10	5 months
C	50	2-4 weeks	2-5	4 months
D	40	2-4 weeks	2-5	3 months
E	30	2-4 weeks	1-5	2 months
F	20	2-4 weeks	1-5	1 month
G	10	2-4 weeks	0.5-5	1 month

† From Table 7

Source: (Vinovention, 2001b)

5.4.5 Monitoring Micro-oxygenation

Micro-oxygenation treatment requires careful and constant monitoring for a number of reasons:

- Wine goes through a complicated development process during micro-oxygenation (Section 5.4.1);
- Each wine responds differently to micro-oxygenation;
- Under-treatment is ineffective and may worsen wine structure; and
- Over-treatment is harmful and may ruin the wine completely.

The most common parameters currently available to monitor micro-oxygenation are summarised in Table 9. Essential monitoring for micro-oxygenation is possible without recourse to very specialised tests.

Table 9: Monitoring of Micro-oxygenation

Essential Monitoring	Intermediate Monitoring	Advanced Monitoring
Temperature Total + Free SO ₂ Taste MLF	Dissolved oxygen (DO) Turbidity Volatile acidity (VA)	Colour indicators Phenolic compounds Acetaldehyde Condensation products <i>Brettanomyces</i>

Compiled from: (Paul, 2002; Lemaire, 2002; Blackburn, 2004; du Toit, 2007b)

It is usually recommended that the temperature be maintained at 15-17°C during micro-oxygenation to regulate oxygen solubility and the speed of the oxidation reactions (Paul, 2002).

High levels of sulphur dioxide (SO₂) will inhibit micro-oxygenation reactions but the concentration of free SO₂ [Free SO₂] is usually maintained above 20 mg/L to prohibit bacterial spoilage (du Toit, 2007b). SO₂ will react with any oxygen that accumulates so SO₂ levels can be used as an indicator of dissolved oxygen when it is not possible to measure dissolved O₂ directly. A fall in [Free SO₂] would suggest that dissolved O₂ was increasing and that, therefore, the rate of oxygen delivery was too high (Blackburn, 2004).

The most important tool for monitoring micro-oxygenation is regular tasting. The frequency of tasting depends on the stage of treatment but should be at least once a week (du Toit, 2007b) and probably daily when nearing the end of treatment (Lemaire, 2002). A team of tasters is preferable to only one person but in small wineries this may not be feasible. The tasting should focus on wine structure (tannins, body, softness) as well as aroma and flavours (fruit, vegetal, aldehydes).

Other tests have a role in monitoring micro-oxygenation and are available in most wineries include basic chromatography to monitor malolactic fermentation (MLF), and volatile acidity (VA) to check for an increase in acetic acid bacteria.

The concentration of dissolved oxygen is a very useful marker of micro-oxygenation therapy (Blackburn, 2004; Devatine *et al.*, 2007) but requires a specialised oxygen meter.

Also, obtaining representative samples and accurate testing of dissolved oxygen is difficult (du Toit *et al.*, 2006b). If dissolved oxygen is measured, the objective would be to maintain concentrations at very low levels.

Ideally for micro-oxygenation turbidity should be below 200 NTUs but measuring turbidity also requires specialised instrumentation.

Research in this area involves detailed analyses of wine colour parameters and the identification and measurement of a large number of different phenolic compounds (see Section 6.3). These investigations require very specialised tests such as UV-visible spectrophotometry, high performance liquid chromatography (HPLC), and mass spectrophotometry (MS).

Because acetaldehyde is a critical marker in the oxidation reactions of interest, investigations are underway to develop a simple method of monitoring acetaldehyde during micro-oxygenation (Carlton *et al.*, 2007). More simplified tests for *Brettanomyces* are also likely to be available in the near future (du Toit *et al.*, 2006b).

PART III: SCIENTIFIC EVIDENCE FOR MICRO-OXYGENATION

CHAPTER 6: UNDERLYING CHEMISTRY OF MICRO-OXYGENATION

Part III of this report seeks to review the available scientific evidence related to the topic of micro-oxygenation. Chapter 7 will summarise the findings of those studies that have evaluated the impact of micro-oxygenation directly. However, in the absence of conclusive evidence from such studies, much of the justification and support for micro-oxygenation is derived from the more general research on phenolic reactions during winemaking and ageing. That area of research is the topic of Chapter 6.

The first section of this Chapter introduces the important phenolic constituents of wine while the second section will describe some of the phenolic reactions that have been shown to be important during winemaking and ageing.

6.1 IMPORTANT PHENOLIC COMPOUNDS IN GRAPES AND WINE

6.1.1 Importance of Phenolic Compounds in Wine

The phenolic compounds are one of the most important constituents of wine, both in terms of their high concentration in wine, and also because of the key role that they play in determining the organoleptic properties of wine (Ribéreau-Gayon and Glories, 1987; Cheynier *et al.*, 1997; Kennedy *et al.*, 2006; Ribéreau-Gayon *et al.*, 2006c). Wine phenols include the anthocyanins and tannins and it is these compounds that are responsible for the colour, structure and mouthfeel of wines (Jones *et al.*, 1999; Monagas *et al.*, 2005; Cheynier *et al.*, 2006; Parker *et al.*, 2007).

It is also the reactions of phenolic compounds that determine the development of wine during maturation and ageing (Cheynier, 2002; Fulcrand *et al.*, 2006; Ribéreau-Gayon *et al.*, 2006a). Polymerisation of monomers as well as condensation reactions between different phenolic compounds produce the characteristic changes associated with ageing: young bright red wines turn red-orange, and harsh young tannins become softer and less astringent.

More recently, it has been identified that it is the anti-oxidant properties of phenolic compounds that are responsible for most of the beneficial health effects of wine (Manzocco *et al.*, 1999; de Beer *et al.*, 2002). By removing toxic Reactive Oxygen Species (oxygen

6.1.3 Categories of Phenolic Compounds in Wine

A basic classification of the important phenolic compounds in wine is shown in Figure 6.

There are two main groups of phenolic compounds:

1. Non-flavonoid phenols; and
2. Flavonoid phenols.

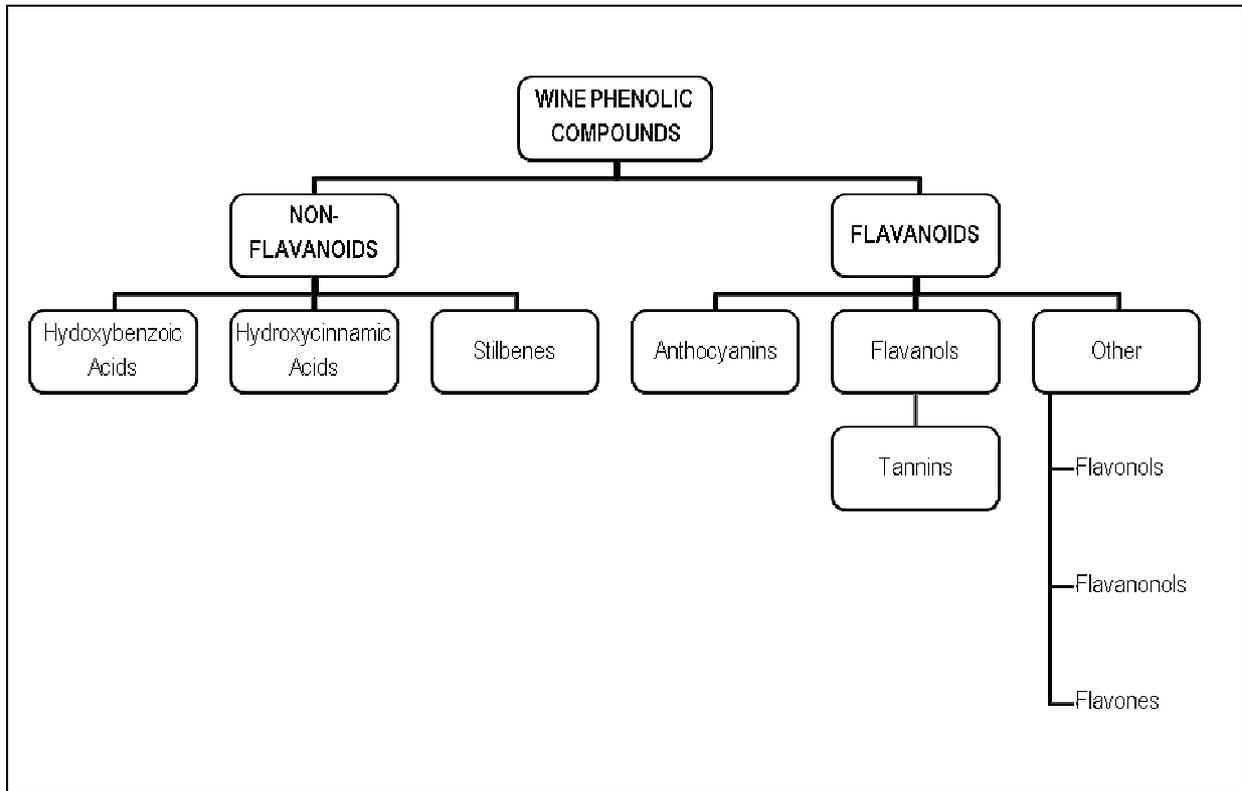


Figure 6: Categorisation of Wine Phenolic Compounds

Drawn from: (Heim *et al.*, 2002; Roussow and Marais, 2004; Parker *et al.*, 2007)

The total concentration and phenolic composition of different types of wines are shown in Table 10.

Table 10: Concentration and Composition of Phenolic Compounds in Wine

Type of Wine	Total Phenolics (Gallic acid equivalents)	Phenolic Composition
<i>Red</i>	1000-2000 mg/l	Mostly flavonoids
<i>White</i>	100-400 mg/l	Mostly non-flavonoids
<i>Rosé</i>	400-800 mg/l	40-60% flavonoids

Source: (Margalit, 2004)

6.1.3.1 Non-Flavonoid Phenols

The non-flavonoid phenols are compounds that usually consist of one phenolic unit and are derived from grape pulp. Therefore, the phenolic composition of free-run juice from both red and white grapes is mostly non-flavonoid phenols of the order of 100-300mg/L (as gallic acid equivalents) (Margalit, 2004). The important non-flavonoid groups and compounds found in wine are summarised in Table 11.

Table 11: Non-Flavonoid Phenols

Class	Structure	Important Members in Wine				
		Name	R ₁	R ₂	R ₃	R ₄
Hydroxybenzoic Acids		<i>p</i> -Hydroxybenzoic acid	H	H	OH	H
		Protocatechic acid	H	OH	OH	H
		Vanillic acid	H	OCH ₃	OH	H
		Gallic acid	H	OH	OH	OH
		Syringic acid	H	OCH ₃	OH	OCH ₃
		Salicylic acid	OH	H	H	H
		Gentisic acid	OH	H	H	OH
Hydroxycinnamic Acids		<i>p</i> -Coumaric acid		H	OH	H
		Caffeic acid		OH	OH	H
		Ferulic acid		OCH ₃	OH	H
		Sinapic acid		OCH ₃	OH	OCH ₃
Hydroxycinnamic Esters		Coutaric acid		H		
		Caftaric acid		OH		
		Fertaric acid		OCH ₃		
Stilbenes		<i>trans</i> -Resveratrol				
		<i>cis</i> -Resveratrol				

Compiled from: (Heim *et al.*, 2002; Ribéreau-Gayon *et al.*, 2006c)

The phenolic acids (**hydroxybenzoic acids** and **hydroxycinnamic acids**) are colourless compounds that have no flavour or odour. In grapes they mainly occur esterified with sugars or alcohols although in wine they may be hydrolysed to their free forms. Certain

phenolic acids act as precursors for the formation of the volatile phenols, a wine fault associated with *Brettanomyces* spoilage. However, the main role of the phenolic acids is to contribute to the anti-oxidant capacity of both red and white wines. For example the tartaric esters of hydroxycinnamic acids (particularly caftaric acid) are centrally involved in oxidation and browning reactions in white wine (Ribéreau-Gayon *et al.*, 2006c).

The other important group of non-flavonoid phenols are the **stilbenes** (Table 11). These phenols have attracted significant attention in recent years because compounds such as *trans*-resveratrol are thought to be responsible for many of the beneficial health effects of red wines (Corder *et al.*, 2006).

6.1.3.2 Flavonoid Phenols

More important in terms of the subject of this report are the flavonoid phenolic compounds. They are responsible for the red pigments, yellow pigments and tannins of grapes and wine. Flavonoids are mostly derived from the skins and seeds of grapes. They share a basic 15 carbon structure (Figure 7) which consists of two phenolic rings (Ring A and Ring B) connected by a short three-carbon chain, though the three carbon chain is frequently closed by oxygen to form an oxygen heterocycle (Ring C) (Heim *et al.*, 2002).

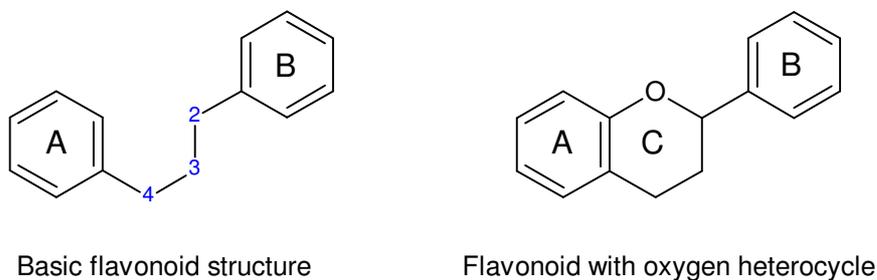
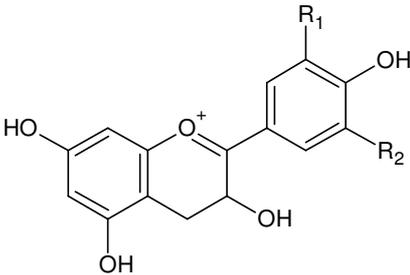
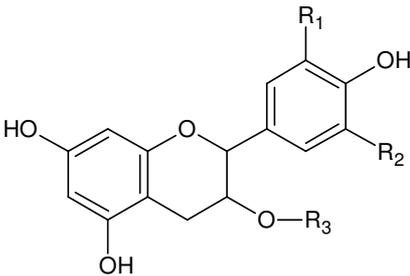
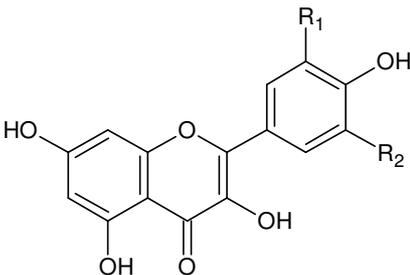
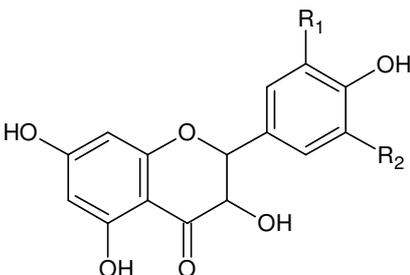
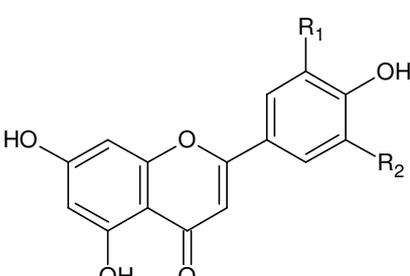


Figure 7: Flavonoid Ring Structure

The flavonoid phenols are organised into a number of different groupings, though the names for the different categories is certainly confusing for the non-expert. The main groups in wine and some of the important members of each group are presented in Table 12. The groups are distinguished by differences in the central ring structure (Ring C) and by substitutions on the B ring. The most important flavonoids in grapes and wine are the anthocyanins, the flavanols, and the flavonols¹.

¹ Notice the subtle difference in nomenclature between flavanols and flavonols.

Table 12: Flavonoid Phenols

Class	Structure	Important Members in Wine			
		Name	R ₁	R ₂	R ₃
Anthocyanadins		Malvidin	OCH ₃	OCH ₃	
		Cyanidin	OH	H	
		Delphinidin	OH	OH	
		Peonidin	OCH ₃	H	
		Petunidin	OH	OCH ₃	
Flavanols (Flavan-3-ols)		(+)-catechin	OH	H	H
		(-)-epicatechin	OH	H	H
		Gallocatechin	OH	OH	H
		Epigallocatechin	OH	OH	H
		Epicatechin-3-O-gallate	OH	H	gallic acid
Flavonols (Flavone-3-ols)		Kaempferol	H	H	
		Quercetin	OH	H	
		Myricetin	OH	OH	
		Isorhamnetin	OCH ₃	H	
Flavanonols (Flavanone-3-ols)		Dihydrokaempferol	H	H	
		Taxifolin	OH	H	
Flavones		Apigenin	H	H	
		Luteolin	OH	H	

Compiled from (Heim *et al.*, 2002; Ribéreau-Gayon *et al.*, 2006c)

a) Anthocyanins

Anthocyanins are the red pigments of grapes and wine. The red colour is due to the C-Ring structure known as a flavylium ion. There are five main anthocyanins in grapes and wine (Table 12) though malvidin (and its derivatives) are the most common. Anthocyanin usually occur as 3-O-monoglucosides or 3-O-acylated monoglucosides in grapes and wine. In strict terminology, anthocyanin is used for the glycosylated form whereas the free forms (aglycones) are called anthocyanidins.

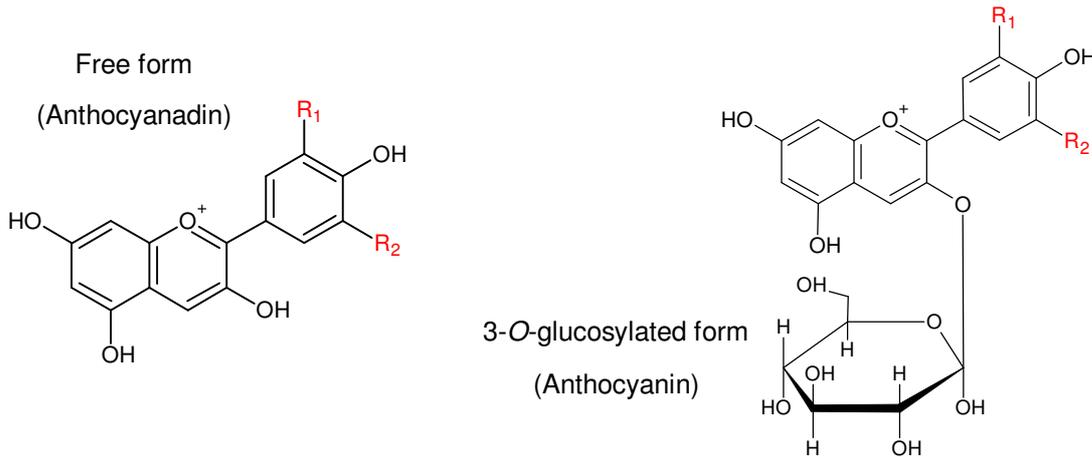


Figure 8: Anthocyanins and Anthocyanidins

The colour of anthocyanins depends on the pH and SO₂ concentration (Figure 9). Anthocyanins actually occur in four different coloured forms in wine:

- Red flavylium ion (A⁺)
- Violet quinoidal base (AO) – which can dissociates to a blue anionic form (AO⁻)
- Colourless carbinol base (AOH)
- Yellow chalcone (C)

Table 13: Distribution of Anthocyanin Forms at Different pH Levels

pH	Percentage in Different Forms					
	Free Anthocyanins			Anthocyanins bound to Tannin		
	A ⁺ [Red]	AO [Violet]	AOH [Colourless]	T-A ⁺ [Red]	T-AO [Violet]	T-AOH [Colourless]
3.0	36%	0%	63%	74%	1%	25%
3.4	18%	0%	82%	53%	8%	39%
3.8	8%	0%	88%	31%	21%	48%

Source: (Ribéreau-Gayon and Glories, 1987)

The equilibrium between these forms depends on the pH (Margalit, 2004; du Toit *et al.*, 2006b). At a typical wine pH of 3.4 only 18% of free anthocyanins are in the red form, whereas 82% are in the colourless carbinol form (Table 13). Anthocyanins also react with SO₂ to produce colourless compounds (Figure 9).

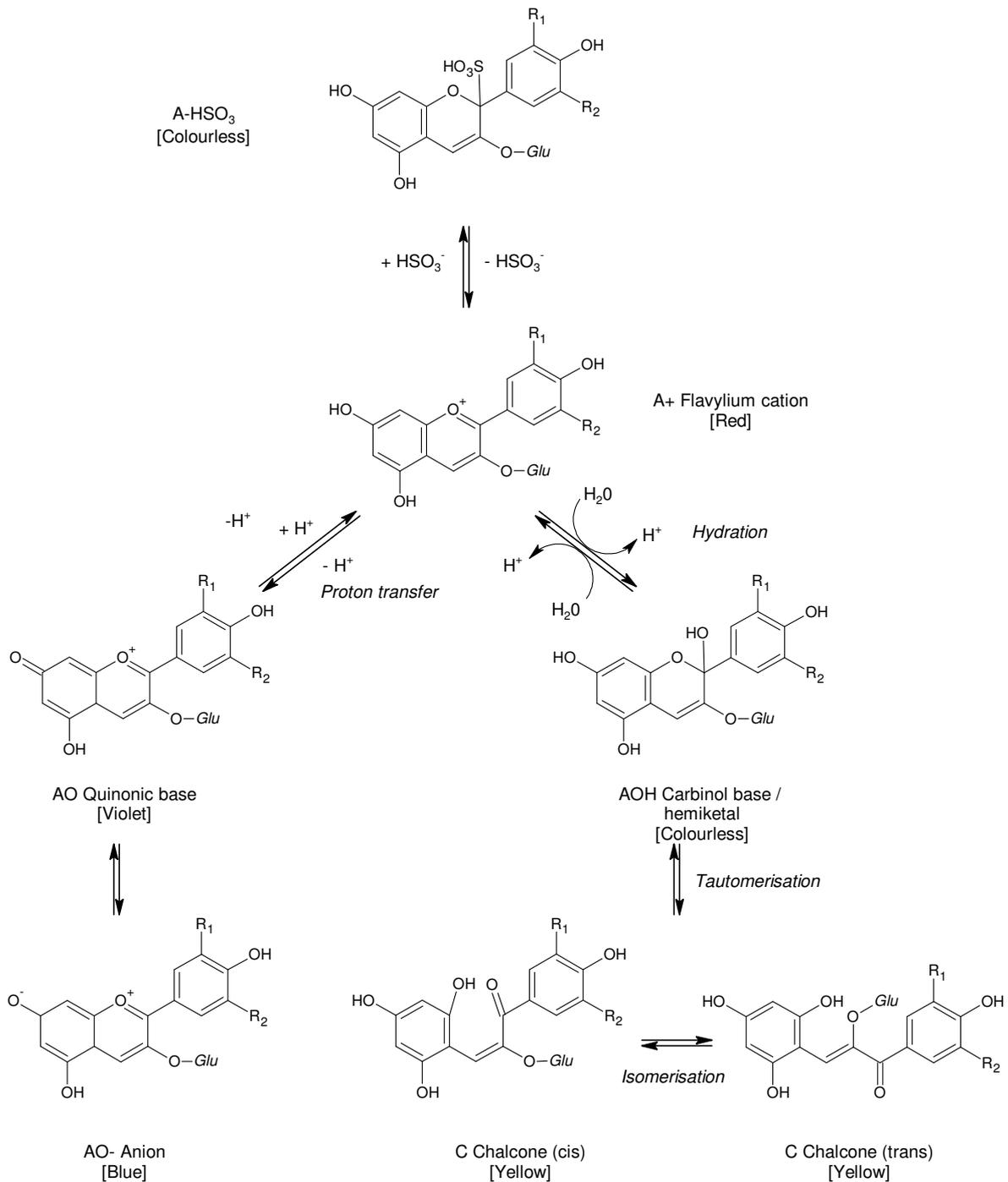


Figure 9: Colour Changes of Anthocyanins with pH and SO₂ Binding

Drawn from (Ribéreau-Gayon *et al.*, 2006c)

Polymeric pigments are produced by reactions between anthocyanins and tannins, and these forms of anthocyanins are much more resistant to SO₂ bleaching and pH changes (Jones *et al.*, 1999). For example, at a pH of 3.4, 53% of polymeric pigments are in the red form (Table 13) which is significantly higher than 18% for the free form.

b) Flavanols and Tannins

Grape tannins are found in the skin, seeds and stems and are important determinants of the astringency and mouthfeel of wine (Kennedy *et al.*, 2006). Astringency is due to tannins forming complexes with salivary proteins (Monteleone *et al.*, 2004). By definition, tannins are water soluble molecules which are capable of reacting with proteins and polyamides. Protein binding requires a very specific molecular weight of between 1000 and 3000 Daltons – smaller molecules aren't able to produce cross-linkages between molecules and larger molecules can't penetrate the active sites of proteins (Margalit, 2004; Herderich and Smith, 2005).

There are two main types of tannins in wine:

1. Hydrolysable tannins:
2. Condensed tannins:

Hydrolysable tannins are gallotannins and ellagitannins which actually originate from wood rather than grapes. They are called gallotannins and ellagitannins because they release gallic acid and ellagic acid on hydrolysis. The most important ellagitannins are vescalagin and castalagin (Ribéreau-Gayon *et al.*, 2006a).

The tannins derived from grapes are known as condensed tannins. The monomeric building blocks of condensed tannins are the flavanols (also known as flavan-3-ols or catechols) (Table 12). The most important flavanol monomers are (–)-epicatechin, (+)-catechin, galocatechin, epigallocatechin and epicatechin-3-*O*-gallate (Monagas *et al.*, 2005; Herderich and Smith, 2005).

The monomeric units, mostly (–)-epicatechin and (+)-catechin, form linkages in a variety of ways to produce dimers, trimers, oligomers and eventually condensed tannins (Figure 10). (In another confusing twist of nomenclature, condensed tannins are also called proanthocyanadins or procyanidins in the literature because they release anthocyanins when heated under acidic conditions). The polymers are distinguished by the length of the chain and the type of inter-flavanic bond formed. For example, Type B dimers have C4-C6 or C4-C8 linkages, while Type A dimers have an additional ether bond between the two

monomers. Oligomers have from 3-10 monomeric units whereas condensed tannins have more than 10 units (Cheynier *et al.*, 2006). Tannins from grape seeds and skins have an average chain length of 10 units whereas skin tannins are usually about 30 units long (Monagas *et al.*, 2005).

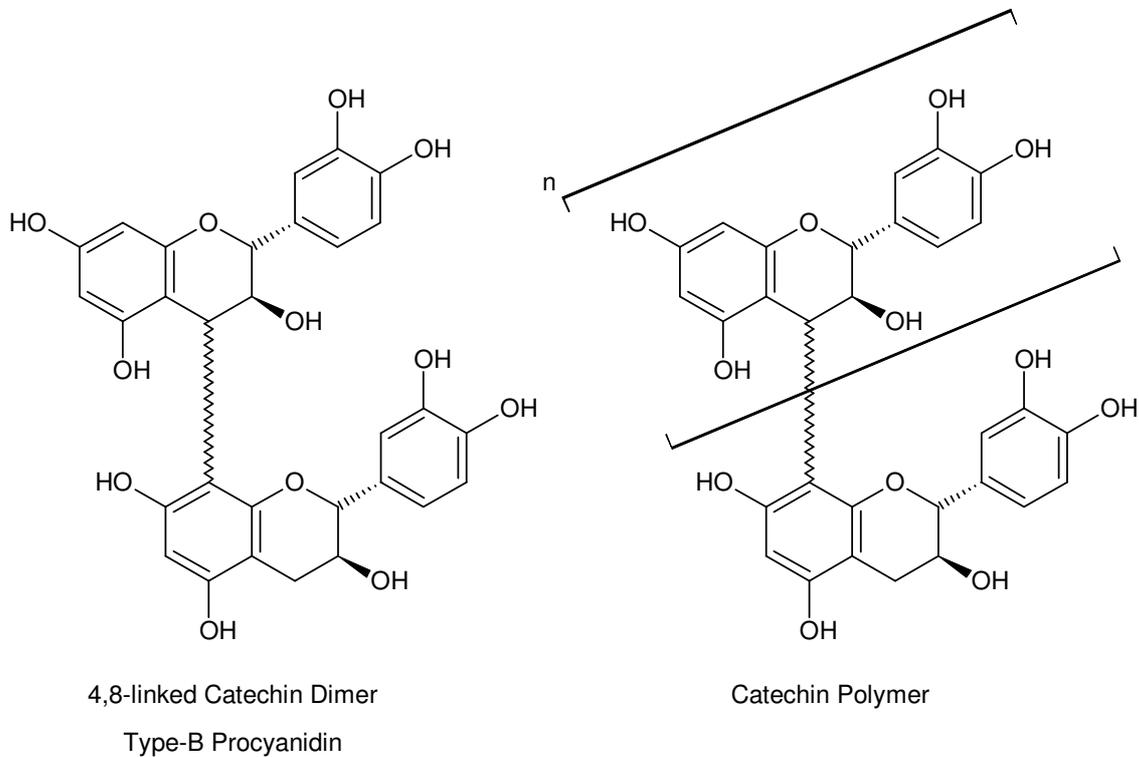


Figure 10: Grape Tannins

c) Flavonols

The flavonols (Table 12) are yellow pigments which are produced in grapes in response to UV radiation (Monagas *et al.*, 2005). Quercetin, myricetin and kaempferol are the most common flavonols in grapes and wine, occurring mostly as 3-*O*-glycosides rather than free aglycones.

These compounds are important as co-pigments for anthocyanins and contribute to wine's anti-oxidant capacity.

6.2 IMPORTANT PHENOLIC REACTIONS OCCURING DURING WINEMAKING AND AGEING

6.2.1 Overview

The developments that occur in red wine during maturation and ageing have been described for centuries:

- There is a decrease in colour intensity over time;
- The colour hue changes from bright red through brick-red to red-orange;
- The astringency decreases and the mouthfeel softens; and
- Tertiary aromas and flavours develop.

However, there has been significant progress in recent years in understanding the chemistry which underlies these observed changes (Monagas *et al.*, 2005; Ribéreau-Gayon *et al.*, 2006a). The changes in colour and mouthfeel, in particular, are due to reactions involving the anthocyanins and tannins in wine (Fulcrand *et al.*, 2006).

Phenolic compounds are relatively reactive compounds due to acid-base reactions of the hydroxyl function and the nucleophilic character of the benzene nucleus.

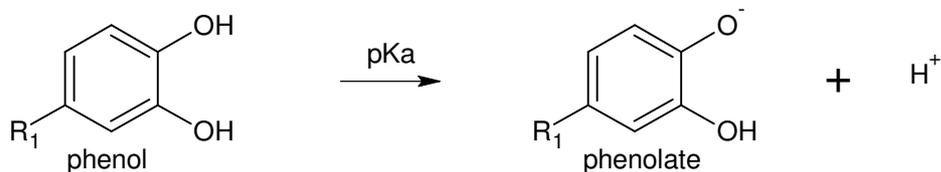
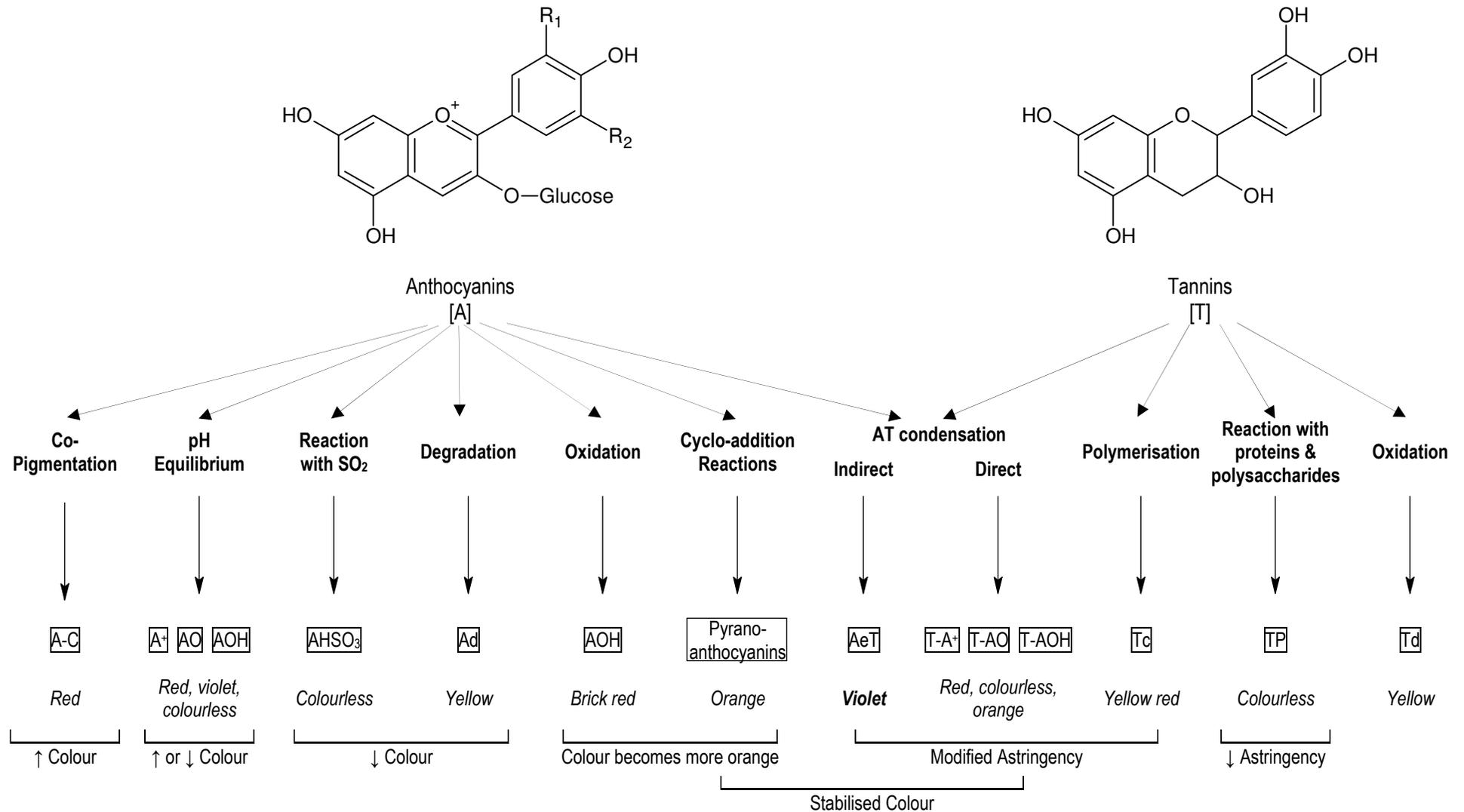


Figure 11: Phenol Acid-Base Reactions

The main reactions of anthocyanins and tannins in wine, and their impact on wine colour and astringency, are summarised in Figure 12. The changes in anthocyanin form and colour with pH, and the reactions between anthocyanins and SO₂ were discussed in Section 6.1.3.2. Tannin-protein interactions and tannin polymerisation reaction were also briefly mentioned in Section 6.1.3.2. Co-pigmentation is a phenomenon observed in wine where the anthocyanins form complexes with other non-coloured organic compounds resulting in colour enhancement or colour shift (Boulton, 2001). Anthocyanins and tannins also undergo degradation reactions, particularly when exposed to high doses of oxygen, resulting in the formation of brown/yellow pigments in wine.

Figure 12: Summary of Key Phenolic Reactions



AC: Co-pigmented complex; A⁺: Flavylium; AO: Quinoidal; AOH: Carbinol; AHSO₃: Anthocyanin-SO₂ complex; Ad: Degraded Anthocyanin; T-A⁺ / T-AO / T-AOH: Polymeric pigments (flavylium / quinoidal / carbinol)
 AeT: Ethyl-linked Anthocyanin-Tannin complex; T-A: Tannin-Anthocyanin complex; Tc: Condensed Tannin; TP: Tannin-polysaccharide; Td: Degraded Tannin

Drawn from (Ribéreau-Gayon and Glories, 1987; Ribéreau-Gayon *et al.*, 2006a)

However, the main focus of this section is on the reactions responsible for the ageing of red wine which are also the reactions that appear to be important in micro-oxygenation. The following key phenolic reactions will be briefly discussed (Monagas *et al.*, 2005):

1. Enzymatic + chemical oxidation;
2. Direct condensation reactions between anthocyanins and tannins;
3. Aldehyde-mediated condensation reactions between anthocyanins and tannins; and
4. Cyclo-addition reactions leading to the formation of pyranoanthocyanins.

6.2.2 Phenolic Oxidation Reactions

Oxidation reactions involve the exchange of electrons.

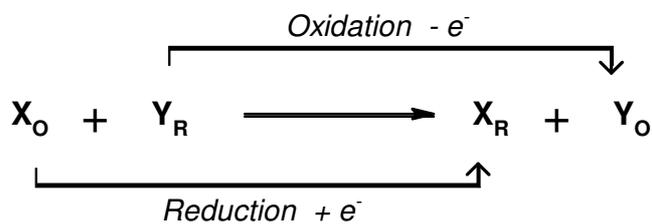


Figure 13: Basic Redox Reaction

Phenolic compounds are the most important oxidation substrates in wine. Phenols are involved in both enzymatic and chemical oxidation reactions which results in the phenols being converted to quinones.

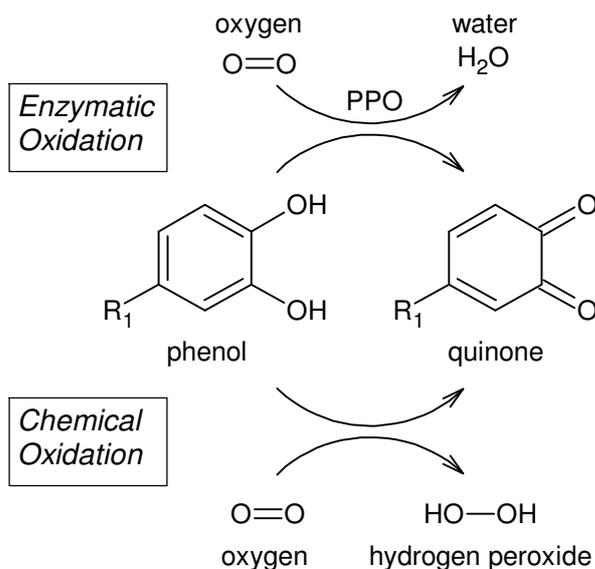


Figure 14: Phenolic Oxidation Reactions

Enzymatic oxidation is produced by oxidases (primarily polyphenoloxidase, PPO, and laccase) derived from grapes. Because oxidases are rapidly degraded and inactivated in must, enzymatic oxidation only occurs early in the vinification process. However, it is an important cause of browning in white wine making. The main phenolic substrates for enzymatic oxidation are the hydroxycinnamic acids and hydroxycinnamic acid esters such as caftaric and coutaric acid.

Chemical oxidation is much slower but plays an important role in wine ageing. The major changes that occur during barrel ageing are primarily due to the slow diffusion of oxygen through the barrel walls and the slow oxidation reactions that occur as a result. It is these beneficial oxidation reactions that micro-oxygenation treatment aims to produce by replicating a similar slow exposure to oxygen.

The main phenolic substrates for chemical oxidation during ageing are the anthocyanins and tannins. They become oxidised through complex coupled reactions which convert the phenols to semi-quinones and quinones (Figure 15). This conversion is coupled to the formation of hydrogen peroxide (H₂O₂) which is an even stronger anti-oxidant and which then, in turn, oxidises ethanol to acetaldehyde (ethanal).

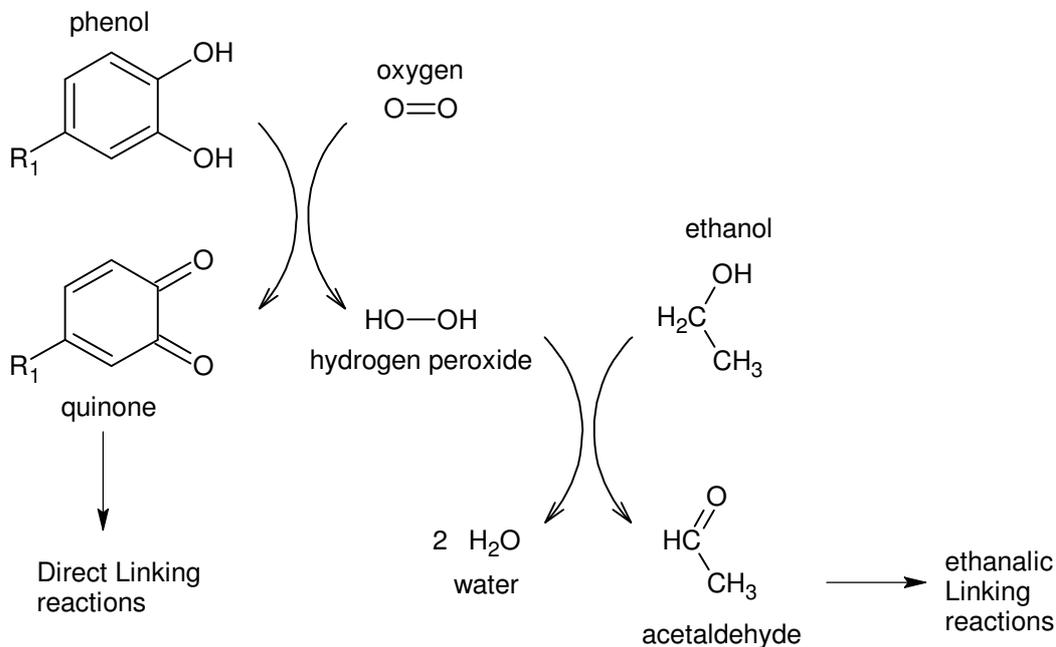


Figure 15: Coupled Chemical Oxidation of Phenolic Compounds

Drawn from : (Kennedy *et al.*, 2006; du Toit *et al.*, 2006b)

As will be seen this reaction is of fundamental importance because the acetaldehyde produced plays a central role in subsequent linking reactions between anthocyanins and tannins (see Section 6.2.4). Acetaldehyde also reacts directly with anthocyanins to produce pyranoanthocyanins (Section 6.2.5). The activated quinone oxidation products can also participate in direct polymerisation and condensation reactions (Figure 15).

The reaction is somewhat more complex than the simple representation in Figure 15 because the chain of coupled redox reactions also involve iron (Fe) and copper (Cu) ions (Danilewicz, 2003; Danilewicz, 2007). Iron is relatively abundant in must and wine but copper may be an important rate limiting cofactor for these reactions. The rate of oxidation reactions in wine is also influenced by a range of other factors including temperature, pH, and the concentration of other anti-oxidants such as ascorbic acid and glutathione.

6.2.3 Direct Condensation Reactions between Anthocyanins and Tannins

Anthocyanins and tannins can react directly with each other to form polymeric pigments (Remy *et al.*, 2000; Dueñas *et al.*, 2006). The tannin molecule is usually in the form of an oligomer. As with free anthocyanins, the resulting polymeric pigments occur in a number of different molecular forms according to pH. However, at wine pH more of the polymeric pigments are in the red form (see Table 13). Because they are no longer able to bind with bisulphite they are also more resistant to SO₂ bleaching.

There are two possible mechanisms (see Figure 16 and Figure 17 below) for the formation of direct anthocyanin-tannin condensation products (Salas *et al.*, 2003; Salas *et al.*, 2004):

1. **Direct A-T reaction:** The anthocyanin is in the flavylium form and forms a C4-C8 A-T linkage with tannin as the upper molecule. The resulting product is a colourless flavene but can be oxidised to the red flavylium form (Figure 16).
2. **Direct T-A Reaction:** This mechanism requires an activated tannin molecule to react with the carbinol anthocyanin form (Figure 17). This time the tannin is the upper molecule and the colourless carbinol product has to be hydrated to produce the red pigmented polymer.

These direct condensation reactions occur very slowly. The direct T-A reaction does not involve oxygen but oxygen is required to produce the red-coloured product in A-T reactions (Remy *et al.*, 2000).

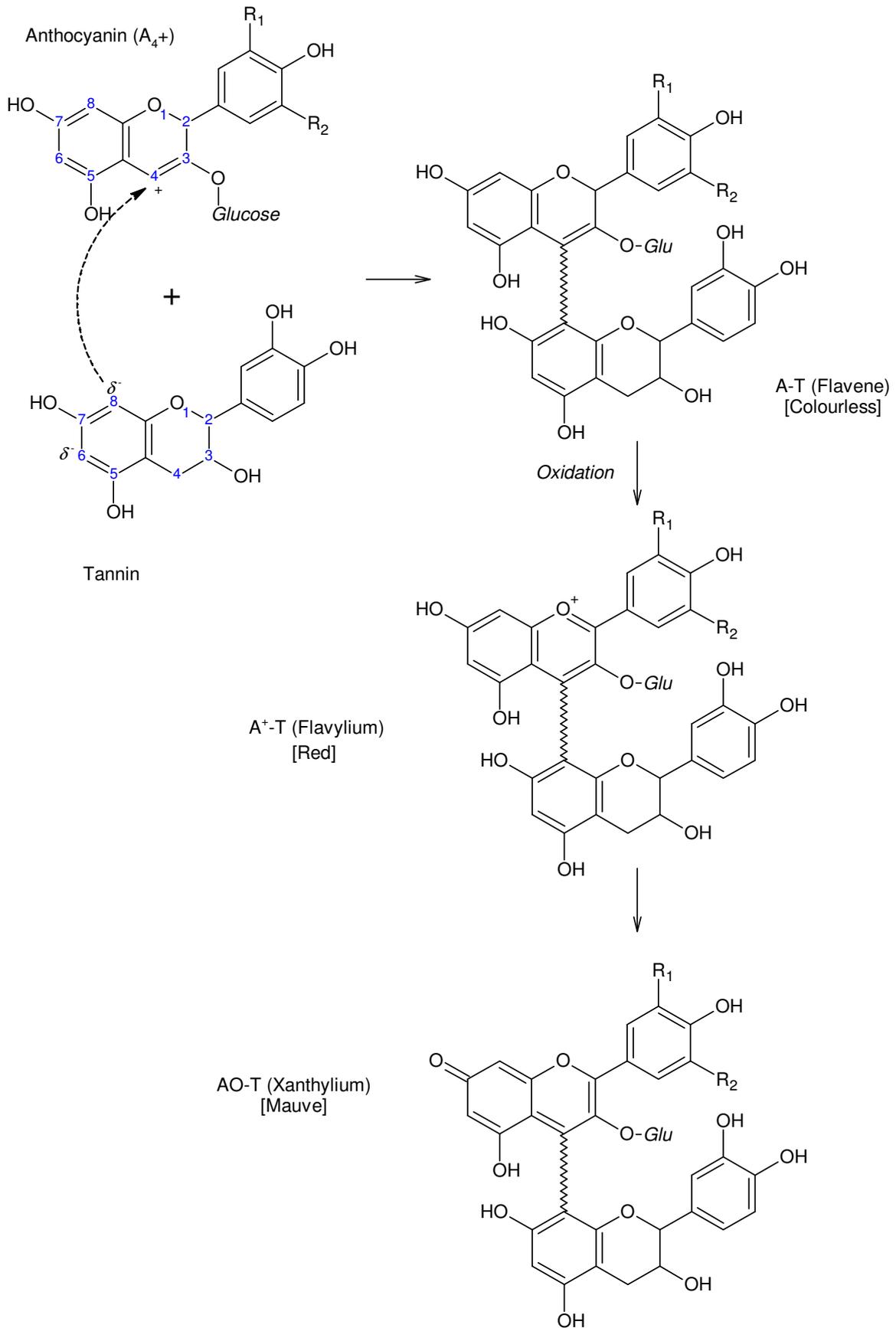


Figure 16: Direct A-T Condensation Reaction

Drawn from (Ribéreau-Gayon *et al.*, 2006c)

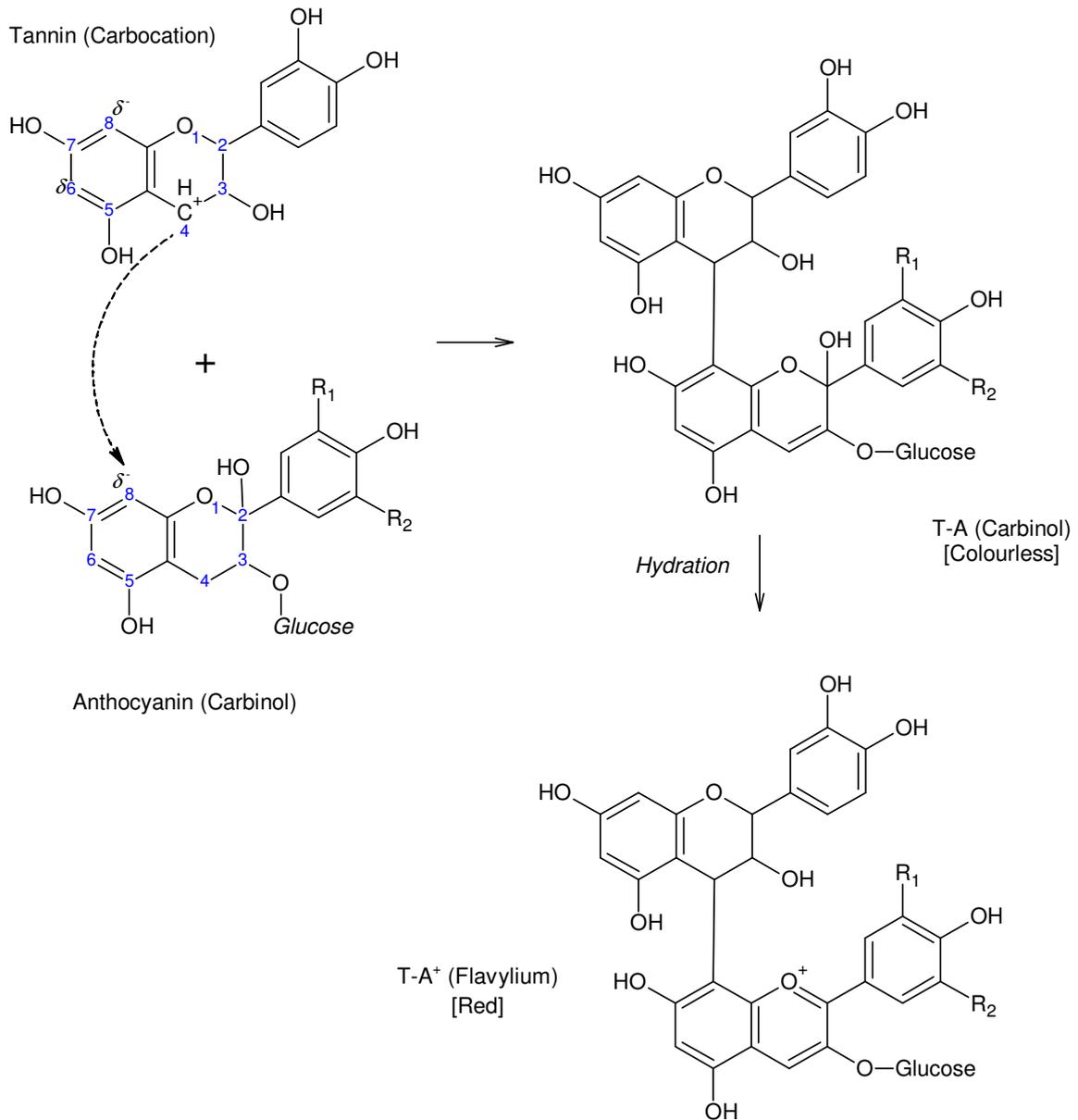


Figure 17: Direct T-A Condensation Reaction

Drawn from (Monagas *et al.*, 2005; Ribéreau-Gayon *et al.*, 2006c)

6.2.4 Aldehyde-Mediated Condensation Reactions between Anthocyanins and Tannins

Indirect reactions between anthocyanins and tannins can also occur. These reactions are mediated by acetaldehyde and other similar molecules (Figure 18). Acetaldehyde is a product of alcoholic fermentation. But as discussed in Section 6.2.2, acetaldehyde is also an important product of the oxidation of ethanol in the presence of phenols (Figure 15). Therefore, this is the most important mechanism for anthocyanin-tannin condensation under oxidative conditions (Saucier *et al.*, 1997). It is also the reaction that would be most critical in producing the beneficial effects of micro-oxygenation treatment.

As shown in Figure 18 the acetaldehyde produced through coupled phenolic oxidation reactions is activated to the carbocation form and then reacts with tannin at C6 or C8. The new carbocation attacks the anthocyanin at C8 resulting in an anthocyanin-tannin complex joined by an ethyl bridge (Wildenradt and Singleton, 1974; Timberlake and Bridle, 1976). The resulting compound is stabilised by deprotonation to form a violet coloured quinoidal base.

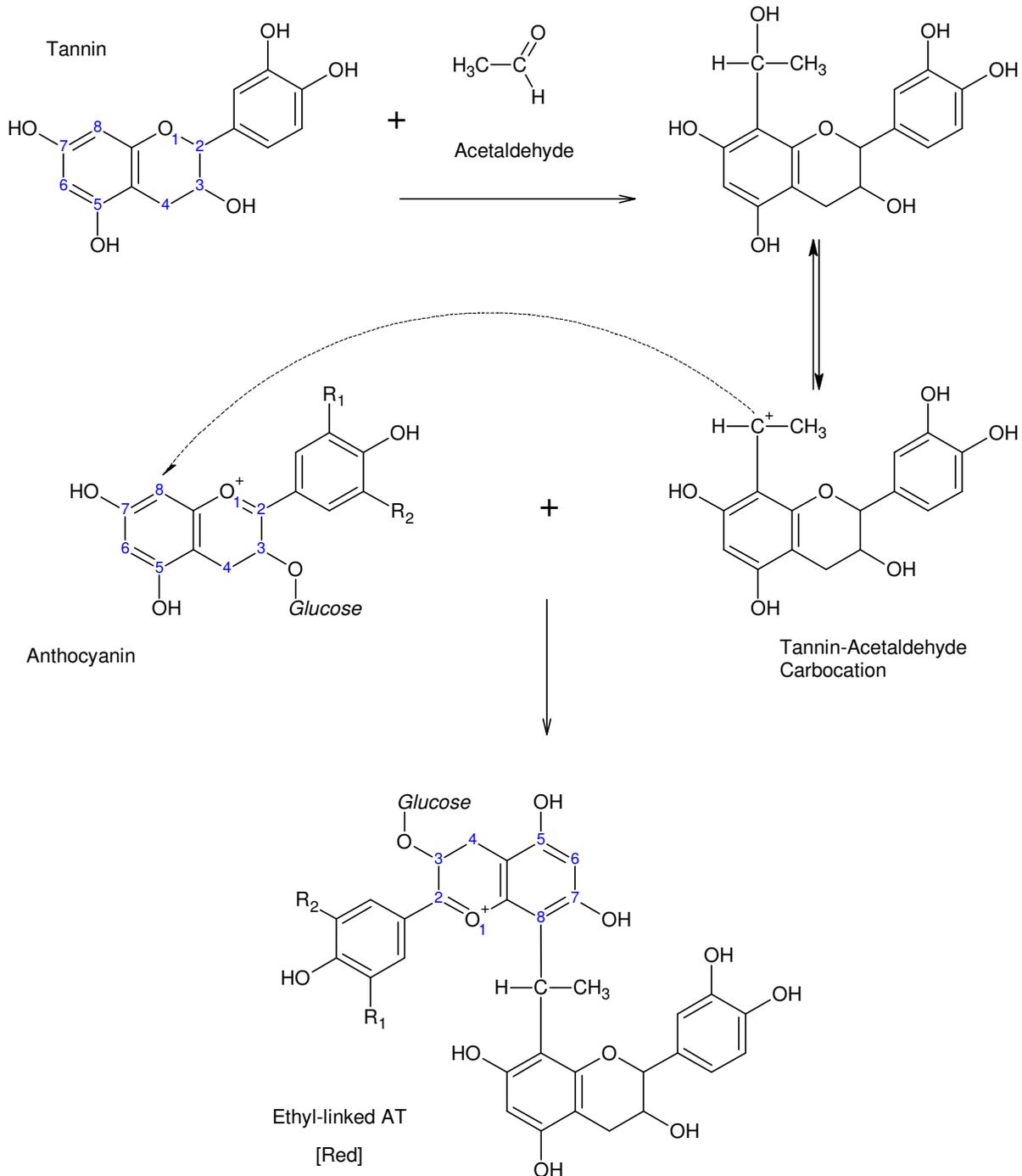


Figure 18: Acetaldehyde-induced Anthocyanin-Tannin Condensation

Drawn from (Monagas *et al.*, 2005; Ribéreau-Gayon *et al.*, 2006c)

The acetaldehyde linking reaction can also occur with the tannin in dimeric or polymeric forms resulting in large polymeric pigments.

The indirect acetaldehyde-mediated condensation reactions occur much more rapidly than direct condensation in the presence of acetaldehyde and so is more important in the production of polymeric pigments. The resulting compounds have higher colour intensity and stability (Es-Safi *et al.*, 1999a; Es-Safi *et al.*, 1999b).

Acetaldehyde is not the only aldehyde that can produce such linkage reactions. For example, it has been shown that glyoxylic acid, produced from the oxidation of tartaric acid, and the furfurals can produce similar condensation reactions (Es-Safi *et al.*, 2002; Monagas *et al.*, 2005).

6.2.5 Cyclo-addition Reactions Leading to the Formation of Pyranoanthocyanins

The last set of important reactions are those that produce pyranoanthocyanins. The pyranoanthocyanins were discovered relatively recently and are formed by the addition of an ethylene bond to C4 or C5 of the anthocyanin C-Ring (Fulcrand *et al.*, 1996; Fulcrand *et al.*, 2006). The added chain is then oxidised to produce a molecule with an additional pyran ring (Figure 19).

The resulting compounds are red-orange which suggests that they may be important in wine ageing, because none of the other products discussed so far are able to explain the typical colour changes that occur with wine maturation (Francia-Aricha *et al.*, 1997). The pyranoanthocyanins are also very stable pigments resistant to changes in pH and SO₂ bleaching.

The pyranoanthocyanins can be formed through a number of different mechanisms. Most important in terms of oxidation and micro-oxygenation are the reactions involving the addition of acetaldehyde to the anthocyanin molecule (Atanasova *et al.*, 2002) but pyranoanthocyanins are also formed by reactions involving pyruvate and vinyl phenols (Figure 19).

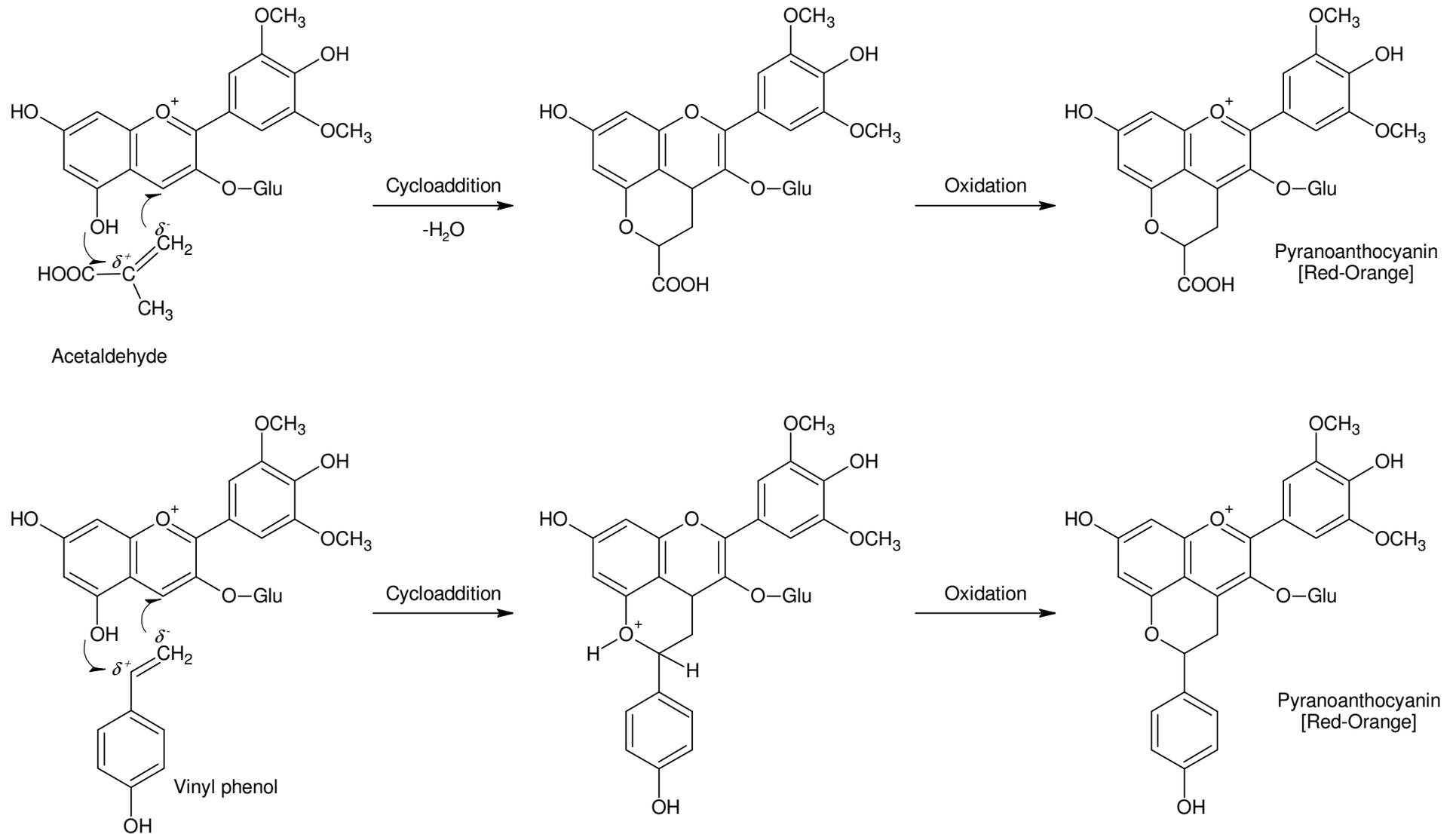


Figure 19: Reactions Producing Pyranoanthocyanins

Drawn from (Monagas *et al.*, 2005)

6.3 THE ANALYSIS OF PHENOLIC COMPOUNDS IN WINE

To some extent, our increased understanding of the reactions of phenolic compounds in wine, as outlined in Section 6.2, is the result of advances in the techniques available for the fractionation and assay of wine phenolic compounds. In research laboratories methods such as high performance liquid chromatography (HPLC) allow the identification and quantification of individual phenolic molecules and mass spectrophotometry is used to elucidate the complex structure of different phenolic derivatives. However, there are also basic spectrophotometric and chemical analysis which can be conducted in winery laboratories to measure key phenolic characteristics.

The vast literature on phenolic analysis will not be presented here. There are a number of more technical texts available for the interested reader (Somers, 1987; de Beer *et al.*, 2004; Herderich and Smith, 2005; Ribéreau-Gayon *et al.*, 2006c; Garcia-Falcon *et al.*, 2007; Roussis *et al.*, 2007; Versari *et al.*, 2008; Seddon and Downey, 2008).

However, a basic appreciation of some of the important phenolic tests and indices is helpful in understanding the technical academic literature. The more common tests for assaying wine phenolic compounds are summarised overleaf in Table 14.

Table 14: Common Tests for the Analysis of Phenolic Compounds in Wine

Category	Test	Measurement Principles
Total Phenols	Folin-Ciocalteu Test	Measure total phenols by reaction of the hydroxyl groups with a phosphor-molybdate reagent which can be measured spectrophotometrically.
	OD ₂₈₀	The benzene molecule characteristically absorbs at 280nm so the optical density at 280nm is a simple measure of [total phenol]. Various corrections have been proposed to provide more accurate estimates.
Anthocyanins	[Anthocyanin]	There is no simple test to measure [total anthocyanin]. Changes in OD ₅₂₀ after HCl addition or SO ₂ bleaching can be used to estimate [anthocyanin]. Both tests measure the sum of [free anthocyanin] + [bound anthocyanin susceptible to bleaching].
	PVP Index	Measures the proportion of free anthocyanins and anthocyanins bound to tannin (T-A forms). Free anthocyanins bind to a PVP column whereas T-A forms do not.
	Ionisation index	Measures the proportion of coloured and uncoloured anthocyanins. Based on the difference in effect of SO ₂ bleaching on wine colour at normal wine pH and at a pH of 1.
	HPLC	High performance liquid chromatography can be used to separate and quantify different anthocyanin compounds.
Tannins	[Tannin] LA Method	Under acid conditions tannins can be hydrolysed to form coloured cyanidins (tannins are also termed procyanidins). The change in colour after addition of HCl is measured at OD ₅₅₀ . Various corrections have been proposed to provide more accurate estimates.
	HPLC	High performance liquid chromatography can be used to separate and quantify different tannin compounds.
	MDP	The mean degree of polymerisation (MDP) indicates the average chain length of the polymeric tannins. Measured by acid depolymerisation in the presence of a nucleophilic agent such as toluene- α -thiol (thiolysis) to identify terminal units.
	HCl Index	Measures the proportion of large polymeric forms. Based on the hydrolysis of tannins in an acid medium where speed of precipitation depends on degree of polymerisation. Values range from 5-40. HCl index > 25 indicates highly polymerised tannins.
	Dialysis Index	Measures the proportion of tannins that do not pass through a dialysis membrane under standard operating conditions. Depends on size and charge of tannin molecules. Values range from 5-30.
	Gelatin Index	Measures the reactivity of wine tannins with gelatin. Used as an indication of astringency. Values range from 25-80.
Wine Colour	Colour Intensity	Total amount of colour in wine. Sum of optical densities at 420nm (yellow), 520nm (red) and 620nm (blue). $CI = OD_{420} + OD_{520} + OD_{620}$
	Colour Stability	Measures change in colour intensity after SO ₂ bleaching.
	Hue	Development of colour towards orange. $Hue = OD_{420} / OD_{520}$
	Colour Composition	Each of three colour components as a proportion of total colour intensity

Source: (Ribéreau-Gayon and Glories, 1987; de Beer *et al.*, 2004; Ribéreau-Gayon *et al.*, 2006c)

CHAPTER 7: SCIENTIFIC EVALUATIONS OF MICRO-OXYGENATION

Chapter 6 reviewed the basic science research that suggests that the intentional exposure of wine to oxygen influences the phenolic composition of wine. It is now well established that oxygenation in the presence of wine phenols results in the production of acetaldehyde which, in turn, would induce anthocyanin-tannin condensation and would react with anthocyanins to form pyranoanthocyanins. These reactions produce more stable polymeric coloured pigments with a concomitant reduction in the concentrations of monomeric anthocyanins and tannins in solution.

However, much of this research has been done on model wine solutions. The dynamics and relative kinetics of these different phenolic reactions as they occur in real wine are likely to be more complex and less predictable. Also, the fact that oxygenation has been shown to stimulate phenolic condensation reactions does not prove that the micro-oxygenation technology, as currently operationalised, is consistently able to produce the desired reactions. Lastly, the basic science considered so far does not explain some of the other stated benefits of micro-oxygenation, such as that the technique is able to increase fruit flavours or reduce unwanted herbaceousness (Table 1).

Clearly further empirical evidence is required to support the claims of the micro-oxygenation industry. This chapter summarises the scientific literature that has attempted to investigate more directly whether or not the various hypothetical and postulated changes can be shown to occur during micro-oxygenation treatment under controlled conditions.

7.1 SEARCH STRATEGY AND IDENTIFIED STUDIES

The objective was to identify and review scientifically rigorous evaluations of micro-oxygenation. The search strategy employed to identify relevant trials is summarised in Table 15.

A number of academic databases were searched for appropriate studies. The Food Science and Technology Abstracts (FSTA) is the main reference for agricultural research but this was supplemented with other scientific databases such as Ingenta that include food and agricultural journals. The main wine journal archives and websites were also interrogated directly. The South African Wine Industry Information and Systems (SAWIS) library maintains their own database of academic and industry references extracted from

their wine magazines and journal subscriptions. This database was also searched. Lastly general web search engines such as Google Scholar and Google Web were also used but few academic articles were identified through those sources.

Table 15: Search Strategy to identify Scientific Micro-oxygenation Studies

Search Databases	Search Terms
Food Science and Technology Abstracts (FSTA) Chemical Abstracts Science Direct Ebsco Host Ingenta Connect Wiley Interscience Springer Link SAWIS library database American Journal of Enology & Viticulture archive Other Food and Agriculture websites Google Scholar Google	Wine Micro-oxygenation Microoxygenation Micro-aeration Oxygenation Oxidation Oxygen Phenolic Tannin

The search terms used are also shown in Table 15. These words were used singly and in combination in order to narrow the search and to ensure that only relevant articles were retrieved. The titles and abstracts obtained from this search strategy were then evaluated to identify controlled scientific trials of micro-oxygenation. The full-text of all likely articles was then obtained from various fulltext databases, procured through the SAWIS library, or purchased directly if not available from other sources. Finally, the complete article was read in order to identify published scientific trials of micro-oxygenation.

The final list of relevant published studies identified by this search strategy is shown in Table 16. A total of 19 English published scientific studies were available for this review. I excluded a number of more anecdotal reports (Table 16). Some of these described winemakers' experiences with micro-oxygenation but not under controlled conditions and usually over a number of years. There are also one or two articles in the excluded list (Zoecklein *et al.*, 2003; Blackburn, 2004) that described accumulated evidence from a number of studies without describing any in sufficient to assess properly. There were also 7 studies published in non-English journals that seemed relevant but that I was unfortunately not able to evaluate.

Table 16: Identified Scientific Studies Evaluating Micro-oxygenation

Anecdotal Reports	Scientific Studies (English Language)	Scientific Studies (Non-English Language)
(Ribéreau-Gayon and Glories, 1987) (Gerland, 2000) (Cheynier, 2002) (Paul, 2002) (Lemaire <i>et al.</i> , 2002) (Loch, 2002) (Lemaire, 2002) (Graham, 2003) (Tibbits, 2003) (Otto, 2003) (Blackburn, 2004) (Zoecklein <i>et al.</i> , 2003) (Waterhouse, 2004) (ICV, 2007)	(Castellari <i>et al.</i> , 2000) (Pour-Nikfardjam and Dykes, 2002) (Pour-Nikfardjam and Dykes, 2003) (Atanasova <i>et al.</i> , 2002) (McCord, 2003) (du Toit <i>et al.</i> , 2006a) (del Carmen-Llady <i>et al.</i> , 2006) (Cano-López <i>et al.</i> , 2006) (Cano-López <i>et al.</i> , 2007) (Pérez-Magariño <i>et al.</i> , 2007) (Tao <i>et al.</i> , 2007) (Rayne, 2007) (Sartini <i>et al.</i> , 2007) (Kovacevic Ganic <i>et al.</i> , 2008) (Ortega-Herás <i>et al.</i> , 2008) (de Beer <i>et al.</i> , 2008) (Cano-López <i>et al.</i> , 2008) (Rivero-Pérez <i>et al.</i> , 2008) (Hernández-Orte <i>et al.</i> , 2009) (Pérez-Magariño <i>et al.</i> , 2009) (Rudnitskaya <i>et al.</i> , 2009)	(Maurizio Ciani, 1997) (Amati <i>et al.</i> , 2000) (Bosso <i>et al.</i> , 2000) (Castel <i>et al.</i> , 2001) (Ferrarini <i>et al.</i> , 2001) (Moutounet <i>et al.</i> , 2001) (Pérez-Magariño and González-Sanjosé, 2002)

7.2 EVALUATION OF IDENTIFIED STUDIES

Rigorous scientific research in this area is difficult for a number of reasons (du Toit *et al.*, 2006a; Sampaio *et al.*, 2007):

- It requires specialised equipment,
- Research cannot be done on small batches. Micro-oxygenation treatment requires large tanks (and therefore large volumes of wine) which makes it very expensive,
- If researchers have to work through commercial wineries they don't have complete control of the experiment;
- Evaluating the impact of micro-oxygenation requires complicated and expensive laboratory tests; and
- Evaluating the actual impact on wine requires sensory testing with is also difficult to do rigorously.

Nevertheless, in comparing the identified studies and their findings I considered a number of key aspects of each study. These evaluation criteria are summarised in Table 17.

Table 17: Important Criteria in Evaluating Scientific Micro-oxygenation Studies

Study objective
Varietals used
Trial design
Existence of control for comparison
Nature of control treatment
Replicates of treatments done
Statistical analysis of differences found between control and MOX treatment wines
Timing of MOX
Equipment and dosing schedule used for MOX
Dose of O ₂ administered
Duration of MOX treatment
End-point parameters evaluated
Methods used for evaluation of end-points
Evaluations occur over time
Economic analysis of comparisons

An important aspect of scientific trial design is the existence of a suitable control group for comparison. Claimed benefits of micro-oxygenation can be demonstrated by showing statistically significant differences between treated and untreated wines. However, it is not completely straightforward to determine the best objective and control group for micro-oxygenation studies. Comparing micro-oxygenated wines with completely untreated wines may show that micro-oxygenation has an effect but would not prove that micro-oxygenation is as effective as current maturation in barrel, for example. A different study design is required to investigate that objective. Similarly, it is not possible to interrogate the common claim that micro-oxygenation is more cost-effective than standard barrel maturation without a detailed evaluation of the costs and benefits of both alternatives. Unfortunately, no such study was identified.

Differences between two wine treatments may occur by chance so statistical analysis is required to prove a real effect. Unfortunately, this is not possible without replicates of each treatment which is difficult to do and increases the costs of the research.

An important difficulty in comparing micro-oxygenation studies is that the treatment conditions are not standardised. The timing, dose, duration and administration of the micro-oxygenation therapy are usually different. Also different varietals, with significantly different phenolic composition, are used for the trials which makes it difficult to generalise about the results.

Another consideration is the end-point parameters used in the studies and whether or not these have been measured using valid and accepted methodologies. Laboratory testing

needs to be based on current best practice and tasting panels need to be properly constituted and analysed correctly. Another significant problem in these studies is the timing of when the endpoints are evaluated. Significant differences that are found immediately after the micro-oxygenation treatment may not persist after subsequent oenological manipulation and maturation which means, in fact, that that impact of the treatment on the final product delivered to the consumer is negligible.

7.3 DESCRIPTION OF IDENTIFIED STUDIES

The key characteristics of the 19 English language scientific evaluations of micro-oxygenation I identified are summarised in Table 18. All of these studies were published in the last decade and there is clearly a rapidly growing literature on the subject since more than half of the articles were published in the last two years.

A wide range of different grape varieties were used in the trials and this is certain to influence the results obtained (Pérez-Magariño *et al.*, 2009). It is encouraging that two of the papers (du Toit *et al.*, 2006a; de Beer *et al.*, 2008) are from South African researchers using local varieties. All of the study designs included a control wine for comparison though the nature of that control differed between studies. Earlier research tended to compare MOX-treated wines against untreated controls whereas the more recent investigations have used more realistic comparison groups.

The timing, dose and duration of micro-oxygenation employed was quite variable. A number of the initial studies investigated MOX treatment after malolactic fermentation (post MLF) whereas, in keeping with current practice, later investigations have used pre-MLF MOX, or treatments both before and after malolactic fermentation. Again, this variation makes it extremely difficult to compare the findings of different studies.

The vast majority of the identified studies focused on changes in colour and phenolic composition of treated wines. There are only three reported studies which have investigated the effect on aroma compounds, three which measured wine anti-oxidant status, and only one which has evaluated the impact on microbiological stability with any rigour (Table 18). At present in the literature there are only five studies which have scientifically evaluated the impact of MOX using sensory panels. Most of the reported studies did have replicates and undertook some statistical analyses although this was usually fairly limited.

Table 18: Summary of Key Scientific Studies on Micro-oxygenation

Reference	Study Characteristics		MOX Treatment		Replicates	Stats	Evaluation					
	Varietal/s	Trial Design	Timing	Dose x Duration			Col	Phen	Aroma	AntiO ₂	Micro	Sens
(Castellari <i>et al.</i> , 2000)	Sangiovese	1. Control 2. Saturation with pure O ₂ every 2Mo 3. Saturation with pure O ₂ every 1Mo	Post AF (No MLF)	6Mo	Quadruplicate	Yes	✓	✓		✓		
(Atanasova <i>et al.</i> , 2002)	Cabernet Sauvignon-Tannat blend	1. Control: saturated with N ₂ 2. MOX		5ml/L x 7Mo	Triplicate	Yes	✓	✓				
(Pour-Nikfardjam and Dykes, 2003)	Pinot noir Cabernet Sauvignon	1. Control 2. MOX	Post MLF	4ml/L/Mo x 2Mo	No	Yes	✓	✓				✓
(McCord, 2003)	Cabernet Sauvignon	1. Control: No O ₂ , no staves 2. No O ₂ + wood staves 3. No O ₂ , + wood segments 4. MOX, no staves 5. MOX + staves 6. MOX + segments	Post MLF	10ml/L/Mo x 1Mo + 5ml/L/Mo x 4Mo	Duplicate		✓	✓	✓			
(du Toit <i>et al.</i> , 2006a)	Cabernet Sauvignon Red blend Pinotage Shiraz	1. Control: No O ₂ , + staves 2. Control: No O ₂ , Oak barrel 3. MOX immediately post MLF 4. MOX 7Mo post MLF	Post MLF	Range of 1.5 / 3.0 / 4.0 mg/L/Mo ? Duration	No	Yes	✓	✓			✓	✓
(del Carmen-Llaudy <i>et al.</i> , 2006)	Cabernet Sauvignon	1. Control: 8Mo barrel + 3Mo stainless steel 2. 3Mo MOX in stainless steel + 8Mo barrel	Post MLF	3 mg/l/Mo x 3 Mo	Triplicate	Yes	✓	✓				✓
(Cano-López <i>et al.</i> , 2006)	Monastrell	1. Control: No O ₂ 2. Low dose MOX 3. High lose MOX	Pre MLF + Post MLF	Low: 5mg/L/Mo x 1Mo + 3mg/L/Mo x 1Mo + 1.5mg/L/Mo x 2wk High: 10mg/L/Mo x 1Mo + 5mg/L/Mo x 1Mo + 2.5mg/L/Mo x 2Wk	Triplicate	Yes	✓	✓				
(Cano-Lopez <i>et al.</i> , 2007)	Monastrell	Continuation of study above. Wines from each of 3 original study arms split in two: 1. Further 6Mo ageing in American oak 2. Further 6Mo ageing in bottle			Triplicate	Yes	✓	✓				
(Pérez-Magariño <i>et al.</i> , 2007)	Mencia Tinta de Toro Tinta del Pais Tempranillo	4 wines, 3 vintages 1. Control 2. MOX	Pre MLF	Total O ₂ dose ranged from 28-43ml/L. over period of 18-23 days	Duplicate	Yes	✓	✓				
(Rayne, 2007)	Merlot Cabernet Sauvignon	1. Control 2. MOX	Pre MLF	Merlot: 24-34ml/L/Mo x 15d Cabernet: 2-44ml/L/Mo x 14d	No	No	✓					
(Sartini <i>et al.</i> , 2007)	Sangiovese	1. Control 2. No O ₂ , wood chips 3. MOX + wood chips 4. MOX + chips + lees	Post MLF	3 ml/L/Mo x 3Mo	Triplicate	Yes	✓	✓				

Table 18: Summary of Key Scientific Studies on Micro-oxygenation

Reference	Study Characteristics		MOX Treatment		Replicates	Stats	Evaluation					
	Varietal/s	Trial Design	Timing	Dose x Duration			Col	Phen	Aroma	AntiO ₂	Micro	Sens
(Kovacevic Ganic <i>et al.</i> , 2008)	Plavac mali	1. Control: under N ₂ 2. MOX	Pre MLF	40.1 ml/L x 39 days	Duplicate	No	✓	✓				
(Ortega-Herás <i>et al.</i> , 2008)	Tinta de Toro Mencia	2 wines over 2 vintages 1. Control: No O ₂ + 12Mo barrel 2. MOX + 12Mo barrel	Pre MLF	According to sensory analysis. 50-60ml/L/Mo x 10d + 20-30ml/L/Mo x 10d	No	No			✓			
(de Beer <i>et al.</i> , 2008)	Pinotage	1. Control 2. Low dose MOX 3. High does MOX	Post AF (No MLF)	Low: 2.5mg/L/Mo High 5.0mg/L/Mo Discrete monthly dose x 2/4/6 Mo	Triplicate	Yes	✓	✓		✓		✓
(Cano-López <i>et al.</i> , 2008)	Monastrell	3 different wines with different [phenolic] 1. Control 2. MOX	Pre MLF + Post MLF	Pre MLF: 10mg/L/Mo Post: 3mg/L/Mo x 2Mo + 1.5mg/L/Mo x ? duration	Triplicate	Yes	✓	✓				
(Rivero-Pérez <i>et al.</i> , 2008)	Tinta de Toro Mencia Tempranillo Tinta del Pais	4 varieties x 3 vintages 1. Control 2. MOX	Pre MLF	According to sensory analysis. 35-47mg/L/Mo x ? duration	Duplicate	Yes				✓		
(Hernández-Orte <i>et al.</i> , 2009)	Tempranillo Cabernet Sauvignon	1. No O ₂ + MLF in stainless steel + 8Mo barrel 2. No O ₂ + MLF in barrel + 8Mo barrel 3. No O ₂ + No MLF + 8Mo barrel 4. MOX + MLF in stainless steel + 8Mo barrel 5. MOX + MLF in barrel + 8Mo barrel 6. MOX + No MLF + 8Mo barrel	Pre MLF	60 ml/L/Mo x 15 days.	Duplicate	Yes			✓			✓
(Pérez-Magariño <i>et al.</i> , 2009)	Mencia Tinta del Pais	1. Control + 4 different wood chips 2. MOX + 4 different wood chips	Pre MLF	Mencia: 25ml/L x 17d Tinta: 31ml/L x 20d	Duplicate	Yes	✓	✓				
(Rudnitskaya <i>et al.</i> , 2009)	Shiraz	Full factorial design with following factors: MOX: 2 levels (No / Yes) Oak chips: 2 levels (No / Yes) Vintage: 3 levels (2004 / 2005 / 2006)	Post MLF	2ml/L/Mo x ? duration	Triplicate	Yes	✓	✓				

MOX: Micro-oxygenation; ml/L/Mo: ml/L O₂ /Month; d: Days; wk: Week; Mo: Month; AF: Alcoholic Fermentation; MLF: Malo-lactic fermentation; Stats: Statistical analysis done; Col: Colour measurement; Phenol: Phenolic assays; Aroma: Aromatic compound assay; AntiO₂: Measured anti-oxidant capacity; Micro: Microbial evaluation; Sens: Performed sensory assessment

7.4 KEY FINDINGS OF IDENTIFIED STUDIES

The key findings of the 19 identified scientific studies on micro-oxygenation are summarised in Table 19. The table shows the significant changes in MOX treated wines relative to control wines, though the timing of the measurements may differ between studies.

7.4.1 Impact on Colour

There are now a number of studies which clearly demonstrate that micro-oxygenation has beneficial effects on wine colour. Colour intensity is significantly increased (or decreased less than controls) by MOX, and treated wines are more resistant to SO₂ bleaching than control wines. These results are consistent with the predicted oxidative reactions discussed in 6.2.2. Micro-oxygenation does appear to result in the production of larger polymeric pigments which preserve wine colour.

Although the postulated formation of pyranoanthocyanins should cause a shift towards orange, the empirical results reveal no clear pattern with regard to changes in wine hue.

7.4.2 Impact on Phenolic Composition

Changes in phenolic composition after micro-oxygenation have also been well studied. Earlier studies found a decrease in the total phenol concentration relative to controls but more recent research has shown no significant differences between treated and untreated wines (Table 19).

One of the most constant patterns in the literature reviewed here is that MOX treatment produces an increase in measured polymeric pigment compounds while free anthocyanins and tannin monomers are decreased. Again this is consistent with the theoretical oxygenation reactions and the observed colour changes.

However, the increase in pyranoanthocyanins that should occur with micro-oxygenation has not been clearly proven. del Carmen-Laudy *et al* (2006) and Pérez-Magariño *et al* (2007) found no difference relative to controls although two recent studies found significantly higher levels of pyranoanthocyanins in treated wines (de Beer *et al.*, 2008; Cano-López *et al.*, 2008). Cano-Lopez (2006) showed an increase in pyranoanthocyanins immediately after micro-oxygenation but a higher rate of breakdown of these compounds in

subsequent barrel maturation (Cano-Lopez *et al.*, 2007) so that the final impact of treatment was reduced.

Wine flavonols (quercetin, myricetin etc – see Table 12) have been consistently reduced by micro-oxygenation treatment in all of the studies that have measured these compounds (Castellari *et al.*, 2000; Sartini *et al.*, 2007; de Beer *et al.*, 2008; Kovacevic Ganic *et al.*, 2008). This is of some concern because flavonols are an important contributor to wine anti-oxidant capacity (Parker *et al.*, 2007).

The impact of micro-oxygenation on tannin polymerisation (as measured by the MDP) is also mixed. Atanasova *et al* (2002) and del Carmen-Laudy *et al* (2006) found an increase in MDP whereas Pour-Nikfardjam & Dykes (2003) showed a decrease in MDP relative to controls. Cano-Lopez *et al* (2008) measured an increase in MDP in one wine that started with a lower phenolic concentration (perhaps suggesting over-treatment with oxygen) but a decreased MDP in wines that were initially more tannic.

Although, most studies demonstrated a decrease in total phenolic or monomeric tannins, there is no consistent evidence on the actual impact of MOX on astringency and mouthfeel. The chemistry of astringency is complex (Gawel, 1998; Francis *et al.*, 2002), and although ageing promotes tannin polymerisation this may well increase rather than decrease astringency (Vidal *et al.*, 2003; Gawel *et al.*, 2007).

7.4.3 Impact on Aroma Compounds

McCord's research in 2003 suggested that micro-oxygenation decreased sulphide reductive flavours (mercaptans) but this has not been confirmed in subsequent studies. Two recent studies measured a large number of aroma compounds in wines treated with MOX (Ortega-Herás *et al.*, 2008; Hernández-Orte *et al.*, 2009) but found no consistent increase in fruitiness or decrease in green herbaceous flavours, which are key claims of the industry. Also, both studies demonstrated that treated wines take up less wood compounds on subsequent barrel maturation (Table 19).

7.4.4 Impact on Wine Anti-oxidant Activity

The decrease in total phenols produced by micro-oxygenation would be expected to decrease total anti-oxidant activity. This may have implications for the claimed health benefits of red wine. To date only two studies has evaluated this directly (de Beer *et al.*, 2008; Rivero-Pérez *et al.*, 2008). De Beer *et al* (2008) found that wine total anti-oxidant

capacity was indeed decreased by micro-oxygenation treatment but Rivero-Pérez *et al* (2008) found no difference. The impact on the health-promoting effects of wine may also be more complex because Rivero-Pérez *et al* (2008) found that DNA damage prevention was increased by MOX treatment (which would be beneficial) but that lipid peroxidation capacity was decreased (which would be harmful).

7.4.5 Impact on Microbiological Growth

Micro-oxygenation may increase the risk of microbial spoilage by aerobic organisms such as acetic acid bacteria and *Brettanomyces*. Only one study was found that evaluated that risk directly (du Toit *et al.*, 2006a). Although there was some evidence of increased acetic acid bacteria and *Brettanomyces* aromas there was no actual spoilage detected in this study.

7.4.6 Impact on Sensory Assessment

Lastly, only a few studies have included rigorous sensory testing by tasting panels (Table 19). The results of these studies are also inconsistent. Some panels reported decreased astringency after micro-oxygenation treatment, but a number found no change, and at least one study seemed to produce an increase in astringency (Pour-Nikfardjam and Dykes, 2003). The panel in Pour-Nikfardjam & Dykes' (2003) evaluation reported decreased herbaceous flavours in treated wines but the sensory trials in the Hernández-Orte *et al* (2009) study found that herbaceousness was increased by micro-oxygenation. In keeping with their analysis of wood aroma compounds, this study also found that wood and toast flavours were decreased in treated wine. Overall, a key concern in the current scientific literature is that treated wines were not always consistently preferred to non-treated wines.

Table 19: Summary of Results from Key Scientific Studies on Micro-oxygenation

Reference	Significant Changes in MOX-treated Wines Relative to Control					
	Colour Parameters	Phenolic Chemistry	Aroma Compounds	Anti-Oxidant Status	Microbial Activity	Sensory Evaluation
(Castellari <i>et al.</i> , 2000)	↑ colour intensity	↑ polymeric pigments ↓ tannin monomers ↓ flavonols		↓ flavonols ↓ trans-resveratrol		
(Atanasova <i>et al.</i> , 2002)	↑ colour intensity ↑ colour stability	↑ polymeric pigments ↓ anthocyanins ↑ MDP				Not done
(Pour-Nikfardjam and Dykes, 2003)	↑ colour intensity → hue	↓ MDP				↓ herbaceousness ↑ astringency
(McCord, 2003)	↑ colour intensity	↓ tannin monomers ↑ polymeric phenols ↓ anthocyanins ↓ flavonols	↓ free mercaptans → dimethyl sulphide			
(du Toit <i>et al.</i> , 2006a)	↑ colour intensity ↑ colour stability	↓ total phenols ↓ anthocyanins ↑ polymeric pigments ↑ HCl index ↑ gelatin index			↑ acetic acid bacteria counts No ↑ volatile acidity ↑ Brett counts	Early MOX: improved Late MOX: worse, ↑ Brett
(del Carmen-Llaudy <i>et al.</i> , 2006)	Lower ↓ colour intensity ↑ colour stability Shift to yellow	↑ polymeric pigments ↑ anthocyanins → pyranoanthocyanins ↑ MDP ↓ astringency (albumin rxn)				Enhanced oak flavours ↓ astringency
(Cano-López <i>et al.</i> , 2006)	↑ colour intensity ↑ colour stability	↑ polymeric pigments ↓ anthocyanins ↑ pyranoanthocyanins			No ↑ volatile acidity	
(Cano-Lopez <i>et al.</i> , 2007)	Colour improvements maintained in both barrel + bottle aged wines	Larger ↓ pyranoanthocyanins with maturation				
(Pérez-Magariño <i>et al.</i> , 2007)	↑ colour intensity ↑ colour stability Shift to orange	↓ total phenols ↑ polymeric pigments ↓ anthocyanins → pyranoanthocyanins				
(Rayne, 2007)	↑ colour intensity					
(Sartini <i>et al.</i> , 2007)	→ colour intensity ↑ colour stability ↓ hue	↓ total phenols ↑ polymeric pigments ↓ anthocyanins ↓ flavonols				

Table 19: Summary of Results from Key Scientific Studies on Micro-oxygenation

Reference	Significant Changes in MOX-treated Wines Relative to Control					
	Colour Parameters	Phenolic Chemistry	Aroma Compounds	Anti-Oxidant Status	Microbial Activity	Sensory Evaluation
(Kovacevic Ganic <i>et al.</i> , 2008)	↑ colour intensity ↓ hue	→ total phenols ↑ polymeric pigments ↓ anthocyanins ↓ flavonols				
(Ortega-Herás <i>et al.</i> , 2008)			→ fruit compounds → herbaceous compounds ↑ fusel alcohols ↑ fatty acids ↓ wood compounds			
(de Beer <i>et al.</i> , 2008)	↓ L* (Lightness) ↓ C* (Chroma) ↓ a* (red/green chromacity)	→ total phenols ↓ tannin monomers ↑ polymeric pigments ↑ pyranoanthocyanins ↓ flavonols		↓ total anti-oxidant capacity		↑ sensory colour ↓ berry ↑ fullness → astringency ↓ overall quality
(Cano-López <i>et al.</i> , 2008)	↑ colour intensity ↑ colour stability → hue	→ total phenols ↓ anthocyanins ↑ ethyl-linked compounds ↑ pyranoanthocyanins ↑ MDP in least tannic wine but ↓ MDP in more tannic wines			No ↑ volatile acidity	
(Rivero-Pérez <i>et al.</i> , 2008)				→ Total anti-oxidant capacity → scavenger activity ↑ DNA damage prevention ↓ lipid peroxidation capacity		
(Hernández-Orte <i>et al.</i> , 2009)			Some differences pre MLF but disappear with MLF + ageing → fruit compounds → herbaceous compounds ↓ acetoin ↓ wood components ↓ volatile phenols			→ before MLF After MLF: ↓ wood + toast ↓ fruit ↑ herbaceousness
(Pérez-Magariño <i>et al.</i> , 2009)	Grape variety + MOX significantly influenced wine colour and phenolic composition but wood chips did not.					
(Rudnitskaya <i>et al.</i> , 2009)	MOX significantly influenced the phenolic composition of wine but only explained about 4.1% of the observed variation in physicochemical parameters. Vintage was the most important determinant.					

↑: Increase; ↓: Decrease; →: No Change; MDP: Mean degree of polymerisation; Brett: Brettanomyces; rxn: reaction. (For interpretation of tests see Section 6.3.)

PART IV: CONCLUSION

CHAPTER 8: CONCLUSION AND RECOMMENDATIONS

Micro-oxygenation is a new, innovative wine-making technique which involves the controlled introduction of low concentrations of oxygen during early wine maturation. It is claimed that micro-oxygenation can reproduce the benefits of barrel-aging but in a much shorter time and at a fraction of the cost. Micro-oxygenation is supposed to result in wines with soft, accessible tannins as well as greater colour stability which is obviously appealing to winemakers vying for a share of the increasingly competitive global market. The technique was originally developed in France but is now used throughout the world including South Africa.

This report has provided details on the practical application of micro-oxygenation and evaluates the available scientific literature on the basic science underlying the technique as well as studies which have attempted to evaluate the impact of micro-oxygenation on wine quality, structure and flavour.

There is reasonable evidence that micro-oxygenation produces enhanced colour and colour stability in current applications. Although micro-oxygenation does produce changes in tannin structure, the actual impact on astringency and mouthfeel are less certain. At present there is little support for the claim that micro-oxygenation reduces green herbaceous flavours in wine. The impact of micro-oxygenation on wine anti-oxidant activity require further study.

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