THE EFFECT OF COLD MACERATION WITH AND WITHOUT SULPHUR DIOXIDE ON PINOT NOIR WINE

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The effects of varying levels of sulphur dioxide (SO_2) on the cold maceration process was investigated with Pinot noir (*Vitis vinifera* L.) wine. The effects of these varying levels on the wines composition and colour parameters were examined.

Cold maceration is a technique whereby grapes are crushed and placed at low temperatures (4 - 10^0C) in the presence 50 - 150 mgL^{-1} SO_2. This process is believed to provide a medium for the extraction of water soluble phenolic compounds, rather than the alcoholic extraction employed in normal fermentations. The extraction of these phenolic compounds was monitored from the juice through to six months of bottle age. The changes were measured using both Spectrophotometric and High Performance Liquid Chromatographic (HPLC) procedures.

Cold maceration wines were found to be not significantly different to the control wine in all compositional parameters other than titrateable acidity which was found to be less than the control for all the cold maceration wines. The unsulphured cold maceration wine was not significantly different from the control wine in any of the spectral measurements except natural degree of ionisation, in which it was higher, and total phenolics, in which it was lower. These results indicate that the cold maceration process alone does not alter the extraction of phenolic compounds. The HPLC analysis of the wine confirmed the spectral results indicating that their were no significant differences in the levels of extraction of anthocyanins.

The sulphured cold maceration wines were significantly greater than the control in visible colour, colour density, total anthocyanins, natural degree of ionisation, ionised anthocyanins and total phenolics. These results followed similar patterns with wine ageing, at six months these wines were still significantly greater in all the measurements apart from natural degree of ionisation. The results for the sulphured cold maceration wines indicates that SO_2 is acting as a solvent for the extraction of phenolic compounds including anthocyanins.

The 50 mgL^{-1} SO_2 cold maceration wine had similar colour and phenolic content to the 100 mgL^{-1} SO_2 cold maceration wine at bottling, at six months the 50 mgL^{-1} SO_2 cold maceration wine still retained a similar colour to the 100 mgL^{-1} SO_2 cold maceration wine but had vastly reduced anthocyanin content. This indicates that for the grapes utilised in this study the most appropriate level of addition at cold maceration would be 50 mgL^{-1} of SO_2. With grapes of differing phenolic content the level of addition required will vary.

KEYWORDS: Pinot noir wine; cold maceration; phenolic; anthocyanin; colour; sulphur dioxide.

If Claret is the queen of natural wines, Burgundy is the King.....

George Saintsbury, 1920

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Chapter One

Introduction

Interest in the variety Pinot noir is high in New Zealand, it is widely believed that New Zealand has the potential to produce varietal wines of international distinction from Pinot noir grapes. Pinot noir is generally recognised as a difficult variety from which to make good quality wine (Smart 1992). Research has therefore been concentrated on this variety due to the sporadic nature of the quality of wine produced. This is a problem which occurs in all new world wine regions where high quality Pinot noir is produced. There is considerable industry interest in better defining/understanding winemaking practices on wine quality.

Recent research conducted at Lincoln university has studied the effects of different pre-fermentation maceration techniques on Pinot noir wine. The research concentrated on two main pre-fermentation maceration methods these were cold maceration and carbonic maceration. The latter method is a time honoured method and has been researched extensively. The former method is a method which has undergone a recently revival in France, as such little research has been undertaken. Previous research (Goldsworthy 1993) demonstrated that cold maceration could result in increased extraction of total anthocyanins and total phenolics. It did not identify whether this was due to sulphur dioxide acting as a solvent for the extraction or as a result of the maceration process, it also did not quantify either the pigment or phenolic components.

Cold maceration is used in winemaking to increase the non-alcoholic extraction of phenolics and aroma/flavour compounds (Goldsworthy 1993). The technique involves holding crushed grapes at low temperatures (~10 ^{0}C) for periods of 7-10 days in a highly sulphured environment (50-150 ppm) (Norman 1992). Concluding this time the grapes are brought up to temperature and inoculated for primary fermentation. This technique alters the sensory profile of the wine with the main aroma attributes being concentrated fruit flavours of plum, blackberry and kirsch with full soft tannins. It also alters the

colour of the resultant wine with wines displaying deeper colours and less browning (Goldsworthy 1993).

This research concentrates on the effects of cold maceration with and without sulphur dioxide on the anthocyanins and phenolics. These affect the colour, colour stability, and taste of the wine.

The sulphur dioxide concentration has important implications on the final colour of the wine, as well as the wines other sensory characteristics. It is known that sulphur dioxide binds with the monomeric anthocyanin pigments forming a colourless bisulphite addition compound, it is not known whether this occurs during the cold maceration process to aid the extraction or the extent of extraction of monomeric anthocyanins which results from the cold maceration without SO_2.

The analysis of the anthocyanins using High Performance Liquid Chromatography (HPLC) is now a fairly routine procedure, this procedure provides information on the anthocyanin profile of any given wine. HPLC is to be utilised in this study to provide information on the pigment profile of the different wines and also amounts of each individual pigment present.

These measurements give an excellent indication of whats happening to the actual pigments but don't provide any information on the colour of the wine. A range of measurements describing wine colour and phenolic content have been determined utilising a spectrophotometer (Somers & Evans 1977).

Another important aspect in a wines colour development is the rate of ageing reactions. A wine is initially constructed from monomeric anthocyanin pigments, with time these condense with a variety of compounds, to produce coloured polymeric pigments. Coloured polymeric pigments are more stable than their monomeric counterparts. This research includes ageing studies to determine the effect of cold maceration on the rate of ageing of the wines colour.

Chapter Two

Review of Literature

2.1 Phenolic compounds in grapes

All phenolic compounds found in Vitis Vinifera are present in red grapes. The amounts which are present vary for individual grape cultivars and also for environmental and seasonal influences (Somers & Vérette 1988). Anthocyanins and procyanidins (tannins) are the most significant phenolics in red wine. The interactions between these two classes of phenolics account for a large part of the colour and flavour in red wine (Ribéreau-Gayon & Glories 1986). Genetic factors account for the presence or absence of anthocyanins and for the 3-monoglucoside character of anthocyanins in red cultivars of *V. Vinifera*. Other *Vitis* species, which are generally unsuitable for the production of fine wines contain the 3,5-diglucoside of the same anthocyanidins (Somers & Vérette 1988). All of the flavonoid components are located in the skins, seeds and vascular tissue, while the smaller non-flavonoid phenolics are also present in the juice vacuoles (Somers & Vérette 1988).

2.1.1 Non Flavonoids

In red wines the non-flavonoids are of less importance than in white wines. The non-flavonoids are generally found in the pulp of the berry, resulting in high levels in white wines where no maceration occurs (Singleton & Esau 1969). Non-flavonoids may also be derived from other sources such as production from yeast and contact with oak. The ethyl and vinyl phenol's may be produced by bacterial action on coumaric acid phenols (Amerine & Ough 1980).

2.1.1.1 Hydroxybenzoic Acids

Hydroxybenzoic acids are structurally derived from benzoic acid, variations in structure are the result of different hydroxylation and methylation patterns on the aromatic ring (Macheix et al 1990). Red wines contain four different hydroxybenzoates that are derived from grapes;

1. *p*-hydroxybenzoic acid
2. Vanillic acid
3. Syringic acid
4. Gallic acid

The first of the hydroxybenzoates above are degradation products of lignin, which are released upon acid hydrolysis (Macheix et al 1990). Red wines which are matured in contact with oak may derive further hydroxybenzoates, the derived amount being dependent upon the treatment which the oak received during seasoning and cooperage (Singleton 1972). A large proportion of hydroxybenzoates found in red wines are present in bound forms, constituting structures such as hydrolysable tannins (Macheix et al 1990). The degradation of these structures results in the appearance of gallic acid and its dimer ellagic acid. Hydroxybenzoates are important in the sensory properties of a wine due to their contribution to hydrolysable tannin.

2.1.1.2 Hydroxycinnamic Acids

In grapes there exists only three derivatives of cinnamic acid these being;

1. *p*-Coumaric acid
2. Caffeic acid
3. Ferulic acid

The hydroxycinnamates do not exist in nature in their free forms, occurring only as esters with tartaric acid (Macheix et al 1990). The winemaking process can lead to high levels of free hydroxycinnamates, this occurs through acid hydrolysis of the tartaric esters, and leads to formation of ethyl esters (Somers & Vérette 1988). The hydroxycinnamates originate in the flesh of the berry and as such constitute most of the phenol content in wines which are made with minimal skin contact. In red wines their importance is decreased due to the extraction of other phenolic compounds from the

skins and seeds. Hydroxycinnamates are important in wines as the main substrate for oxidative browning reactions (Somers & Vérette 1988).

2.1.2 Flavonoids

These are the more important of the two classes of phenolics for red wines. Flavonoids contribute to the colour and flavour of red wines but not the aroma. They are found in the skins, seeds and vascular tissue of the berry. The flavonoids are all based on a C_{15} skeleton (Macheix et al 1990).

2.1.2.1 Flavan-3-ols

This class of phenolic compounds contains two of the most important phenols found in red wines; (+)-catechin and (-)-epicatechin. These compounds are based upon a 5,7,3',4'-tetrahydroxyflavan-3-ol unit (Singleton and Esau 1969). Other flavan-3-ols do exist, such as (+)-gallocatechin, (-)-epigallocatechin, and the 3-gallate esters of epicatechin and epigallocatechin, however these additional compounds are not found in appreciable amounts in wines (Singleton 1988). In the base structure carbon's 2 and 3 are asymmetric, so for catechin and epicatechin there are four structural stereoisomers. Unfortunately the isomers can crystallise in more than one form making identification by crystalline means difficult (Singleton & Esau 1969). Of the four stereoisomers two dominate (+)-catechin and (-)-epicatechin. The polymeric forms of (+)-catechin and (-)-epicatechin constitute the procyanidin class of phenolic compounds (cf section 2.1.2.3).

2.1.2.2 Anthocyanins

The anthocyanins have long been of interest due to their role in the colouration of natural products. The colours they are capable of range from red to purple. The variation in their colouration is due to the extent of hydroxylation, methylation and glycosylation of the base structure (Lee & Hong 1992). The base structure for the anthocyanins is the 3,5,7,3´ -tetrahydroxyflavylium cation. The degree of hydroxylation and methylation which occurs on the flavylium cation determines the type of anthocyanin (Goodwin &

Mercer 1983), these also influence the colouration, with increasing methylation the colour of the molecule moves towards red, while with increasing hydroxylation the colour moves towards blue. The glycosidation of the flavylium cation occurs at the 3, 5, and 7 positions (Strack & Wray 1989). The non-glycosidated molecule (aglycone) is referred to as an anthocyanidin, these occur very rarely in nature; however they are often measured as they are common artefacts of isolation processes (Strack & Wray 1989). Glycosidation of the flavylium cation confers solubility and stability to the pigment (Brouillard 1982). Monosaccharides are the most common sugars for the glycosidated derivatives, of these the most frequently occurring are glucose, galactose, arabinose and rhamnose (Macheix et al 1990). Wines produced from *V. vinifera* grapes have only the 3-monoglucosides, while wines produced from hybrids and non-classical varieties may also contain the 3,5-diglucosides (Singleton 1988). Most of the anthocyanins which are present in grapes and wine are also partially acylated, there are exceptions to this the most notable being Pinot noir. The acylation occurs on the 6-hydroxyl of the 3-linked glucose (Singleton 1988), the phenolic hydroxyl groups are not involved in the esterification (Ribéreau-Gayon 1972). Compounds responsible for the acylation include p-coumaric, caffeic, ferulic or sinapic acids and sometimes p-hydroxybenzoic, malonic or acetic acids (Lee & Hong 1992).

2.1.2.3 Tannins

There are two different types of tannin found in wine

1. Flavonoid
2. Non-flavonoid

Detailed analysis with chromatographic procedures generally cannot identify the structures of the more complex tannin compounds, while considerable difficulty is encountered even measuring the simplest structures.

Flavonoid

This class is more commonly termed procyanidin or condensed tannin. The term procyanidin is used because on heating with acid these polymeric compounds hydrolyse to cyanidin (Lea et al 1979). These procyanidin units are made from repeating units of

(+)-catechin and (-)-epicatechin joined together by acetaldehyde. The simplest of procyanidins are dimeric in nature, with the most common being the 4 → 8 linked. These are procyanidins B1, B2, B3, and B4 with lower concentrations of the corresponding 4 → 6 linked isomers (Porter 1989). Higher oligomers are formed through successive addition of further flavan-3-ol units (Porter 1989). In the grape berry procyanidins occur up to tetramers (n = 1 - 3) while in wine they may exist up to octomers (n = 8) or even higher.

Non-Flavonoid

These are more commonly referred to as either gallotannins or hydrolysable tannins, these are constructed from either gallic acid or its dimer ellagic acid. The simplest of these being an ester of gallic acid and glucose (Porter 1989). Gallotannins increase there degree of condensation by self-esterification between gallic acid units (Porter 1989). If subjected to acid hydrolysis the gallotannins will degrade to produce gallic acid, ellagic acid and glucose.

2.2 Extraction of phenolic compounds

2.2.1 Extraction of phenolics from seeds.

A large proportion of phenolic compounds found in wines originate in the seeds. The main phenolic compounds in seeds are gallic acid, (+)-catechin, (-)-epicatechin, and the procyanidins B_1, B_2, B_3, and B_4. The extraction of phenolics from the seeds is the result of two phenomena which follow one another during maceration:

1. A dissolution of each phenolic compound at a cellular level in the seeds
2. The diffusion of each phenolic compound into the must/wine.

(Oszmianski et al 1986). The main influences in the extraction are temperature, SO_2 levels, ethanol concentration and duration of maceration (Oszmianski et al 1986). The individual effects of either alcohol or SO_2 on extraction are always small, when combined however the diffusion of phenolics increases. The diffusion of phenolics

increases when the temperature is raised to 35^0C, however if the temperature is increased to around 70^0C there is no effect on the extraction (Oszmianski et al 1986). Another effect which the increased temperatures can induce is an increase in gallic acid levels as a result of the degradation of gallotannins. The greatest extraction occurs when the maceration conditions incorporate alcohol, SO_2, and temperature (~ 35^0C) (Oszmianski et al 1986). A high level of extraction of phenolics from seeds is generally undesirable as these often constitute the most bitter and astringent of tannins. Kovac and Co-workers (1992) found that with addition of seeds, the number of brown polymers increased, while red monomers and polymers decreased (Kovac et al 1992). Anthocyanin levels also increased, this is thought to be due to the increased content of catechins and procyanidins which are available for co-pigmentation (Kovac et al 1992). The level of extraction of phenolics from the seeds can be controlled at the crusher, by adjustment of the rollers in the crusher to increase or decrease the percentage of whole berries. The extraction of phenolics from the seeds decreases with increasing content of whole berries, at the same time fermentation time is increased as is the fruit character of the wine (Zoecklein 1991).

2.2.2 Extraction of phenolics from stems

Another source of phenolics in wine are the stems. The additions of stems can increase the concentration of both catechins and proanthocyanidins in wines, while not altering the wines colour measures (Kovac et al 1992). The stem tannins are of a different structure to those found in the skins. They have been found to be "rougher" and more astringent than skin tannins (Riberau-Gayon & Glories 1986). Despite these facts mature stems are still sometimes added to Pinot noir (Drouhin 1991). As a result of the increased phenolic content colour stability is increased. If a high percentage of stems are added then they can contribute a spicy character, however if the stems are torn or damaged ie during crushing/destemming then they can add stemmy notes to the aroma as well as increasing the bitterness of the wine (Zoecklein 1991).

2.2.3 Conditions affecting Maceration

The extraction of phenolic compounds from red grapes occurs during both the maceration and fermentation of the grapes. Various factors can alter the amount of extraction, these include length of maceration, temperature, SO_2 levels, and pH of the grapes.

2.2.3.1 Cold maceration

This winemaking technique has a long history in Burgundy (France) with Henri Jayer reportedly using the technique regularly from 1976 (Matthews 1990b). It is only in recent history that the technique has become more commonly used both within Burgundy and in New World areas such as Oregon, California and New Zealand. In Burgundy the technique has been revived by Guy Accad, an oenological consultant who has implemented the technique in a range of wineries (Matthews 1990a). In Oregon the technique is often referred to as "Cold Soaking" (Heald & Heald 1993). Cold maceration involves holding partially destemmed (50 - 75 %) grapes for a period of 5 - 10 days at a temperature of 8 - 15 ^{0}C, with levels of sulphur dioxide between 100 - 150 mgL^{-1} (Norman 1992). The main aim of this pre-fermentation maceration is to extract colour and aroma before alcohol begins to act as the solvent of extraction. It is believed by Accad that alcohol extraction is unselective and leaches out undesirable compounds (Norman 1992). In line with these beliefs fermentation temperatures are kept to below 30 ^{0}C, and the wine is pressed off skins at the conclusion of fermentation (Heald & Heald 1993, Matthews 1990b, Norman 1992)). In cold maceration the cold temperatures are employed to inhibit fermentation, while the high levels of SO_2 both help to inhibit fermentation and also as a solvent to augment extraction of phenolic compounds (Norman 1992). Altering the temperature of maceration from 4 - 10 ^{0}C has been found to provide no significant effects on either extraction of phenolics or the sensory profile of the wine (Goldsworthy 1993). Wines made using this technique are marked by deep opaque colours, aromas of plums and kirsch and rich, concentrated fruit flavours braced by full soft tannins (Matthews 1990b). Critics of the wines made using the technique say that although the wines have more structure they have less finesse and elegance (Drouhin 1991, Matthews 1990b)

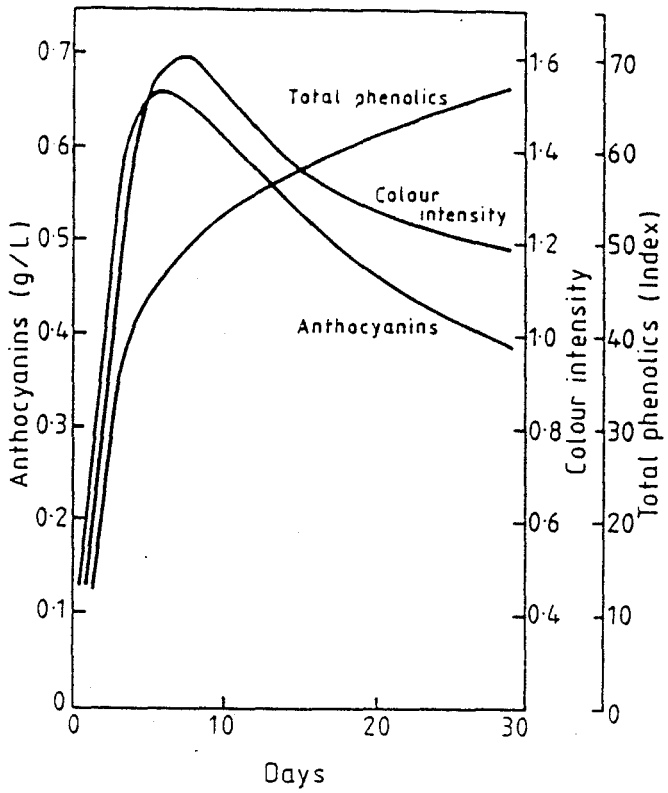

Figure 2.1 Effect of maceration on the evolution of anthocyanins, colour intensity and total phenolics (Ribéreau-Gayon & Glories 1986)

2.2.3.2 Skin contact time

All coloured material ie anthocyanins are located in the skin as a result of this if the juice is pressed off the skins without maceration then the juice doesn't contain any colour . The basis of this is used in sparkling wine production with the cultivar Pinot noir. Red wines therefore require a degree of skin contact to obtain their colour. The most important aspect is the length of time that the must is in contact with the skins. Maximum anthocyanin content is reached at approximately halfway (3 - 4 days, fig. 2.1) through fermentation (Berg & Akiyoshi 1956). This is due to the increased extraction of anthocyanins with increasing ethanol concentration.

Phenolic extraction increases with increasing contact time (fig 2.1). An example of this is the tannin content of the wine which is directly proportional to the length of the contact time (Steans 1987).

Studies (Bissell et al 1989, Somers and Evans 1986) have shown that colour stability is also directly related to the length of maceration. Singleton and Trousdale (1992) when investigating the interaction between anthocyanins and tannins found that anthocyanins combine with condensed tannins to increase the amount of polymeric phenol's retained in the wine. Therefore the longer the wine is retained on the skins the greater the amount of tannin present for the anthocyanins to bind with, leading to wines with better colour stability (Singleton & Trousdale 1992).

Decline in Anthocyanin concentration.

If the maceration period is longer than the three or four days required for maximum anthocyanin concentration then the amount of anthocyanins drops over the following days. A number of proposals have been forwarded explaining the decline.

1. Anthocyanin equilibria, changes in the equilibrium between coloured and non-coloured forms due to both pH shifts and SO_2 additions.
2. The fixation of extracted anthocyanins onto the solids in the ferment, which are subsequently racked off (Ribéreau-Gayon 1982).
3. Polymerisation of anthocyanins with tannins to small non-coloured polymers which may or may not be later oxidised back to coloured polymers (Bakker et al 1986)
4. Co-pigmentation, Self-association

2.2.3.3 Temperature

The temperature of maceration vastly influences the extraction of phenolics from the skins. A linear increase in Pinot noir colour as measured at 520 nm is seen with fermentation temperature increasing from 15^0C to 33^0C (Bissell et al 1989). At temperatures of around 60-70^0C (thermovinification), most of the available anthocyanins can be extracted in 15-30 minutes (Bissell et al 1989). One of the side effects of this extremely fast extraction is that it leads to extremely poor colour stability, as the tannins required for achieving good colour stability are not extracted during this

short period (Singleton & Esau 1969). Another possibility for the poor colour stability is that the commercial pectinases which are added after thermovinification have a glycosidase side activity, this cleaves the glycosides from the anthocyanin rendering it unstable. Another technique to extract colour is to increase the temperature of the must once fermentation is complete, or nearing completion. By increasing the temperature to ~ 40^0C increased extraction is effected but without infringing upon the colour stability. This must be done at the conclusion of fermentation as yeast strains won't ferment at this temperature (Reeves 1988).

2.2.3.4 Sulphur Dioxide Extraction

The addition of sulphur dioxide during red winemaking is generally shunned due to the negative effects that it can have upon pigment equilibrium (Section 2.3.1). The addition of SO_2 at the beginning of maceration can increase the extraction of anthocyanins and phenolic compounds (Dallas & Laureano 1994). The extraction using SO_2 is quite specific with some anthocyanins more susceptible than other to this form of extraction. The addition may also slow the formation of polymeric pigments during the wines maturation (Dallas & Laureano 1994). Some care needs to be exercised over the level of addition as excess sulphur can alter the sensory profile of the wine, and also possibly inhibit molalactic fermentation.

2.2.3.5 Solid to liquid ratio

The influence of the ratio of liquid phase to solid phase during maceration has been reported in France as modifying the quality of Pinot noir wines, extraction of juice varying from 55% in a dry year through to 80% in a wet year (Seigrist & Legisle 1982). Experiments conducted at Beaune in France using sensory panels found that the best wine, with substantial ageing potential was made with a juice proportion of 60%. Wines however that were made with juice proportions of 65% - 72% (which corresponds to "normal" vintages) were found to be of good quality and wines made above this (72%) level were found to be of poorer quality (Siegrist & Leglise 1982). One way suggested of adjusting this ratio is to remove juice prior to fermentation, thus obtaining the most advantageous ratio (Dupuy 1984).

2.2.3.6 Pressing Regime

The wine can be pressed off the skins at any stage during the skin contact period, generally there are three times when this is done.

1. Prior to dryness
2. At dryness
3. At some point past dryness

These all lead to characteristic wines. If the wine is pressed off before dryness it tends to have good initial colour, little astringency, low total phenol's and a floral aroma. They are also light in body, complexity and (Zoecklein 1991). It is also sometimes practised as a blending option for Cabernet Sauvignon producers who need to back blend a high "fruit" content into their wine (Zoecklein 1991).

Wines are pressed off at dryness for two reasons. The first being that at the completion of fermentation the wine still possesses some dissolved CO_2 which will protect the wine from oxidation during the pressing cycle. The second reason is that some wines do not benefit from an extended maceration period, this is especially true of reds with a touch of unripeness or "greenness", this characteristic intensifies with extended maceration (Zoecklein 1991).

The last option involves an unspecified length of post fermentation maceration, this is the most common option. Extended maceration creates more body, complexity, depth of colour and colour stability (Zoecklein 1991). It also increases the ageing potential of the wine. Maceration length can be extended by gassing the headspace with CO_2. The length of maceration is governed by numerous factors, two of the most important are fruit maturity and development of the wine (Zoecklein 1991)

2.2.3.7 Cap Management

The management of the cap is singularly important for both colour and phenol extraction as most of the skins and stems reside in the cap. There are a variety of cap management techniques used these include

- Submerged cap & autofermentation
- Rototanks

- Pumping over
- Plunging

Submerged Cap

A submerged cap is when the cap is held under the fermenting must by header boards, later versions included a riser tube so that warm must could circulate from the bottom of the tank to the top. It enabled a slow extraction of phenolics from the must. An autofermenter is a device which automatically performs this function by irrigating the skins at regular intervals. The process allows rapid extraction of colour. It is actually an automated form of the submerged cap technique. The most widely distributed of these is the Ducellier Autofermenter (Rankine 1989).

Rototanks

These consist of a horizontal cylinder which can roll in either direction. Inside the tank is a spiral flange which moves the skins. This system also enables rapid extraction of maximum colour, The must is distributed in a thin layer around the circumference of the tank, this leads to high levels of extraction (up to 30% more) in short periods (Zoecklein 1991). An advantage of the system is that it draws off the pomace without manual manipulation (DuPay 1984).

Pumping over

This technique involves the pumping of wine from the bottom of the vat and spraying it over the top of the cap (Rankine 1984). The main flaw with pumping over is that of uneven leaching of the skins, due to the fact that the juice flows through fissures in the cap leaving a high proportion of skins untouched. Care must therefore be taken when pumping over to rinse the entire cap in an even manner. A modification to the traditional technique involves the use of a sprinkler or splash plate to ensure the even irrigation of the entire cap.

Plunging

This final technique is used most frequently due to the gentle and thorough nature of the extraction. It involves the punching down at regular intervals of approximately 6 hours,

of the cap and thereby mixing the cap into the wine. This can be done either by hand or mechanically.

The general trend for all cap management techniques is towards gentler extraction of the phenol's. The main problem with the techniques of autofermentation and rotovinification is the shortened ageing potential of wines produced using these techniques, due to the reduced period of maceration achieved with these two techniques. The move towards the most gentle extraction techniques has seen a move towards using a mixture of pumping over and punching down, this helps to reduce the extraction of harsh, bitter and aggressive tannins (Zoecklein 1991).

2.3 Evolution of Red wine Colour

Red wine undergoes two distinct phases of ageing the first of these occurs while the wine is ageing after fermentation in either oak barrels or stainless steel tanks. This is generally referred to as the maturation stage. The second stage begins once the wine is bottled and is in a reductive environment, often referred to as the conservation stage. Most of the evolution that occurs in a wines colour occurs within the first year or two after vinification (Somers and Vérette 1988). Most of the initial colour in a wine is due to monomeric anthocyanins, during wine ageing these monomers are gradually displaced by more stable, darker pigments. At the conclusion of fermentation there is already as much as 35% polymeric pigment in the wine (Bakker et al 1986). There is a gradual transition from monomeric anthocyanins through oligomers to polymeric pigment. This transition is accompanied by increasing resistance to bleaching by both pH change and SO_2 concentration (Bakker et al 1986).

2.3.1 Pigment Equilibria

The anthocyanins as mentioned earlier are the coloured pigments in wine. A number of factors effect both the total concentration of anthocyanins and also the amount of anthocyanins which are important for the colour of the wine. The pH of the wine and the level of free SO_2 are the main factors altering the pigment equilibria and hence the

number of anthocyanins found in coloured form. The weakly acidic nature of wines results in instability of the flavylium cation, the intense colouration of wine is therefore due to stabilisation of anthocyanins. The two effects which play important roles in this stabilisation are self-association and co-pigmentation.

2.3.1.1 pH

Anthocyanins exist in an equilibrium which is pH dependent.

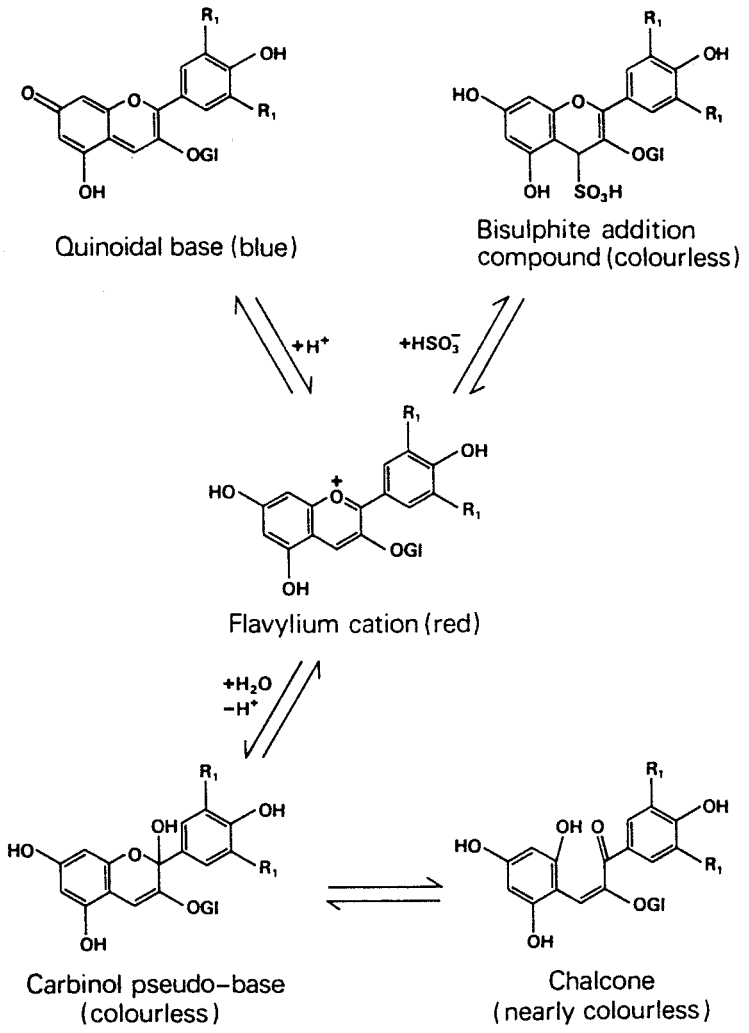

Figure 2.2 Anthocyanin equilibrium; effects of changes in SO_2 and pH (from Somers and Vérette 1988)

As can be seen in the diagram the only coloured anthocyanin is the flavylium cation (fig 2.2). There are five anthocyanin monoglucosides which occur in wine, these are delphinidin-3-monoglucoside, petunidin-3-monoglucoside, peonidin-3-monoglucoside, cyanidin-3-monoglucoside and malvidin-3-monoglucoside. Of these the most important

is malvidin as it can account for as much as 90% of the monomeric pigment in (appendix 4.10). Malvidin has a pKa of around 4 (Macheix et al 1990), this means that at this pH half of the anthocyanins are red and the other half are colourless. Anthocyanins exhibit intense red colouration at pH's of between 1 and 3, this corresponds to the pH range where the flavylium cation is dominant (Macheix et al 1990). By increasing the pH structural transformations occur which result in either colour loss or the formation of undesirable colour. Therefore by increasing the pH to between 4 and 6.5 the flavylium cation is converted to a quinoidal base which is blue, this slowly hydrates to the carbinol base giving rise to progressive decrease in colour (Macheix et al 1990). The result of lowering the pH on wine colour is to increase both the wine colour density and hue. The hue increases as a result of increasing red pigments while the density increases as a result of increasing absorption at 520 nm (Iland & Bruer 1986).

2.3.1.2 Effect of SO_2

When SO_2 is added to a finished red wine it reacts with the red flavylium cation converting it to a colourless bisulphite addition compound (Iland & Bruer 1986), this is illustrated in figure 4. The bleaching effect of free SO_2 on anthocyanins is moderated in wine by the presence of other SO_2-binding compounds, viz. acetaldehyde, pyruvic acid, α-keto glutaric acid, sugars, with all of which free SO_2 is also in equilibrium (Somers & Vérette 1988). SO_2 itself exists in an equilibrium in wine and of which only the bisulphite ion condenses with anthocyanins. In increasingly acidic solution the concentration of bisulphite ion decreases as it is converted to the undissociated acid. So even though colour disappears on addition of SO_2 the condensation reaction is reversible and with time colour will slowly reappear as SO_2 levels decrease (Ribéreau-Gayon 1974). The addition of SO_2 to achieve a level of 20 mgL^{-1} free SO_2 results in a decrease in wine colour density of 17% (Iland & Bruer 1986). Minimum usage of SO_2 is now recommended throughout vinification due to its perceived negative effects on both wine colour and sensory profile. If SO_2 is added prior to fermentation then the SO_2 will bind with acetaldehyde, this bound acetaldehyde may later be utilised by malolactic bacteria thereby releasing SO_2 into the wine (Iland & Bruer 1986). Another source of SO_2 during fermentation is yeast, certain yeast strains actually produce significant amounts of SO_2

SO_2 on wine colour occurs when the wine is young with high levels of both free SO_2 and monomeric pigments.

Both of the above effects have less effect on wine colour as the wine ages and is increasingly composed of polymeric pigment. In wine the effect of sulphur dioxide on colour is much more important than the effect of changing pH (Iland & Bruer 1986).

2.3.1.3 Self-Association

The mechanism of self-association was first described by Asen et al in 1972. They based there assumption on the fact that at a pH of 3.16 the absorbance of cyanidin-3,5-

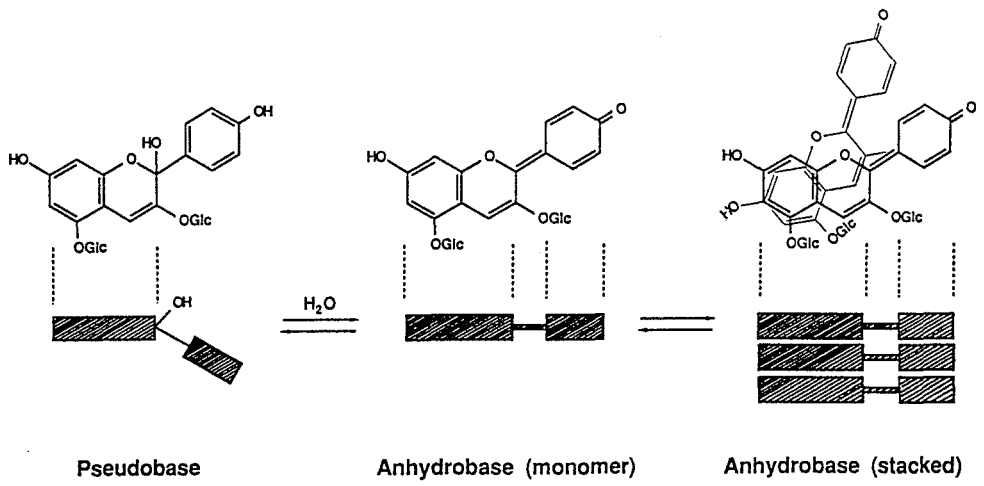

Figure 2.3 Vertical stacking of anthocyanins
(from Goto & Kondo 1991)

diglucoside at λ_{max} increased 300 times while the concentration only increased 100 times (10^{-4} to 10^{-2}). This is a large deviation from Beer's Law and indicated that the flavylium cation was undergoing self-association (Asen et al 1972). Initially there was some discussion as to whether the molecules were stacked horizontally or vertically. Work done by Hoshino, Matsumoto, and Goto in the early 1980's using circular dichroism (CD) and ^1H NMR showed that the molecules stacked vertically with either a left or right handed screw depending upon anthocyanin (fig 2.3, Goto & Kondo 1991). The vertical stacking employs hydrophobic interactions to overcome the anthocyanins electrostatic

electrostatic repulsions. The anthocyanins form the hydrophobic core which is surrounded by hydrophilic glucose moieties (Hoshino 1992). This structure prevents the hydration of the anthocyanidins, inhibiting subsequent ionisation and resultant loss of colour (Hoshino 1992). As the concentration of anthocyanin increases the magnitude of the absorbance also increases, this indicates that with increasing concentration there is enhanced formation of stacked molecules (Goto & Kondo 1991). The colour intensification occurs as a result of a hypsochromic shift of the λ_{max}, this means that the λ_{max} moves to a shorter wavelength and as a results a more purple colour. This hypsochromic shift is a direct result of self-association. At wine pH the flavylium ion is in equilibrium with the quinoidal bases (fig. 2.2), self-association suppresses the formation of these bases due to the structure protecting the anthocyanin from hydration, thus giving rise to a hypsochromic shift (Goto & Kondo 1991). An increase in temperature dissociates the anthocyanins, which initiates destacking of the molecule. This results in a reduction of the wines colour measures as a result of ionisation of the anthocyanins (Hoshino 1992).

2.3.1.4 Co-Pigmentation

This phenomenon involves either bonding between anthocyanins or the formation of complexes between anthocyanins and other colourless phenolic molecules which play the role of copigment (Macheix et al 1990). The copigmentation depends on the type and concentration of the anthocyanin, on the type and concentration of the flavone, and on the pH and temperature of the solution (Goto & Kondo 1991). Anthocyanins which contain an aromatic acyl group form much stabler copigmentation complexes with flavones than unacylated anthocyanins. A copigmentation is possible with both the anhydrobase as well as the flavylium cation, these both proceed non-stoichiometrically and form a bluish-purple colour (Goto & Kondo 1991). Copigmentation forms vertical stacks similar to those found in self association, this time however these consist of hydrophobic stacking between the aromatic nuclei of the anthocyanin and the flavone. The stacking can be further stabilised by hydrogen bonding of the hydrophilic sugar moieties (Goto & Kondo 1991). Copigmentation as with self-association has an associated wavelength shift, this time bathochromic, this is a shift in λ_{max} towards longer wavelengths. As mentioned above various factors effect the copigmentation

reactions. One of these factors is pH, maximal colour enhancement of cyanin upon addition of chlorogenic acid occurs at a pH of 3.6 (Mazza & Brouillard 1990). Another factor is the concentration of pigment and copigment, as with self-association increasing concentrations of pigment result in increased molecular stacking. This however is dependent upon the pigment structure, for instance malvin shows more increase in copigmentation with increasing concentration than cyanin does. The same results are also noted for increasing concentration of copigment (Mazza & Brouillard 1990). The colouration from copigmentation is also strongly effected by ethanol concentration, increasing ethanol concentration strongly effects the degree of copigmentation (Mazza & Brouillard 1990). Using the vertical stacking model for copigmentation the main forces which bind the complex together are hydrophobic, in the absence of water copigmentation doesn't occur. As the ethanol concentration increases and water concentration decreases the copigments dissociate resulting in a decrease in colour intensity (Mazza & Brouillard 1992).

2.3.2 Tannins

Tannins are the result of the condensation and polymerisation of procyanidin. The condensation and polymerisation dependent upon conditions gives rise to various products. If there is air available then oxidative condensation of procyanidin occurs, tannins of a pale yellow colour with maximal astringency are produced (Ribéreau-Gayon & Glories 1986). If the conditions are anaerobic then non-oxidative condensation occurs producing condensed tannins of a yellow-red colour, with diminished astringency. These condensed tannins may undergo further polymerisation resulting in highly condensed tannins, which may become sufficiently large to precipitate (Ribéreau-Gayon & Glories 1986). These highly condensed tannins also display decreased astringency. A particularly important condensation reaction involves the condensation of tannins and anthocyanins to produce coloured condensation products. These coloured condensation products are more resistant to decolourisation by both pH shifts and SO_2 additions. At the same pH value the coloured condensation products have a greater proportion of molecules in the coloured form than do monomeric anthocyanins, the condensation products also have less molecules in uncoloured forms (Ribéreau-Gayon & Glories 1986). While this additional colouring is important, equally important is the

increased stability of the colour of these anthocyanin-tannin condensations. Other reactions which procyanidins may undergo are condensations with proteins and polysaccharides. This reaction seems to contribute to the suppleness of the wine produced, while also inactivating the astringency of the tannin.

2.3.3 Conservation and Ageing

Polymerisation during conservation and ageing is largely uncontrolled. A diverse range of polymerisation processes are likely to occur, creating the potential to produce an enormously diverse range of polymeric compounds in ageing wine (Wilson & Allen 1994). Two mechanisms which are proposed to be responsible for changes which occur in the phenolic composition are:

1. An anaerobic mechanism, which is slow at temperatures of less than 20^0C.
2. A semi-aerobic mechanism, which also functions at temperatures of less than 20^0C (Ribéreau-Gayon & Glories 1986).

Various arguments are proposed for and against each of these mechanisms.

Anaerobic reactions are said to involve the direct condensation of anthocyanins and tannins. This requires the acid catalysed degradation of polymeric procyanidin into a reactive colourless flavenyl intermediate (Somers & Evans 1986). This produces numerous carbocation species which would be available for attack by a variety of phenolic and other nucleophillic reagents, the principal nucleophillic reaction sites on phenolic compounds are the vacant 6- and 8- positions. This form of degradation is largely randomised with the reaction occurring with all types of polymerised procyanidins. Only anthocyanins in the coloured flavylium form could contribute to the formation of coloured molecules. These molecules must after condensation be oxidised from the colourless to the coloured flavylium structure (Somers & Vérette 1988). As these molecules do not occur within the grape Somers (1971) was lead to conclude that the condensation reaction occurred very rapidly, this condensation has subsequently been found to occur at vastly reduced rates (Timberlake & Bridle 1976), and not with all pigments (Bishop & Nagel 1985). Recent work conducted by Thorngate and Singleton (1994) looking at the condensation of monoglucosidic anthocyanin with catechin and

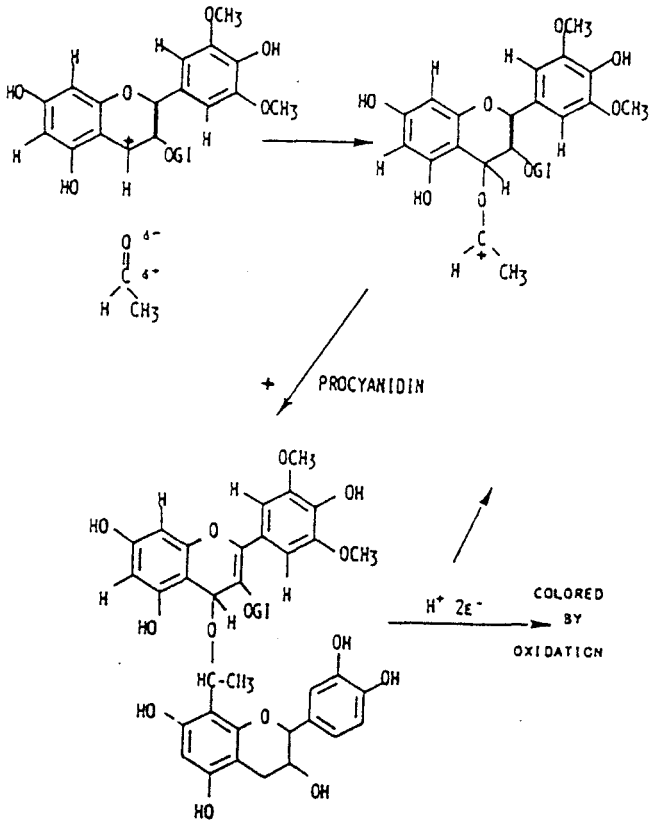

Figure 2.4 Condensation of anthocyanins and tannins, with acetaldehyde.
(from Ribéreau-Gayon & Glories 1986)

The semi-aerobic mechanism involves the condensation of anthocyanins and tannins via a Baeyer condensation reaction which involves acetaldehyde. The acetaldehyde is produced by the coupled autoxidation of ethanol and phenolic compounds. The autoxidation involves the oxidation of ortho-diphenols to quinones with the subsequent production of hydrogen peroxide, the hydrogen peroxide then oxidises ethanol to acetaldehyde (Wildenradt & Singleton 1974). The acetaldehyde is used in the Baeyer condensation reaction where it forms the bridge between two phenolic units. This reaction occurs only when oxygen is present, as without oxygen their can be no autoxidation of ethanol to acetaldehyde. Any acetaldehyde present at the conclusion of fermentation is strongly bound to SO_2. So for this reactions to occur more acetaldehyde needs to be produced during maturation and conservation, this could be the case if

fermentation is strongly bound to SO_2. So for this reactions to occur more acetaldehyde needs to be produced during maturation and conservation, this could be the case if ageing was conducted in the oxidative conditions found in barrel ageing. Even when this is considered there have still been reports of Baeyer condensations occurring in wine during reductive conservation (Somers & Evans 1986). If the procyanidins are only slightly condensed then the semi-aerobic mechanism will react these to form stable coloured pigments, if however the semi-aerobic reaction occurs with highly polymerised pigments, then unstable pigments are formed which may precipitate (Ribéreau-Gayon & Glories 1986). Anthocyanin monomers play an important part in limiting polymer size, the glucose molecule is the essential component required for the limitation. The incorporation of the glucose molecule into the polymer increases both the solubility of the polymer and decreases the reactivity of the polymer to further polymerisation (Wilson & Allen 1994). While the above mechanisms are the two main mechanisms proposed for ageing reactions other mechanisms and factors also affect both the rate of these reactions and the formation of other ageing compounds in wine. The pH of the wine exerts an influence over the speed at which these reactions occur, at lower pH's where concentration of the flavylium cation is high the reactions proceed quickly. As the pH increases reaction speed decreases, the quantity of each compound produced also alters with changing pH (Garcia-Viguera et al 1994). A factor which reduces the rate of the ageing reactions is SO_2, this also reduces the degree of polymerisation. If acetaldehyde is present these SO_2 effects no longer occur, due to strong binding between acetaldehyde and SO_2 (Picinelli et al 1994). The temperature at which these reactions occur is fundamentally important for their reaction rates (Somers & Pocock 1990). By reducing the temperature to less than 20^0C the rate of the ageing reactions is reduced, and the reactions occur slowly. An increase in the temperature therefore increases the rate of ageing and decreases the wines ageing potential (Somers & Pocock 1990).

2.4 Measurement of phenolic compounds in wine

2.4.1 Analysis by Chromatography

A large proportion of the initial chromatographic work which was conducted on the analysis of phenolics was done by Paper Chromatography. The advent of Thin Layer Chromatography allowed the use of different stationary phases, which meant new separation mechanisms could be employed. Open column chromatography was trialed next, this meet with very limited success, mainly due to the low resolving power of the technique. Phenolic compounds have also been separated using Gas Chromatography which while it has some problems allowed reasonably accurate determinations. The most common form of chromatography used today for the separation and identification of phenolic compounds is High-Performance (Pressure) Liquid Chromatography. The main advantage of HPLC over other chromatographic systems for phenolic separations is that it provides excellent qualitative and accurate quantitative analysis in one and the same operation (Harborne 1989). The identification of phenolic compounds by chromatography can sometimes be delicate since standards for complex forms (esters and glycosides) are not always available for comparison as reference standards.

2.4.1.1 High-Performance Liquid Chromatography

High performance liquid chromatography (HPLC) from liquid chromatography, early in the development of liquid chromatography it was realised that column efficiency could be vastly increased by decreasing the particle size of the packing (Skoog 1984). Modern HPLC columns have particles in the 3 - 10 μm range, these smaller particles dramatically reduce the plate height. This reduction in plate height increases the apparent number of plates in the column, by increasing the apparent number of plates the resolution increases (Skoog 1984). Some other ways in which enhanced resolution can be achieved are low viscosity solvent phase, elevated temperatures and alterations in solvent phase composition.

Analysis of phenolic compounds is conducted using partition chromatography under reversed phase (RP) conditions. Normal phase chromatography consists of a highly

polar stationary phase combined with a relatively non polar solvent system, using these conditions the least polar component is eluted first. Reverse phase chromatography consists of a non polar stationary phase and a relatively polar mobile phase, under these conditions the most polar component appears first (Skoog 1984). The stationary phase is usually bonded silica, while the mobile phase is usually constructed from water, an organic modifier and an acid. In the stationary phase the most commonly used columns are prepared from rigid silica, however polymer based columns are gaining in usage as they can cover a wider pH range. The desired column packing (C_{18}, C_8 etc) is then bonded to these base compounds (Skoog 1984). The initial analysis of phenols were carried out using isocratic systems for their elutions (Wulf & Nagel 1979), this quickly changed to binary and even ternary gradient elution systems as more complexz analysis were performed (Lamuela-Raventós & Waterhouse 1994). The solvents used usually need to be acidified to suppress ionisation of the acid group (Wulf & Nagel 1979). In general most classes of phenolics can be determined by HPLC using direct injection, in some instances however it is desirable to use some form of preliminary extraction either to remove interfering substances ie for procyanidin analysis, or to concentrate the sample.

Separation Techniques

There are two main types of separation which are employed in preparation for analysis by HPLC. The first is solid phase extraction which generally divides the phenolics into discreet groups. The second technique which is commonly used is open column chromatography, this is generally used to divide the phenolics into two separate groups - monomeric and polymeric phenolic compounds. In some instances HPLC itself has been used as a preliminary separation technique although this will not be discussed here (Escribano-Bailón et al 1992).

Solid Phase Extraction

This technique involves the use of chromatographic sorbents with different chemical selectivity's to allow the components of interest to either be retained or unretained (Millipore 1993). The most commonly used cartridges are C_{18} Sep-Pak cartridges

(Waters Assoc.) these are compression packed, disposable "mini-columns" (Millipore 1993). Salagoïty-Auguste and Bertrand (1984) used these cartridges to separate neutral and acidic phenolic compounds in red wine by changing the pH prior to extraction by ethyl acetate, unfortunately the extraction efficiency for procyanidins was low (Oszmianski et al 1988). Jaworski and Lee (1987) also used a C_{18} Sep-Pak cartridge with methanol as a solvent, the fractionation worked well under these conditions, except methanol was not exclusively selective towards the procyanidins and flavanols, with the anthocyanins also eluting (Jaworski & Lee 1987). At present with the solvents being used this separation technique is still not selective towards procyanidins and flavanols, further work is being carried out on different solvents for their extraction (Oszmianski et al 1988). This technique unfortunately is also unsuccessful at resolving the anthocyanins into a discrete group, it does however separate polymeric anthocyanins from the monomeric, although further investigation is required to identify exactly what is happening (Oszmianski et al 1988).

Open Column Chromatography

Open column chromatography can be used to isolate a specific phenol or group of phenols. Typical packing's for these columns include adsorbent's, gel filtration materials, partition supports, or ion exchange or other resins capable of separation (Singleton 1988). The number of theoretical plates provided by these packing's is generally low, resulting in incomplete separations. As such their major use is in the partial separation and concentration of samples prior to HPLC or other chromatographic techniques (Singleton 1988). The majority of preparative work for phenolics has been done using dextran based gels, the most common of which is Sephadex. Two types of Sephadex exist, both of which have been used for phenolic separations. The G series which is produced by cross-linking dextran with epichlorohydrin, has been used to attempt separations by size exclusion chromatography (Somers 1968, Cacho & Castells 1995). While Sephadex LH-20 gel which is a hydroxypropyl modified dextran, acts primarily as a hydrogen bond acceptor with phenols. It does not act by size exclusion chromatography in the molecular size range of the phenols (Singleton 1988). Somers (1968) used a Sephadex-G25 Fine column for phenolic separation, this column separated the phenols into gross fractions of monomeric nonacylated, monomeric

acylated, and polymeric pigments (Somers 1968). Somers (1968) proposed that use of gels which were more loosely cross-linked may be able to provide better separation (Somers 1968). Further studies on size exclusion have confirmed that Sephadex G gels separate phenolics into two fractions, one containing polyphenols which are larger than the pore size of the column and the other containing those which are smaller (Cacho & Castells 1991). By variation of the pore size of the gel used differing degrees of polymerisation can be detected (Cacho & Castells 1991). As mentioned above Sephadex LH-20 acts as a hydrogen bond acceptor, the relative absorption of phenolics then depends upon the number of phenolic hydrogens per molecule. Polymeric polyphenols will be more strongly adsorbed than monomeric phenols, these can be quantitatively measured by elution with an appropriate solvent (Kantz & Singleton 1990). This technique still separates the phenolics, into a polymeric fraction and a monomeric fraction. The polymeric pigments which occur in aged wine tended to have lower adsorptive properties on Sephadex LH-20 than the condensed tannins found in young wines, making separations of aged red wines more complicated (Kantz & Singleton 1990).

Non Flavonoids

The quantitatively more important members of this group such as caftaric, coutaric and gallic acids are generally determined individually using reversed phase HPLC. The hydroxycinnamates are usually determined at wavelengths near 320 nm and the benzoates near 280 nm, while gallic acid is usually determined around 265 nm (Singleton 1988). The analysis can be either by direct injection (Lamuela-Raventos & Waterhouse 1994, Achilli et al 1993), or with some form of separation (Oszmianski & Lee 1990, Oszmianski et al 1988, Jaworski & Lee 1987). The most common form of pre-separation for this class of compounds has been through the use of C_{18} Sep-Paks. A simple isocratic solvent system consisting of acetic acid and water (5:95) (Jaworski & Lee 1987) can be used for the quantitation of these simple phenolics after pre-separation. Using a direct injection method such as the one used by Lamuela-Raventos & Waterhouse (1994) a more complex solvent system is used, in this instance this was a ternary elution, with a Novapack C_{18} reverse phase column. A common binary isocratic system consists of Formic acid and water (5:95) as one solvent and acetonitrile and

methanol (5:95) as the other (Van Sumere 1989). Early work in this field used ultraviolet (UV) detectors, this has been somewhat displaced now by the photodiode array (PDA) detector. This is capable of measuring over a wide range of wavelengths simultaneously which enables easier peak identification. Various other detectors are suitable for the detection of simple phenolic acids these include electrochemical detectors (Achilli et al 1993), and detectors which employ eiother amperometric or polarographic methods (Van Sumere 1989).

Anthocyanins

As with analysis of non-flavonoid compounds the dominant HPLC method is reverse phase chromatography using a C_{18} column. Anthocyanins have maxima in the wavelength region 500 - 550 nm, no interference from other phenolic compounds is experienced in this region as none of the phenolic compounds show absorption here (Strack & Wray 1989). A low pH solvent is essential to the separation of anthocyanins, at a pH of 2.5 substantial amounts of both the flavylium cation and the colourless carbinol base are present (cf section 2.3.1.1). If separated on a chromatogram at this pH each pigment would appear as a broad peak, they are not resolved into two separate peaks because the two forms are continually interchanging. If the pH is shifted to 1.5 the flavylium cation is dominant (96%) (Somers 1971), and the separation of pigments produces sharp peaks. Recommendations for most RP-C_{18} columns, state that columns are stable down to a pH of 2, below this the octadecylsilyl groups may be hydrolysed from the silica backbone, decreasing the efficiency of the column. Despite these recommendations most separations which have been conducted on these columns at pH's less than 2 have reported minimal degradation of the column (Wulf & Nagel 1978, 1979, Lamuela-Raventós & Waterhouse 1994). The use of non-silica polymeric columns which are stable from pH 1 to 13, is growing rapidly (Hong & Wrolstad 1990, Fernandez-Lopez et al 1992, Spanos & Wrolstad 1987)). Gradient elution is the preferred solvent system for separating complex mixtures of anthocyanins which are structurally similar (Lee & Hong 1992). The organic modifiers are usually acetonitrile or methanol, the nature of the organic modifier has a dramatic influence on the nature of the separation. Better resolution and less retention time can be achieved by the addition of Butylamines in the mobile phase (Drdak et al 1990), while the addition of 15% acetic

acid in the mobile phase can also increase selectivity (Spanos & Wrolstad 1987). Detection of anthocyanins is usually carried out using PDA detectors so that structural elucidation is possible (Lee & Hong 1992). Other detectors such as FAB-MS or NMR may also be connected to give further structural information. In RP-chromatography the anthocyanins elute according to their polarity and the stereochemistry of the compound (Strack & Wray 1989), the elution order is delphinidin, cyanidin, petunidin, peonidin and malvidin.

Tannins

Most HPLC techniques which investigate the quantity of tannin present use some form of preliminary extraction to separate the polymeric compounds from the monomeric (cf seperation techniques). The polymeric compounds which constitute the majority of the tannin class can be separated using HPLC without preliminary extraction, but they in general elute as an ill defined late eluting peak (broad hump). The simpler dimeric and trimeric procyanidins have been separated by various researches (Escribano-Bailón et al 1992, Rigaud et al 1991, Kovac et al 1992), these have all used some form preliminary extraction prior to elucidation by HPLC. As of yet no researchers have managed to separate or identify polymeric compounds which incorporate anthocyanins, or the larger of the polymeric compounds. This is an area of ongoing interest, with research going into isolating and identifying these compounds.

2.4.2. Spectrophotometry

Spectrophotometric measurement of wine phenolics provides information on a wide range of phenolic compounds. In red wines spectral measurement can provide information concerning total phenolic levels as well as a wide range of colour measures including colour density and hue, anthocyanin content, chemical age indices and degrees of ionisation. For the measurement of phenolic compounds in wine absorption spectroscopy is used, this consists of five parts

1. A stable source of radiant energy
2. A transparent container for holding the sample
3. A wavelength selector

4. Photoelectric detector
5. Signal processor and readout

(Skoog 1984) Absorbance spectroscopy is governed by two basic laws, the Lambert law and Beer's law. The basis of the Lambert law is that absorbance is directly proportional to the path length through the solution and the concentration of the sample (Skoog 1984). While Beer's law states that absorbance is a linear function of concentration (Somers & Vérette 1988). In white wines and juices Beer's law is followed thus allowing dilution of the sample at similar pH to occur. In measurement of red wines Beer's law is not obeyed, this is due to both the equilibrium that pigments exist in and also to self-association and co-pigmentation effects. Beer's law is obeyed at high dilution in red wine, requiring that measurement be made on undiluted samples (Somers & Vérette 1988).

2.4.2.1 Spectral Evaluation of Red Wine

As mentioned above the spectral evaluation of red wine falls into two main categories, total phenolics and colour measurement. The measurement of phenolics in red wines by spectrophotometry is extremely complex as there are numerous factors which influence the measurement, these are described below.

Total Phenolics

The measurement of total phenolics underwent a revolution when it was discovered that the Folin-Ciocalteau measurement was overestimating the phenolic content due to the reactivity of non-phenolic compounds with the phosphomolybdate-tungstate reagent (Somers & Ziemelis 1985). Spectral measures of total phenolics take place at 280 nm, this is the λ_{max} for the main phenolic components of red wine. A background correction factor for non-phenolic absorption can be found by heavy fining using p.v.p the corrected measurement is then,

$$\text{Total Phenolics} = (E_{280} - E_{280}^{p.v.p}) \text{ a.u.}$$

(Somers & Evans 1977). Initially the correction was given by Somers & Ziemelis (1972) as 3.9 (S.D. $\pm$ 0.2, $p < 0.001$), however using the p.v.p fining on wines from the Canterbury region (N.Z.) the correction factor was found to overestimate the

background absorbance under these conditions and was consequently reduced to 3 (Steans pers. Com. 1994).

Colour Measurement

Red wine colour is an integration of contributions from monomeric anthocyanins and polymeric pigment forms (Somers & Evans 1977). The pigments found in wine all have similar visible absorbance spectra in aqueous acid solution with λ_{max} in the region 525 - 535 nm and λ_{min} at around 420 nm (Somers & Vérette 1988). It is in the measurement of the colour of red wine that Beer's law is not followed, for this reason samples must be measured undiluted. There are six main measurements which are made, these provide information for the following descriptors which attempt to estimate wine colour (Somers & Vérette 1988);

Wine Colour Density = $E_{420} + E_{520}$, this provides a descriptor of the depth of colour of the wine, originally the measurement was just E_{520} but this was found to not describe aged wines (Ribéreau-Gayon 1982). E_{420} measures the browning of the wine and inclusion of it therefore allows measurement of aged wines.

Wine Colour Hue = E_{420}/E_{520}, this describes the colour or tint of the wine, the visual equivalent of this is viewing the edge of a tilted glass of wine

Degree of ionisation of anthocyanins (α) = $\frac{E_{520} - E_{520}^{SO_2}}{E_{520}^{HCl} - \frac{5}{3}.E_{520}^{SO_2}}$ x 100 %, this attempts to measure the amount of anthocyanins which are actually coloured at a wines pH, or more specifically the amount of anthocyanins in flavylium form. The measurement includes both coloured monomeric and polymeric pigments (Somers & Vérette 1988).

Degree of ionisation of anthocyanins found after abolishing SO_2 effect on wine colour

(α') = $\frac{E_{520}^{CH_3CHO} - E_{520}^{SO_2}}{E_{520}^{HCl} - \frac{5}{3}.E_{520}^{SO_2}}$ x 100 %, the difference between this measurement and the previous one, lies in the use of $E_{520}^{CH_3CHO}$. This measurement restores colour to the SO_2-bleached pigments, the measurement of $E_{520}^{CH_3CHO}$ in young wines where a proportion of

pigment is bound to SO_2 can be much higher than E_{520}, with age as SO_2 becomes less of a factor in pigment equilibria, there is generally no difference between the two measures (Somers & Vérette 1988).

Total anthocyanins (mgL^{-1}) = $20\left(E_{520}^{HCl} - \frac{5}{3}E_{520}^{SO_2}\right)$, the E_{520}^{HCl} measurement attempts to account for all of the anthocyanins by eliminating equilibria and concentration effects (cf section 2.3.1), while $E_{520}^{SO_2}$ measures the SO_2 resistant pigments which are assumed to be polymeric in nature (Somers & Vérette 1988).

Ionised anthocyanins (mgL^{-1}) = $\frac{a}{100}$ x (Total anthocyanins)

= $20\left(E_{520} - E_{520}^{SO_2}\right)$, this provides an indication of the amount of ionised anthocyanins

Chemical age indices (i) $\frac{E_{520}^{SO_2}}{E_{520}^{CH_3CHO}}$

(ii) $\frac{E_{520}^{SO_2}}{E_{520}^{HCl}}$

(Somers & Evans 1977). The chemical age indices attempt to estimate the amount of polymerisation. The measurements and descriptors were based upon analytical assumptions, most of which have subsequently been shown to be correct. The assumption that the contribution of polymers in newly made wine is 0 - 5 % has been shown to be incorrect, at the conclusion of fermentation as much as 20 - 35 % of the pigments are already polymerised (Bakker et al 1986). The measurement at 520 nm with the addition of SO_2 ($E_{520}^{SO_2}$), requires that monomeric pigments are bleached, while the polymeric pigments which have greater insensitivity to bleaching, are not bleached providing the residual colour which is measured (Somers & Evans 1977). The high nature of the polymeric pigment even at this early stage leads to overestimation of all the colour descriptors which $E_{520}^{SO_2}$ is involved with. This has been shown to be the case in investigations in which these spectral measures are compared against data obtained from HPLC analysis of the pigments (Bakker et al 1986, Rivas-Gonzalo et al 1992).

Chapter Three

Materials and Methods

3.1 Winemaking protocol

3.1.1 Grape Selection

Pinot noir grapes (*Vitis Vinifera*, clone 10/5) grown at St Helena, Canterbury, New Zealand, were hand harvested on the 19 April 1995. Grapes of high health were selected ie free from fungal disease, bird damage and rot. These were hand harvested directly into 20L food grade plastic buckets, and transported to Lincoln University for allocation of replicates and crushing/destemming.

3.1.2 Grape Processing

The fresh fruit was crushed and then destemmed using a Zambelli crusher/destemmer directly into 12 separate 20L plastic buckets. These were ramdomly allocated their replicates,

T1:R(A), (B), (C) were assigned as control wines.
T2:R(A), (B), (C) COM wines with 0 mgL^{-1} SO_2
T3:R(A), (B), (C) COM wines with 50 mgL^{-1} SO_2
T4:R(A), (B), (C) COM wines with 100 mgL^{-1} SO_2

Juice samples from each of the 12 replicates were taken and frozen for later compositional analysis.

3.1.3 Control Wines

The three replicates of the control treatment (conventional fermentation, T1, 0 mgL^{-1} SO_2) were warmed to 15^0C and innoculated with *Sacharomyces Cerevisia* (EC-1118, Lallemand Australia), the treatments were placed into a controlled temperature room at 22^0C. The wines were plunged twice daily twenty times each, the wines temperature and ^0Brix were measured for the fermentation period. This was done to give an indication of the wines rate of fermentation and as a pointer to when the wines had finished fermentation.

3.1.4 Cold Maceration Wines

The three replicates of T2 (0 mgL^{-1} SO_2) were placed into a temperature controlled room at 4^0C, no SO_2 additions were made. For the replicates of T3, 50 mgL^{-1} SO_2 was added and for T4, 100 mgL^{-1} SO_2 was added prior to placement in a temperature controlled room at 4^0C.

The macerating juice was left in the 4^0C temperature controlled room for six days. At the end of this period, juice samples were again taken from each replicate and analysed as previously. The replicated treatments were warmed overnight to 15^0C before innoculation with *S. cerevisia* yeast (EC-1118, Lallemond Australia). The plunging and measuring of temperature was carried out as for the control wines.

3.1.4 Wine Management.

Fermentation rate was monitored by measurement of cap temperature and sugar levels. Sugar levels were measured using a ^0Brix calibrated hydrometer. The cap was plunged 2 - 3 times daily at regulated intervals. As fermentation concluded (dropping cap temperature, decreaseing sugar levels), clinitest tablets (Miles Australia) were used to determine when sugar content was approximately 0.25%. When the sugar levels drop to this level the fementation is deemed to have concluded. The treatments were pressed using a 20L hydro pressure press, with a maximum pressing pressure of 1.5 bar.

Samples were taken at the conclusion of fermentation and frozen for later full compositional analysis. An addition of 50 mgL^{-1} of SO_2 was made at this stage to inhibit malaolactic fermentation. The treatments were then placed at 4^0C for cold stabilisation, during this time a CO_2 blanket was maintained over the wines. The wines were racked once off the lees and 30 mgL^1 SO_2 was added to prevent oxidation. After 3 months of cold stabilisation the wines were once again racked and 20 mgL^{-1} SO_2 added prior to filtration and bottling.

Diatomaceous earth was added to the wine and the wine filtered through a Whatman GF/A filter. Wine was bottled using a Sartorius pressurised bottling system. The wine moved through a MFS GF/A filter followed by Whatman GF/C and GF/F filters, finally the wine passed through a Sartorius 1.2µm membrane filter into 0.5% SO_2 rinsed 375 ml bottles. The wines were placed into a 12^0C cellar for storage, and sampled as required for analysis. All analyses were carried out on filtered finished wine.

3.2 Chemical Analysis

Juice and wine analysis were conducted in duplicate by standard methods. Soluble solids were determined as ^{0}Brix by a hand held ATAGO (Japan) PR10 digital refractometer. Titratable acidity and pH were analyzed using a Metrohm 670 Titroprocessor programmed with the method of Iland et al (1993). Malic acid in the juice samples was measured using a Boehringer (Mannheim, West Germany) enzyme assay kit in conjunction with a Phillips PU 8625 UV/VIS spectrophotometer. The finished wines were analysed for alcohol, all organic acids and sugars using an organic acid HPLC technique (cf section 3.3.1).

3.3 Chromatographic Analysis

3.3.1 HPLC Analysis of Sugars and Organic Acids

A Waters discovery system (Waters Assoc., Milford, Mass., U.S.A) equipped with a Waters model 600-ms solvent delivery system, along with Waters model 717plus autosampler, 490E programmable mulitwavelength detector and a Waters R401 differential refractometer all controlled using Millenium 2010 Chromatography Manager (Waters Assoc., Milford, Mass., U.S.A). Two 300 mm x 7.8 mm Aminex HPX - 87H ion exclusion columns (Bio-Rad Laboratories) were used in series, with a Waters Sugar-Pak II used to protect the column. Chromatograms were run at 27^0C using a Spectra-Physics SP 8792 column heater with a flow rate of 0.45 mL/min and an injection volume of 2 µL. The mobile phase was 0.002 N H_2SO_4, distilled water from a Nanopure reagent water system was used with a resistivity of not less than 17 megohms. All standards, samples and solvents were filtered through a 0.45 µm filter. Detection of Malic and Tartaric acid was conducted using the 490E multiwavelength detector set at 210 nm, with the R401 differential refractometer used for the detection of glucose, fructose, glycerol, acetic acid and ethanol. All samples were analysed in duplicate.

3.3.2 HPLC Analysis of Anthocyanins

Anthocyanins were separated, identified and quantified using HPLC with a PDA detector. All standards, samples, and solvents were filtered through a 0.45 µm filter prior to analysis. A Waters discovery system (Waters Assoc., Milford, Mass., U.S.A) equipped with a Waters model 600-ms solvent delivery system, along with Waters model 717plus autosampler and a Waters 996 photodiode array detector all controlled with Millenium 2010 Chromatography Manager (Waters Assoc., Milford, Mass., U.S.A) were used. A Waters 8 x 10 RCM cartridge holder was used in conjunction with a 8 mm x 100 mm Nova-Pak C_{18}, (Waters Assoc., Milford, Mass., U.S.A.) column and a Nova-Pak C_{18} guard column (Waters Assoc., Milford, Mass., U.S.A). Chromatograms were run at ambient temperature with a flow rate of 1 mL/min and an injection volume of 20

μL. The solvents used for separation were: A = 100% CH_3CN and B = 3.6% H_3PO_4, with initial conditions of 10 min at 6% A and 94% B, 45 min linear gradient to 20% A. The column was reconditioned in 100% B for 10 mins, this was followed by equilibration for 5 mins under the starting conditions. Anthocyanin detection was conducted at 520 nm, with a bandwidth of 20 nm. All samples were analysed in duplicate.

The anthocyanins were identified using both spectral characteristics and elution order. Detection of anthocyanins was conducted using a photo-diode array detector (PDA), enabling an entire spectrum to be taken for each of the anthocyanins as they eluted. The spectral characteristics of the anthocyanins are dependent both on pH and on the nature of the solvent, direct comparison of spectral characteristics with those published in the literature may be inappropriate (Hong & Wrolstad 1986). A malvidin standard was used for identification and quantification of malvidin-3-monoglucoside. In reversed-phase chromatography, the pigments elute in order of their polarity: 1) delphinidin-3-monoglucoside; 2) cyanidin-3-monoglucoside; 3) petunidin-3-monoglucoside; 4) the 3-monoglucoside of peonidin; and 5) the major vinifera anthocyanin, malvidin-3-monoglucoside (Wulf & Nagel 1978). Upon determination of malvidin (by spectral comparison) this elution order was employed to identify the remaining anthocyanins (figure 4.1). The anthocyanins were quantified using malvidin-3,5-diglucoside (Sigma Chemical Co. St. Louis) as an external standard.

3.4 Spectrophotometric Analysis

Spectrotometric analysis was used to determine both total phenolics and colour parameters. Colour parameters include colour density and hue, anthocyanin content, polymeric pigment and chemical age indicies. The method of Somers and Evans (1977) was used, in which the above parameters are estimated through use of pH, SO_2 and acetaldehyde differentials. All samples were analysed in duplicate.

3.5 Statistical Analysis

Analysis of results was conducted using the statistical package "Minitab", version 10.1 (Minitab Inc.: Pennsylvania, USA). One way analysis of variances (ANOVA) were calculated. Statistical analyses for the determination of significant differences between the treatment means was conducted using Fisher's Least Significant Difference (LSD) test.

Chapter Four

The effect of Cold Maceration on the composition and colour of Pinot noir Wine.

4.1 The effect of Cold Maceration with varying sulphur dioxide level on Pinot noir wine at the conclusion of bottling

The compositional analysis of juice both pre and post cold maceration are found in table 4.1, while the results of the compositional analysis at bottling are found in table 4.2. The effects of the different treatments on the spectral and HPLC measures are reported in tables 4.3 and 4.4. In addition graphical presentation of all results are shown in appendices 4.1 through to 4.10.

4.1.1 Results and discussion

4.1.1.1 Compositional analysis of juice

The control must was significantly greater ($p \leq 0.001$) in TA than the COM must. The 50 mgL^{-1} SO_2 COM must was significantly less than both of the COM musts in TA. These differences in TA tie in with the differences in pH, the control must was significantly lower ($p \leq 0.001$) in pH than the COM must. These differences in TA and pH can be attributed to the COM process. An increase in the potassium (K^+) concentration will lead to increased TA's and may also decrease the pH, this increase in K^+ concentration is not usually a problem during or after fermentation because of the

increase in ethanol concentration in which the K^+ ions are unstable.The conditions of cold maceration are such that no ethanol is present and K^+ ions are stable.

The sugar level in the 50 gL^{-1} SO_2 COM must was significantly less ($p \leq 0.001$) than both the control and unsulphured COM musts, while the 100 mgL^{-1} SO_2 COM must was significantly greater ($p \leq 0.001$) in sugar level than all of these musts. This higher reading for the 100 mgL^{-1} SO_2 COM may be due to experimental error as the sugar level in the control wine is the level which all of these wines initially had prior to maceration. The decreased level of sugar in the 50 mgL^{-1} SO_2 must was of some concern as it indicates that some form of fermentation may have begun.

The level of malic acid was lowest ($p \leq 0.05$) in the 50 mgL^{-1} SO_2 COM must, while the other two COM musts were higher in malic acid than the control. This decrease in malic acid as well as the decrease in the sugar level of the 50 mgL^{-1} SO_2 COM must, indicates that fermentation may well have begun in this wine at some point prior to the completion of cold maceration. Tests conducted on frozen samples from the end of cold maceration contained no alcohol.

There were significant differences ($p \leq 0.001$) in the level of total phenolics between all the treatments. The level was highest in the 100 mgL^{-1} SO_2 COM must and decreased with decreasing SO_2 addition, the must with the lowest phenolic content was the control. This is an early indication that SO_2 is acting as a solvent for the extraction of phenolic compounds

The COM wine which had no SO_2 added prior to cold maceration was observed to have a mold growth on the surface of the must, which was later identified as being *Botrytis cinerea*, the mold growth was accompanied by an "earthy" aroma. The mold growth resulted from the "isolated" conditions during the cold maceration, the buckets of this treatment were sealed with no SO_2, and left untouched for the entire cold maceration period. The surface of the COM wine with 100 mgL^{-1} of SO_2 was noted as being distinctly bleached in comparison to the other COM wines, this bleaching disappeared upon stirring.

4.1.1.2 Compositional analysis of wines at bottling

The normal level of tartaric acid in table wines ranges from 2 - 5 gL^{-1} (Rankine 1989). Even though there were no statistically significant differences in the level of tartaric acid between the treatments, there was an obvious trend. The level was highest in the control wine and lowest in the COM wine with no SO_2 added, and increased with increased SO_2 addition (appendix 4.1).

The normal level of Malic acid in wine ranges from trace - 5 gL^{-1} (Rankine 1989). The wines were stopped from going through malolactic fermentation (MLF) in which malic acid is converted to lactic acid, this resulted in wines with malic acid levels near the top of the normal range of malic acidity. Even though the wines were stopped from entering MLF the COM wines all had lower malic acid levels than the control (appendix 4.1), this phenomena has been noted previously in COM wines (Goldsworthy 1993). The level of malic acid at bottling decreased by between 20 and 40% from the level prior to fermentation. Yeast from the *Saccharomyces* family have been shown to decompose between 3 and 45% of malic acid during fermentation (Rankine 1966, Radler 1992). Reservations were still held however that a partial MLF may have occurred. Lactic acid is important as an indicator of whether MLF has begun, only trace amounts of lactic acid were detected in any of the wines

The glycerol levels present in the wines fell well within the normal range (1 - 15 gL^{-1}) for red wines (Rankine 1989). Glycerol can be produced by two mechanisms; the first being a result of mold (*Botrytis*) metabolism, where glycerol is a by-product from the breakdown of glucose and fructose (Zoecklein et al 1989, Donèche 1992), while the second mechanism is due to alcoholic fermentation where glycerol is produced as a by-product of the breakdown of dihydroxyacetone phosphate (Zoecklein et al 1989, Bisson 1992). The first mechanism is of interest because of the mold growth observed on the unsulphured COM wine, this wine had a higher level of glycerol than the control wine but lower than the other two COM wines (appendix 4.1), indicating that very little spoilage occurred as the result of the mold growth. Glycerol is also noted as contributing to the sensory perception of body in certain wines (Zoecklein et al 1989), and may contribute to the slight perceived sweetness of the fruit in Pinot noir wines.

The alcohol levels of the wines were not significantly different. Although the levels are low the ^{0}Brix of the grapes was also reasonably low and no chaptilisation was employed. There were only trace amounts of both fructose and glucose left after fermentation, indicating that fermentation had gone to completion.

Normally wine pH ranges from 3.0 through to 3.6 (Rankine 1989), while all the wines fall close to this pH range, they are extremely low. The optimal pH for a red wine is between 3.3 and 3.6 after it has been through MLF (Rankine 1989), so considering these wines have not been through MLF the pH as expected is lower than this optimal range. There were also large decreases in the pH of all the COM wines between cold maceration and analysis at bottling. During the process of cold stabilisation, reductions in pH and TA occur due to the generation of one free proton per molecule of potassium bitartrate (KHT) precipitated, maximum reductions are usually of the order of 0.2 pH units and 2 gL^{-1} TA (Zoecklein et al 1989). The control wine is within these reduction limits, the COM wines however had much greater reductions in pH, with an average reduction of 0.41 pH units. The large change in pH for COM wines is linked to the increase in pH noted during cold maceration, this was the result of higher levels of potassium and consequently KHT than in conventionally vinified wines, these higher levels of saturation lead to higher levels of precipitation.

There were significant differences in the TA levels of the wines. The control and 100 mgL^{-1} SO_2 COM wine were significantly greater ($p \leq 0.05$) than the 0 and 50 mgL^{-1} SO_2 COM wines. The decrease seen between the TA of the juice and the TA of the wine (av. = 1.781 gL^{-1}) corresponds to the reduction in TA which is associated with cold stabilisation, for TA this reduction is usually less than 2 gL^{-1} (Zoecklein et al 1989).

The HPLC technique used for analysis of organic acids and sugars was capable of detecting acetic acid. Acetic acid under the described HPLC conditions has a detection threshold of 1 gL^{-1}, a check was conducted to verify that the acetic acid levels were not above this threshold. One of the wines was analysed in duplicate using the technique described by Iland et al (1993) for volatile acidity (V.A.). The measurement of volatility acidity refers mainly to acetic acid (Iland et al 1993). The analysed wine had a volatile

acidity of 0.5 gL^{-1}, well under the threshold of the HPLC technique, also indicating that the wine had an acceptable level of V.A..

At the conclusion of fermentation there were significant differences in total SO_2 levels (table 4.5) between all of the treatments, with the levels fairly consistent to the amount added prior to cold maceration. The only level which differed from this was the control which although having no SO_2 added had a total SO_2 level of 12.7 mgL^{-1}, this is the result of SO_2 production by the yeast (Rauhut 1992, Zoeckein et al 1989). Normally, *S. cerevisiae* yeast produce between 10 and 30 mgL^{-1} of SO_2, the exact amount produced being dependent upon strain and the degree of clarification of the must (Rauhut 1992). The SO_2 content in all the wines was low enough at the conclusion of fermentation not to inhibit MLF. A free SO_2 level of 10 - 20 mgL^{-1} or a total SO_2 level of > 50 mgL^{-1} will inhibit MLF, especially at the low pH's of these wines where molecular SO_2 is more predominant (Henick-Kling 1988).

By bottling the SO_2 content is usually fairly stable, the control and COM wines with 0 and 50 mgL^{-1} SO_2 had significantly less total SO_2 than the 100 mgL^{-1} SO_2 COM wine. This difference can be attributed to the extra SO_2 added during the cold maceration period, as all the wines had the same additions of SO_2 during their maturation prior to bottling. These levels of SO_2 are well under the New Zealand legal requirements (<200 mgL^{-1}, Rankine 1989) for a dry red wine.

4.1.1.3 Spectral analysis

The control and COM wine with 0 mgL^{-1} SO_2 were significantly lower ($p \leq 0.001$) in colour density than both the other two COM wines. This difference was also clearly visible between the wines with the two sulphured COM wines being substantially darker in colour than the control and unsulphured COM wine. There were no significant differences in wine colour hue between any of the treatments.

The amount of total monomeric anthocyanins was greatest in the COM with 100 mgL^{-1} SO_2 wine, this had significantly ($p \leq 0.001$) more anthocyanins than any of the other wines, the COM 50 mgL^{-1} SO_2 wine was also significantly higher ($p \leq 0.001$) in

anthocyanin content than both the control and 0 mgL^{-1} SO_2 COM wine. The anthocyanin content in the wine increased with increasing SO_2 addition at the cold maceration stage. This indicates that the SO_2 is acting as a solvent for the extraction of anthocyanins, and also that decreasing the temperature alone does not aid in the extraction of anthocyanins.

The degree of ionisation of the anthocyanins (α) represents the percentage of anthocyanins found in coloured form at wine pH. There were significant differences ($p \leq 0.05$) between treatments in α, with the COM treatments having higher α values than the control. In red wines SO_2 is found loosely bound to anthocyanin molecules, this can dramatically influence the amount of anthocyanins which are present in coloured forms, especially when the wines are young with high levels of SO_2 affecting colouration. The natural degree of ionisation (α') uses the fact acetaldehyde binds more strongly with SO_2 than the anthocyanins do, releasing the anthocyanin bisulphate compounds for measurement. There were significant differences between the control and all of the cold maceration wines, the control was significantly lower ($p \leq 0.05$) in α' than all the cold maceration wines. This result indicates that in the COM wines there is a greater amount of anthocyanins present as the coloured flavylium cation, regardless of the amount of anthocyanins extracted. These results agree with the literature (Somers & Evans 1977), indicating that young wines with high SO_2 content are more accurately represented by α' than α. The differences between the measurements are thought to be related to the amount of SO_2 added after fermentation (Somers & Evans 1977), however all of these wines received identical doses of SO_2 throughout there conservation. The differences in this case are more likely to be due to the different levels of anthocyanins extracted, these different levels would result in varied amounts of the anthocyanin bisulphate compound forming.

There were significant differences between the treatments in the level of total phenolics, with the unsulphured COM wine being significantly less ($p \leq 0.001$) than the control which in turn is significantly less ($p \leq 0.001$) than both the sulphured COM wines. The 100 mgL^{-1} SO_2 COM wine was significantly greater ($p \leq 0.001$) than the 50 mgL^{-1} SO_2 COM wine in phenolic content. Levels of total phenolics range from 20 - 100 a.u. in normal red wines (Somers & Evans 1977), these wines were at the lower end of that scale, the wines had not however been in oak which may markedly increase both the

colour and phenolic content. The results obtained for total phenolics also indicate that SO_2 is acting as a solvent for the extraction of phenolics, and that decreasing the temperature alone slows phenolic extraction.

The chemical age index attempts to measure the amount of polymeric pigment in the wine, in young wines it is near zero while in old wines it approaches one (Somers & Evans 1977). The control and 50 mgL^{-1} SO_2 COM wines were significantly greater ($p \leq 0.05$) than the 100 mgL^{-1} SO_2 COM wine, while the 50 mgL^{-1} SO_2 COM wine was not significantly different from any of the other treatments. This measurement is influenced by free SO_2, and is relatively unstable in young red wines while becoming more reliable with bottle age.

4.1.1.4 HPLC analysis

There were significant differences in the levels of anthocyanins found in all treatments except for petunidin-3-monoglucoside. Levels of Delphinidin-3-monoglucoside and cyanidin were significantly lower ($p \leq 0.001$) in the control and unsulphured COM wines than the two sulphured COM wines. For these pigments the 50 mgL^{-1} SO_2 COM wine was significantly less than the 100 mgL^{-1} SO_2 COM wine. The results for peonidin, malvidin and total monomeric anthocyanins all follow the same significance, with the control and unsulphured COM wines significantly less ($p \leq 0.05$) than both the sulphured COM wines.

These results indicate that use of SO_2 in the COM process nearly doubles the extraction of monomeric anthocyanins (appendix 4.10). The extraction with SO_2 does not alter the amount of each individual anthocyanin relative to the total monomeric anthocyanin content for each wine. The HPLC results were actually higher than the spectral results for total anthocyanins, this was unexpected as previous studies (Bakker et al 1986, Rivas-Gonzalo et al 1992) have determined that spectral measures overestimate the anthocyanin content. This is due to partial bleaching of oligomeric pigments, which leads to overestimation of the anthocyanin content (Bakker et al 1986). These studies both used varieties with relatively high phenolic and anthocyanin contents, while this research was based upon Pinot noir, a variety with a low phenolic pool. The experimental wines had low phenolic levels as a result of both variety and the fact that they did not have any

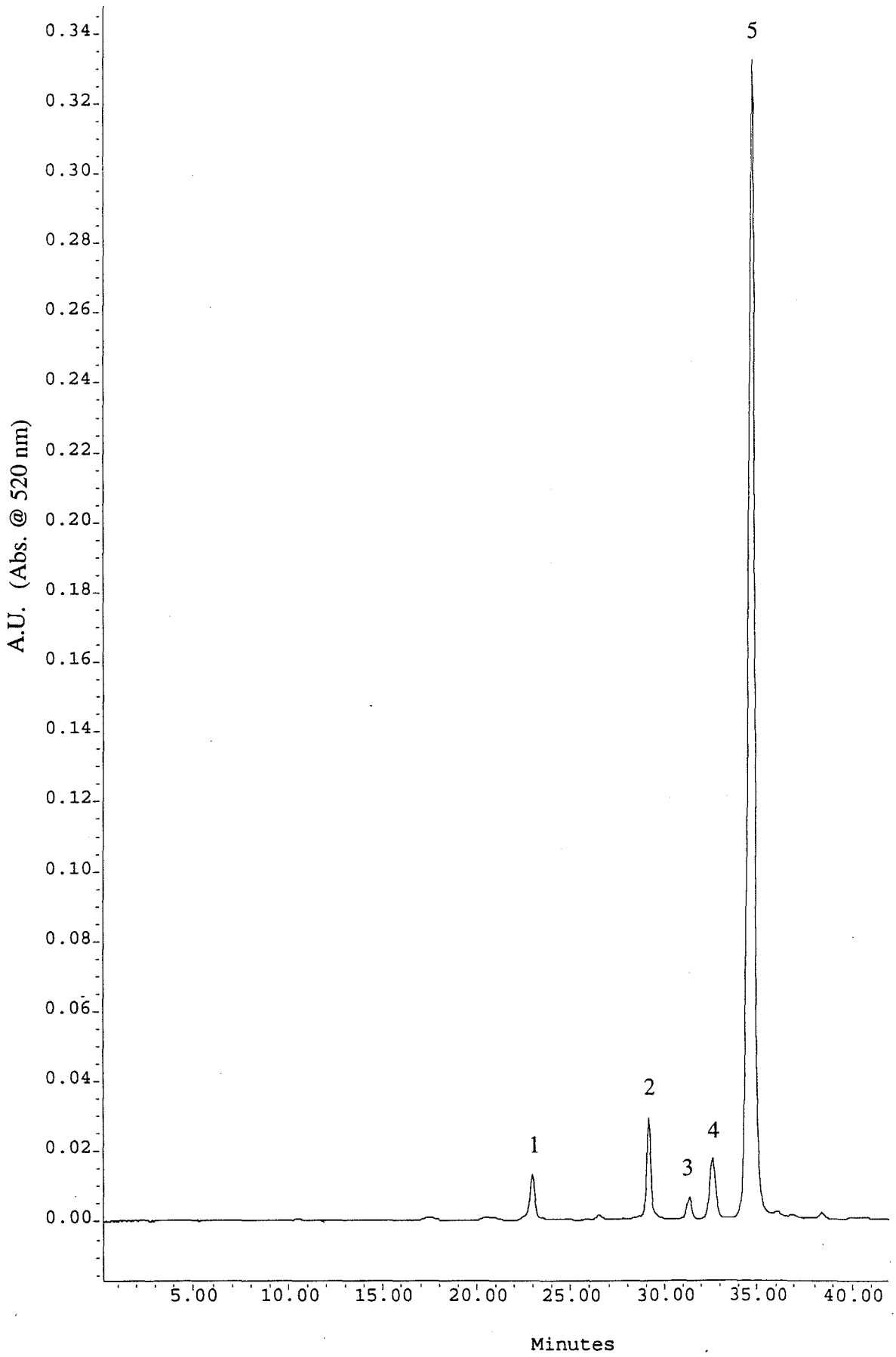

Figure 4.1. HPLC chromatogram of anthocyanins in Pinot noir wine. Peak Identification: 1, delphinidin-3-monoglucoside; 2, cyanidin-3-monoglucoside; 3, petunidin-3-monoglucoside; 4, peonidin-3-monoglucoside; 5, malvidin-3-monogucoside.

oak contact. Another effect of this low phenolic pool is that there is not as much interference from phenolic compounds at 520nm in the spectral measurements The wines all had very low chemical age indexes, therefore only minimal polymerised pigments.

4.1.2 Conclusions

The composition of the must at the conclusion of cold maceration had altered significantly. The maceration had lowered the TA of the wines and increased the pH, this is believed to be as a result of increased potassium ions in the must. The levels of malic acid also increased. The only must not to conform was the must which received 50 mgL^{-1} SO_2. This must is thought to have begun fermenting prior to the conclusion of the cold maceration. The maceration increased the phenolic content of all the cold macerated wines, with the level of phenolics increasing with increasing SO_2 addition.

The compositional analysis at bottling showed that there were very little differences in the wines composition, the only difference being lower TAs for the cold maceration wines. This indicates that the COM process has minimal effects on final wine composition.

Observation of the colour of the wines indicated that there was an increase in visible colour (red-purple hue) in both of the sulphured COM wines. Spectral analysis of the wines showed that the sulphured COM wines were significantly greater than the control and COM wine with no SO_2, in wine colour density, total monomeric anthocyanins, natural degree of ionisation and total phenolics. From these results it is possible to conclude that the SO_2 used during the COM process is acting as a solvent for the extraction of all phenolic compounds including anthocyanins, the phenolic extraction increases with increasing SO_2 concentration during the COM period.

HPLC analysis also found that the sulphured COM wines were significantly higher in anthocyanin content than the control and unsulphured COM wine. HPLC analysis also confirmed that the COM procedure doesn't alter the pigment profile of the wine. The amount of anthocyanins estimated by the spectral method was less than the amount

determined by HPLC analysis. This is due to both the low phenolic content and chemical age indexes of the wines.

Table 4.1 Compositional analysis of Pinot noir juice

	Control[1]	Cold Maceration[2] 0 mgL^{-1} SO_2	Cold Maceration[2] 50 mgL^{-1} SO_2	Cold Maceration[2] 100 mgL^{-1} SO_2	p value[4]
TA[3]	10.84^{d}	9.44^{b}	8.63^{a}	9.74^{c}	<0.001
pH	3.17^{a}	3.42^{b}	3.40^{b}	3.45^{b}	<0.001
Sugar (^{0}Brix)	18.65^{a}	18.70^{a}	16.75^{b}	20.9^{c}	<0.001
Malic acid (gL^{-1})	5.18^{a}	6.69^{b}	3.59^{c}	6.31^{ab}	0.012
Total phenols	9.44^{a}	11.31^{b}	14.56^{c}	19.29^{d}	<0.001

Mean values within the same row designated with a different letter differ significantly (Fishers $LSD_{0.05}$)

[1]Sample taken on 20/4 (prior to fermentation)

[2]Samples taken on 27/4 (prior to fermentation but after cold maceration)

[3]as gL^{-1} tartaric acid

[4]F ratio

Table 4.2 Compositional analysis of Pinot noir wines at bottling

	Control	Cold Maceration 0 mgL^{-1} SO_2	Cold Maceration 50 mgL^{-1} SO_2	Cold Maceration 100 mgL^{-1} SO_2	P value[3]
Tartaric acid[1]	4.23	2.81	3.61	3.39	0.053
Malic Acid[1]	5.16	3.87	4.10	4.05	0.146
Glycerol[1]	6.18	6.58	6.77	6.71	0.233
Alcohol (%)	9.79	10.41	10.72	10.25	0.264
pH	2.99	3.05	3.01	2.98	0.217
TA[2]	8.44^{a}	7.66^{b}	7.53^{b}	7.84^{a}	0.035

Mean values within the same row designated with a different letter differ significantly (Fishers $LSD_{0.05}$)

[1]gL^{-1}

[2]as gL^{-1} tartaric acid

[3]F ratio

Table 4.3 Spectral analysis of Pinot noir wines at bottling

	Control	Cold Maceration 0 mgL^{-1} SO_2	Cold Maceration 50 mgL^{-1} SO_2	Cold Maceration 100 mgL^{-1} SO_2	p value[1]
Wine colour density	1.51[a]	1.68[a]	2.41[b]	2.39[b]	0.001
Wine colour hue	0.59	0.53	0.60	0.57	0.175
Total anthocyanins (mg/L)	176.14[ab]	174.64[a]	194.24[b]	254.22[c]	<0.001
α	8.76[a]	10.94[ab]	13.96[b]	10.46[a]	0.046
α´	20.56[a]	26.63[b]	26.59[b]	25.82[b]	<0.001
Ionised anthocyanins (mg/L)	15.40[a]	19.00[a]	26.57[b]	26.70[b]	0.003
Total phenolics (a.u.)	22.27[b]	20.15[a]	24.09[c]	26.06[d]	<0.001
Chemical age index ii)SO_2/HCl	0.020[a]	0.017[ab]	0.020[a]	0.015[b]	0.023

Mean values within the same row designated with a different letter differ significantly (Fishers $LSD_{0.05}$)

[1]F ratio

Table 4.4 HPLC analysis of Pinot noir wines at bottling

	Control	Cold Maceration 0 mgL^{-1} SO_2	Cold Maceration 50 mgL^{-1} SO_2	Cold Maceration 100 mgL^{-1} SO_2	p value[1]
Delphinidin-3-monoglucoside	4.47[a]	5.23[a]	8.30[b]	12.67[c]	<0.001
Cyanidin-3-monoglucoside	10.80[a]	11.74[a]	16.84[b]	22.40[c]	0.002
Petunidin-3-monoglucoside	1.92	2.41	3.01	3.19	0.168
Peonidin-3-monoglucoside	9.77[a]	12.47[a]	19.54[b]	20.90[b]	<0.001
Malvidin-3-monoglucoside	209.93[a]	208.38[a]	347.84[b]	338.45[b]	0.002
Total monomeric anthocyanins (mgL^{-1})	237.12[a]	240.22[a]	395.51[b]	397.59[b]	0.002

Mean values within the same row designated with a different letter differ significantly (Fishers $LSD_{0.05}$)

[1]F ratio

Table 4.5 Sulphur dioxide content of Pinot noir juice and wine made by Cold Maceration

Time of Analysis	Sulphur Dioxide (mgL^{-1})	Control	Cold Maceration 0 mgL^{-1} SO_2	Cold Maceration 50 mgL^{-1} SO_2	Cold Maceration 100 mgL^{-1} SO_2	p value[1]
End of Fermentation	Free SO_2	1.50[a]	0.00[b]	0.00[b]	0.00[b]	<0.001
	Total SO_2	12.77[a]	0.27[b]	8.80[c]	38.67[d]	<0.001
Bottling	Free SO_2	18.93	21.60	14.93	20.27	0.409
	Total SO_2	53.60[a]	53.87[a]	49.87[a]	75.47[b]	0.002

Mean values within the same row designated with a different letter differ significantly (Fishers $LSD_{0.05}$)

[1]F ratio

4.2 The effect of Cold Maceration with varying sulphur dioxide level on Pinot noir wine, after four months storage

The effect of four months storage was determined for each of the wines by subjecting them to further spectral and HPLC analyses, the results of these analyses are reported in tables 4.6 and 4.7. In addition graphical presentation of all results are shown in appendices 4.1 through to 4.10.

4.2.1 Results and discussion

4.2.1.1 Spectral analysis at four months

At four months wine colour density was significantly greater ($p \leq 0.001$) in the two sulphured COM wines. There was no significant difference between the two sulphured COM wines. The 100 mgL^{-1} SO_2 COM wine was significantly greater ($p \leq 0.05$) in hue than the control and 0 mgL^{-1} SO_2 COM wine. The 50 mgL^{-1} SO_2 COM wine was not significantly different to the control or 0 mgL^{-1} SO_2 COM wine, although it was lower than both of these wines.

The 100 mgL^{-1} SO_2 COM wine had significantly more ($p \leq 0.001$) anthocyanins than any of the other wines. At bottling the 50 mgL^{-1} SO_2 COM wine had greater anthocyanin content than both the control and unsulphured COM wine (tables 4.3, 4.4) after four months bottle age the anthocyanin level in the 50 mgL^{-1} SO_2 COM wine was not significantly different from the control and 0 mgL^{-1} SO_2 COM wine.

At four months there was some significance in the degree of ionisation (α), with the 50 mgL^{-1} SO_2 COM wine being significantly higher ($p \leq 0.05$) than the control and 0 mgL^{-1} SO_2 COM wine. The natural degree of ionisation (α') showed more significant trends, with both of the sulphured COM wines being significantly greater ($p \leq 0.001$) than the control and unsulphured COM wines. At bottling the 0 mgL^{-1} SO_2 COM wine had

significantly greater (table 4.3) α' than the control, while at four months there is no significant difference in α' between these wines. The amount of ionised anthocyanins follows a similar significance, with the two sulphured COM wines again being greater ($p \leq 0.001$) than the control and unsulphured COM wines. This higher degree of ionisation of the sulphured COM wines could be partly responsible for their higher colour densities.

The level of total phenolics in the wines at four months was significantly greater ($p \leq 0.001$) in the two sulphured COM wines than the control and unsulphured COM wines. In addition the 100 mgL^{-1} SO_2 COM wine was significantly greater than the 50 mgL^{-1} SO_2 COM wine. The trends for this measurement have remained the same as at bottling.

The 50 mgL^{-1} SO_2 COM wine had a significantly greater chemical age than the 100 mgL^{-1} SO_2 COM wine, while both of these wines were significantly greater than the control and unsulphured COM wine in chemical age. Though the level of anthocyanins in the 50 mgL^{-1} SO_2 COM wine had dropped significantly, its wine colour density has remained high. This can be attributed to two factors, the first being its high ionised anthocyanin content, while the second is its high chemical age. This high chemical age indicates a greater degree of polymerisation, polymeric pigments have a greater proportion of molecules in coloured forms than monomeric anthocyanins, there is also less uncoloured polymeric pigment (Ribéreau-Gayon & Glories 1986). Combining these factors with the decreased level of anthocyanins has resulted in increased colour density.

4.2.1.2 HPLC analysis at four months

Petunidin-3-monoglucoside was the only anthocyanin which had no significant differences between the treatments. The 100 mgL^{-1} SO_2 COM wine was significantly greater ($p \leq 0.001$) in delphinidin content than the other three wines, in which there was no statistical difference. Cyanidin was also significantly greater ($p \leq 0.001$) in the 100 mgL^{-1} SO_2 COM wine, the 50 mgL^{-1} SO_2 COM wine was significantly greater than the control but not statistically different to the 0 mgL^{-1} SO_2 COM wine. The 0 and 50 mgL^{-1} SO_2 COM wines were greater ($p \leq 0.001$) in peonidin content than the control wine, while the 100 mgL^{-1} SO_2 COM wine was greater ($p \leq 0.001$) in peonidin than these two

wines. Malvidin and total monomeric anthocyanins follow similar patterns, the 100 mgL^{-1} SO_2 COM wine is the only wine which now contains significantly more monomeric anthocyanins than either the control or 0 mgL^{-1} SO_2 COM wines. With the 50 mgL^{-1} SO_2 COM wine not being statistically different to the control and 0 mgL^{-1} SO_2 COM wines.

The HPLC results confirm those of the spectral analysis in that the 100 mgL^{-1} SO_2 COM wine has retained its greater monomeric anthocyanin content after four months bottle age.

4.2.2 Conclusions

The visible colour was again greater at four months in the sulphured COM wines, the 100 mgL^{-1} SO_2 COM wine however did have a slightly more purple hue than the other wines. The two sulphured COM wines were again significantly greater in the spectral measurements of colour density, ionised anthocyanins, total phenolics and chemical age than the control and unsulphured COM wine.

The 50 mgL^{-1} SO_2 COM wines anthocyanin content had decreased to the point where it was no longer significantly different to the control and 0 mgL^{-1} SO_2 COM wines. Its increased colour density seems to be relying upon higher ionised anthocyanin content and increased chemical age.

The HPLC analysis of the wines showed that there was greater monomeric anthocyanin content in the 100 mgL^{-1} SO_2 COM wine. This is in agreement with the results obtained with the spectral analysis, again the results followed the unusual trend where the spectral measurements underestimate the anthocyanin content as measured by HPLC.

Table 4.6 Spectral analysis of Pinot noir wines after four months storage

	Control	Cold Maceration 0 mgL^{-1} SO_2	Cold Maceration 50 mgL^{-1} SO_2	Cold Maceration 100 mgL^{-1} SO_2	p value[1]
Wine colour density	1.73[a]	1.70[a]	2.73[b]	2.93[b]	<0.001
Wine colour hue	0.682[a]	0.693[a]	0.6[ab]	0.57[b]	0.017
Total anthocyanins (mg/L)	142.10[a]	154.06[a]	145.923[a]	195.320[b]	<0.001
α	12.06[a]	10.62[a]	17.92[b]	14.87[ab]	0.036
α´	29.45[a]	29.63[a]	34.15[b]	33.94[b]	<0.001
Ionised anthocyanins (mg/L)	16.98[a]	16.28[a]	25.08[b]	28.92[b]	<0.001
Total phenolics (a.u.)	19.19[a]	18.99[a]	20.66[b]	23.21[c]	<0.001
Chemical age index ii)SO_2/HCl	0.020[a]	0.024[a]	0.056[c]	0.039[b]	<0.001

Mean values within the same row designated with a different letter differ significantly (Fishers $LSD_{0.05}$)

[1]F ratio

Table 4.7 HPLC analysis of Pinot noir wines after four months storage

	Control	Cold Maceration 0 mgL^{-1} SO_2	Cold Maceration 50 mgL^{-1} SO_2	Cold Maceration 100 mgL^{-1} SO_2	p value
Delphinidin-3-monoglucoside	4.27[a]	4.99[a]	5.70[a]	10.38[b]	<0.001
Cyanidin-3-monoglucoside	8.49[a]	10.12[ab]	10.86[b]	16.31[c]	<0.001
Petunidin-3-monoglucoside	2.09	2.11	2.07	2.60	0.145
Peonidin-3-monoglucoside	8.23[a]	10.59[b]	11.43[b]	15.45[c]	<0.001
Malvidin-3-monoglucoside	180.70[a]	187.11[a]	184.56[a]	243.60[b]	<0.001
Total monomeric anthocyanins (mgL^{-1})	203.79[a]	214.91[a]	214.62[a]	288.34[b]	<0.001

Mean values within the same row designated with a different letter differ significantly (Fishers $LSD_{0.05}$)

[1]F ratio

4.3 The effect of Cold Maceration on Commercial and Experimental Pinot noir wine, after six months storage

The wines analysed up to this point have been purely experimental, with no incorporation of factors such as malolactic fermentation or oak ageing in the winemaking because of their effects upon the wines aroma, taste, colour and phenolic content. In this section wines are analysed which were all made from the same grapes both experimentally and commercially. The spectral results of the experimental wines are reported in table 4.8, while the results for the oaked experimental COM wine and the two wines produced by St. Helena are reported in table 4.9

4.3.1 Winemaking Protocol

Three additional wines were analysed, the first was made using a mixture of the 50 and 100 mgL^{-1} SO_2 COM wines which subsequently underwent both malolactic fermentation and oak ageing. The other two wines were made by St. Helena Estate, both of these wines were made with the same grapes as the experimental wines, and are also approximately six months old. Of the two wines produced by St Helena one was effectively a control, undergoing a normal fermentation with 10 days skin contact. The second wine was a cold maceration wine which underwent a 4 day COM with 50 mgL^{-1} SO_2 at a temperature of 13 ^0C, this wine also had 10 days of skin contact. Both of these wines were racked into 3 month old barriques, within 1 week of completing fermentation where they underwent malolactic fermentation. Analysis of the St. Helena wines was conducted on barrel samples.

4.3.2 Results and discussion

4.3.2.1 Spectral analysis of experimental wines at six months

The wine colour density at six months is still significantly greater ($p \leq 0.05$) in the two sulphured COM wines. Wine colour hue was significantly less ($p \leq 0.05$) in all three of the COM wines.

The level of total anthocyanins was greatest ($p \leq 0.001$) in the 100 mgL^{-1} SO_2 COM wine, while the 50 mgL^{-1} SO_2 COM wine had a significantly higher ($p \leq 0.001$) anthocyanin content than both the control and 0 mgL^{-1} SO_2 COM wine, At four months there was no significant difference between these three wines. The control and unsulphured COM wines anthocyanin levels dropped by ~ 36% between the four and six month analysis while the sulphured COM wines dropped by only 13%.

The degree of ionisation (α) and natural degree of ionisation (α') were both greatest ($p \leq 0.05$) in the unsulphured COM wine. There was no significant difference ($p \leq 0.05$) between the control and both of the sulphured COM wines in either α or α'. The huge increases observed in the ionisation of the unsulphured COM wine have increased its colour density, to a level greater but still not significantly different to the control wine. The level of ionised anthocyanins was greatest ($p \leq 0.05$) in the 100 mgL^{-1} SO_2 COM wine and decreased with decreasing SO_2 addition, the control wine had the lowest level of ionised athocyanins.

Total phenolics was significantly greater ($p \leq 0.001$) in the two sulphured COM wines, this trend has been obvious throughout the wines ageing (appendix 4.9).

At six months the most interesting changes have occurred in the chemical age index, with both the control and unsulphured COM wines increasing dramatically. The 100 mgL^{-1} SO_2 COM wine had a significantly lower ($p \leq 0.001$) chemical age than the other three wines. The low chemical age of this wine agrees with findings from other research (Dallas et al 1995, Picinelli et al 1994), where wines with higher levels of SO_2 were found to form polymeric pigment at retarded rates. The large increases observed in chemical age for the control and unsulphured COM wine help to explain some of the other readings obtained for these wines at six months, namely, vastly decreased anthocyanin contents, and increased colour density and ionisation measurements.

4.3.2.2 Spectral results of Oaked experimental wine and St. Helena wines

The St Helena COM wine was significantly greater ($p \leq 0.05$) than the St Helena control wine in colour density, total anthocyanins, ionised anthocyanins and total phenolics. The

COM wine was also significantly less ($p \leq 0.05$) in chemical age. This indicates that the COM process employed by St. Helena was successful in extracting more anthocyanin and phenolic compounds, once extracted the extra phenolic compounds have contributed to the wines increased colour density.

The spectral measurements for the COM wine and control wine follow exactly the same patterns and trends as those observed in the experimental wines. These results although not in a replicated trial indicate that the results obtained with the experimental wines are applicable to commercial winery situations.

The results from the oaked experimental wine illustrate the influence of oak upon colour, this wines colour density was vastly greater than the experimental wines and yet the main difference is oak ageing. The influence of the more oxidative storage conditions can be seen in both the wines high chemical age and low anthocyanin content.

4.3.3 Conclusions

At six months both of the sulphured COM wines still retained greater colour density than the control and unsulphured COM wine. The control and unsulphured COM wine chemical age indexes increased dramatically, this was also reflected in the lowered anthocyanin content of these wines.

The 50 mgL^{-1} SO_2 COM wine had similar colour density to the 100 mgL^{-1} SO_2 COM wine while having increased chemical age and decreased anthocyanin content. These indicate that the addition of 50 mgL^{-1} SO_2 during the COM process can have the same effects on wine colour density as a 100 mgL^{-1} SO_2 addition. The anthocyanins in the 50 mgL^{-1} SO_2 COM wine were stabilised at an earlier age than the 100 mgL^{-1} SO_2 COM wine in which the higher SO_2 content has had inhibiting effects on polymerisation reactions.

Commercially made COM wines indicate that the technique has strong merits for improvement of colour in commercial situations. The oaked experimental wine indicates the large influence of oak on both colour density and stabilisation of a wines colour.

Table 4.8 Spectral analysis of Pinot noir wines after six months storage

	Control	Cold Maceration 0 mgL^{-1} SO_2	Cold Maceration 50 mgL^{-1} SO_2	Cold Maceration 100 mgL^{-1} SO_2	p value[1]
Wine colour density	1.83^{a}	2.12^{a}	2.52^{b}	2.53^{b}	0.006
Wine colour hue	0.72^{a}	0.66^{b}	0.66^{b}	0.62^{b}	0.010
Total anthocyanins (mg/L)	99.38^{a}	86.41^{a}	126.59^{b}	168.99^{c}	<0.001
α	15.44^{a}	21.70^{b}	18.39^{ab}	14.33^{a}	0.032
α´	37.26^{a}	44.73^{b}	34.34^{a}	34.52^{a}	0.001
Ionised anthocyanins (mg/L)	15.08^{a}	18.78^{ab}	22.18^{bc}	24.05^{c}	0.006
Total phenolics (a.u.)	15.23^{a}	13.89^{a}	24.31^{b}	26.28^{b}	<0.001
Chemical age index ii)SO_2/HCl	0.056^{a}	0.069^{a}	0.062^{a}	0.040^{b}	0.001

Mean values within the same row designated with a different letter differ significantly (Fishers $LSD_{0.05}$)

[1]F ratio

Table 4.9 Commercial and Oaked Experimental Pinot noir wine analysis

	St Helena Control	St Helena Cold Maceration wine	Oaked Experimental Wine
Wine colour density	3.47	5.16	3.84
Wine colour hue	0.73	0.64	0.69
α´	33.29	26.34	27.75
Total anthocyanins (mg/L)	85.91	187.75	84.5
Ionised anthocyanins (mg/L)	21.45	39.45	20.4
Total phenolics (a.u.)	29.80	32.83	25.15
Chemical age index ii)SO2/HCl	0.160	0.104	0.198

Mean values within the same row designated with a different letter differ significantly (Fishers $LSD_{0.05}$)

[1]F ratio

Chapter Five

Conclusions

5.1 Chemical Composition of Wines

The cold maceration process resulted in musts which had a distinctly different composition to the control must, of importance was reduced TA and increased pH, malic acid and total phenols. The increased pH and reduced TA are linked to uptake of potassium ions during the maceration. The phenol content of the musts increased with increasing SO_2 addition to the must.

Wines made using the Cold Maceration process were not significantly different to the normally vinified wines in any attribute apart from TA, in which they were lower. The cold maceration process does not effect the final composition of the wine.

The sulphur dioxide content in the finished wines was similar in the control, 0 and 50 mgL^{-1} SO_2 COM wines but significantly greater in the 100 mgL^{-1} SO_2 COM wine. The sulphur level in the 100 mgL^{-1} SO_2 COM wine at fermentation was low enough not to effect malolactic fermentation.

5.2 Spectral and HPLC analysis

The spectral analysis of the wines at bottling revealed that the sulphured COM wines had significantly greater wine colour density, total anthocyanins, natural degree of ionisation (α') and total phenolics. The HPLC analysis at bottling also indicated that the sulphured COM wines were significantly greater in anthocyanin content than the control and unsulphured COM wine. The composition of the anthocyanins in each wine was not altered by either the level of extraction of anthocyanins or the cold maceration process.

The sulphur dioxide added at Cold Maceration acted as a solvent for the extraction of both anthocyanins and other phenolic compounds.

The spectral analysis after four months storage indicated that the sulphured COM wines were again greater than the control and unsulphured COM wines in colour density, ionised anthocyanins, total phenolics and chemical age. The 50 mgL^{-1} SO_2 COM wines colour stabilised at a faster rate than the other wines. The HPLC results again confirm the spectral results in that the 100 mgL^{-1} SO_2 COM wine has the greatest monomeric anthocyanin content.

At six months the control and unsulphured COM wines colour were stabilised, this is seen by the decreased anthocyanin content and increased chemical age of these wines. The sulphured COM wines still retained greater colour density, anthocyanin and phenolic content. The high sulphur levels present in the 100 mgL^{-1} SO_2 COM wine at bottling were still affecting the amount of polymerisation, low chemical age indicates a low degree of polymerisation.

There were differences in the visible colour of the wines right throughout the wines conservation, the two sulphured COM wines had increased visible colour (red-purple hue) from both the control and unsulphured COM wines.

The sulphur dioxide which is added during cold maceration acts as the solvent for the extraction of anthocyanins and other phenolic compounds from the must. The level of addition is important for the ageing characteristics of the monomeric pigments. At high levels (100 mgL^{-1} SO_2) of addition formation of polymeric pigment is reduced. With sulphur addition during cold maceration a higher wine colour density is obtained. In this study the optimum sulphur addition was found to be 50 mgL^{-1}, at different levels of phenolic extraction different optimum sulphur levels will be required for the most efficient extraction. The commercial COM wine and oaked experimental wines indicate that cold maceration in commercial situations has the same dramatic influence on a wines colour as found with these experimental wines.

5.3 Further Research

The area of cold maceration still requires further research, while the effects of temperature (Goldsworthy 1993) and SO_2 on colour measures have been investigated, other areas of research could include the use of macerating enzymes for the extraction of colour prior to fermentation, whether this occurs at cold temperatures is debatable as enzymes usually have reduced activity at lower temperatures.

Problems were encountered during this research with the 50 mgL^{-1} SO_2 COM must which appears to have begun fermentation, future research could investigate the native microbes and their development during cold maceration.

The commercial and oaked experimental wines demonstrated that oak ageing plays an important role in both colour development and ageing, the influence of the oak on colour parameters could be more thoroughly researched, and also incorporated into an overall study on the effects of oak ageing, ie unoaked vs oaked over longer periods of time.

The HPLC analysis yielded invaluable information on the anthocyanin structure of the wine, it did not however yield information on the polymeric compounds. This area is of significant interest as understanding ageing reactions and how they contribute to a wines ageing is largely unexplored.

A technique which furthers the concept of cold maceration is to freeze the must, this is often used in the fruit juice industry to increase juice yields. This could foreseeably have application in the wine industry to improve colour extraction from the must, prior to normal fermentation.

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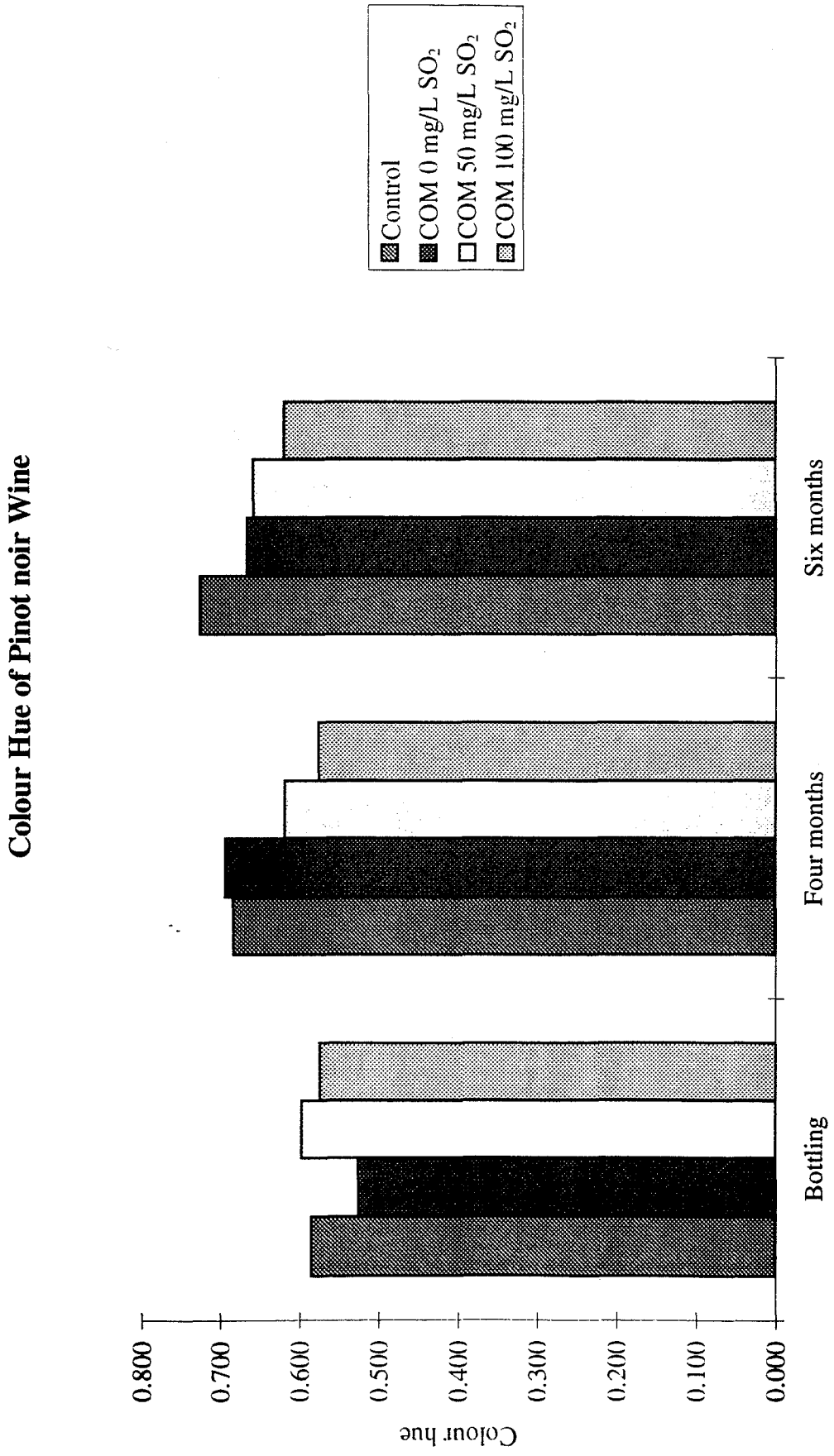
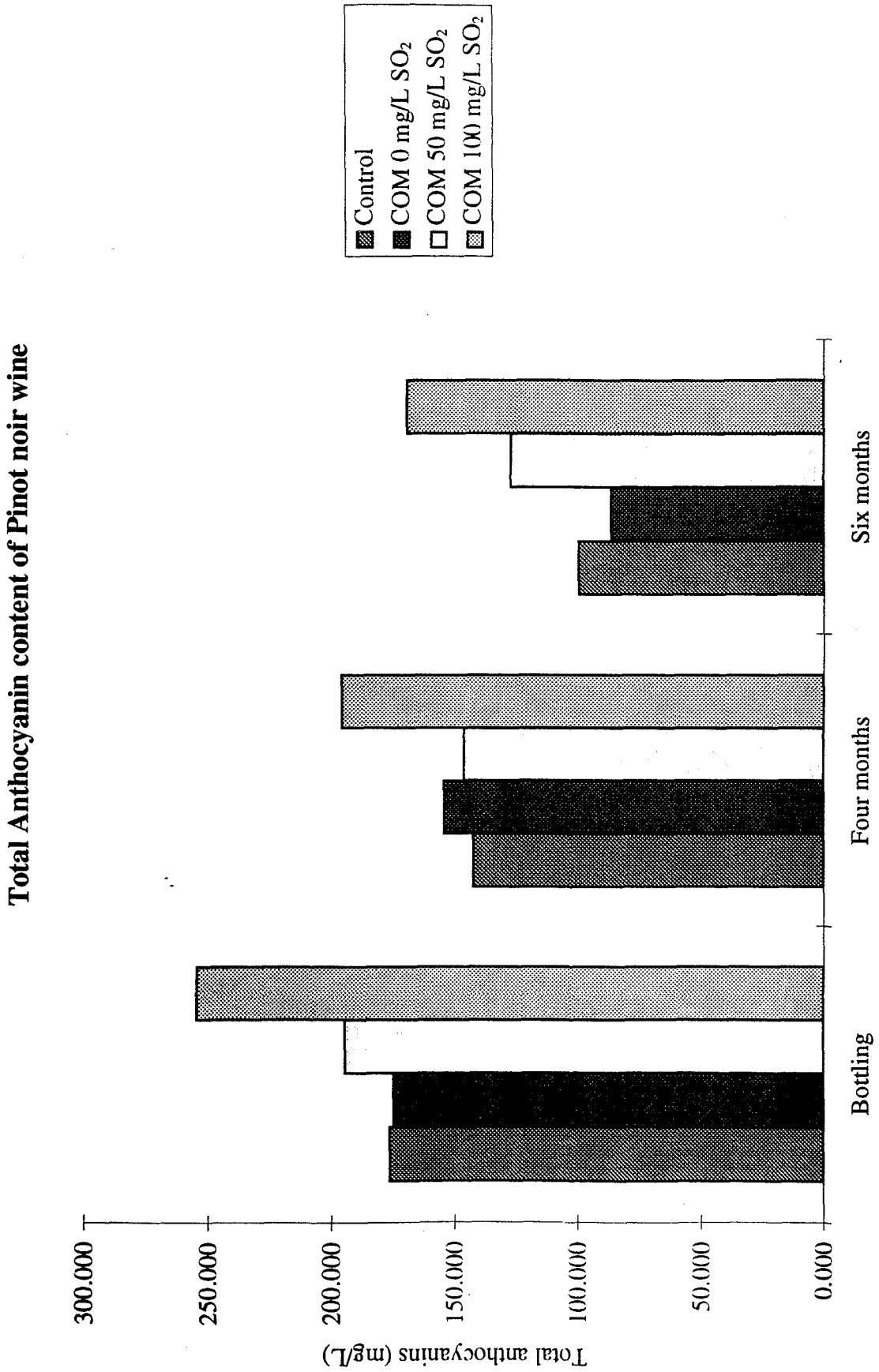
Compositional Analysis of Pinot noir Wine

Colour density of Pinot noir wine

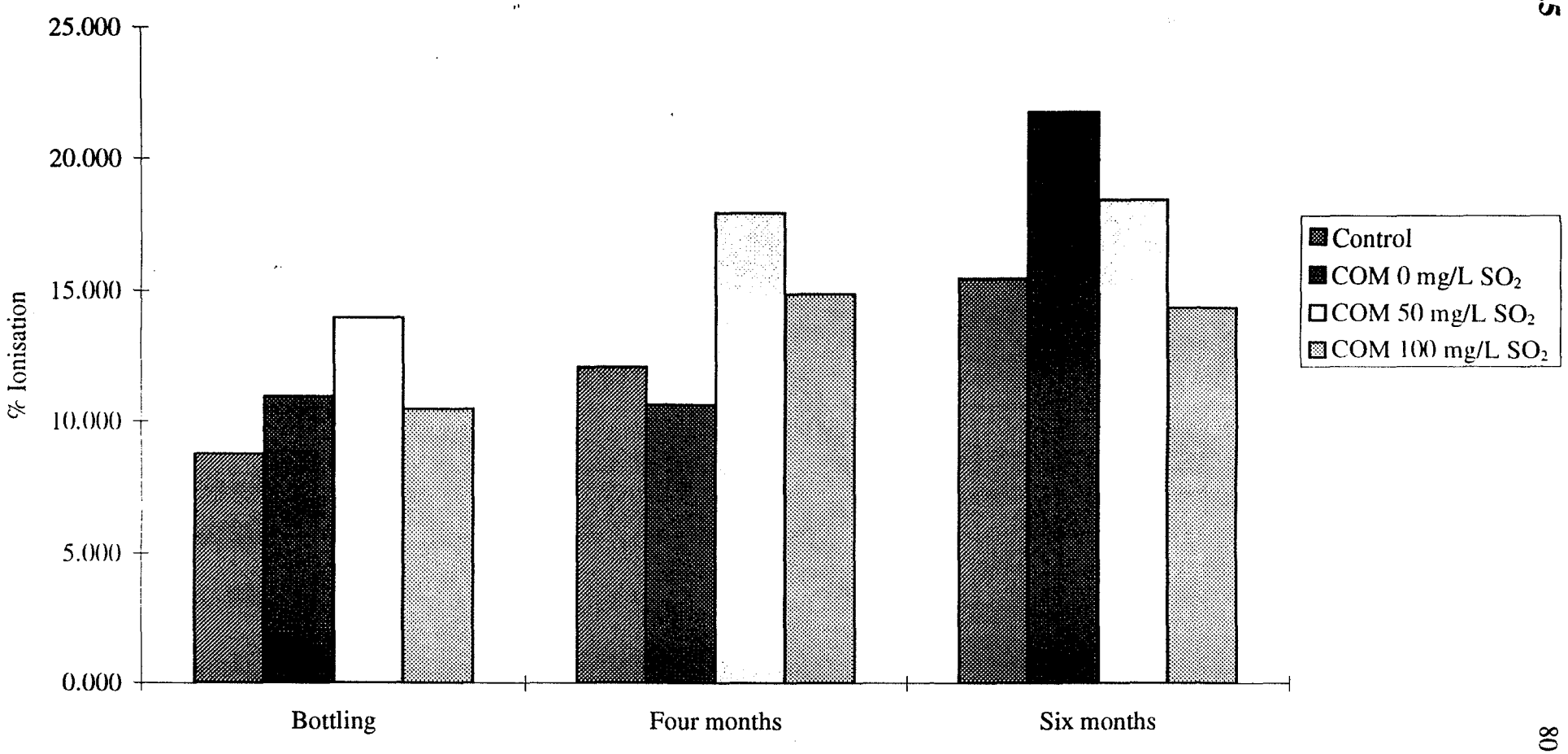
Appendix 4.3


Colour Hue of Pinot noir Wine
Control
COM 0 mg/L SO2
COM 50 mg/L SO2
COM 100 mg/L SO2
Colour hue
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
Bottling
Four months
Six months

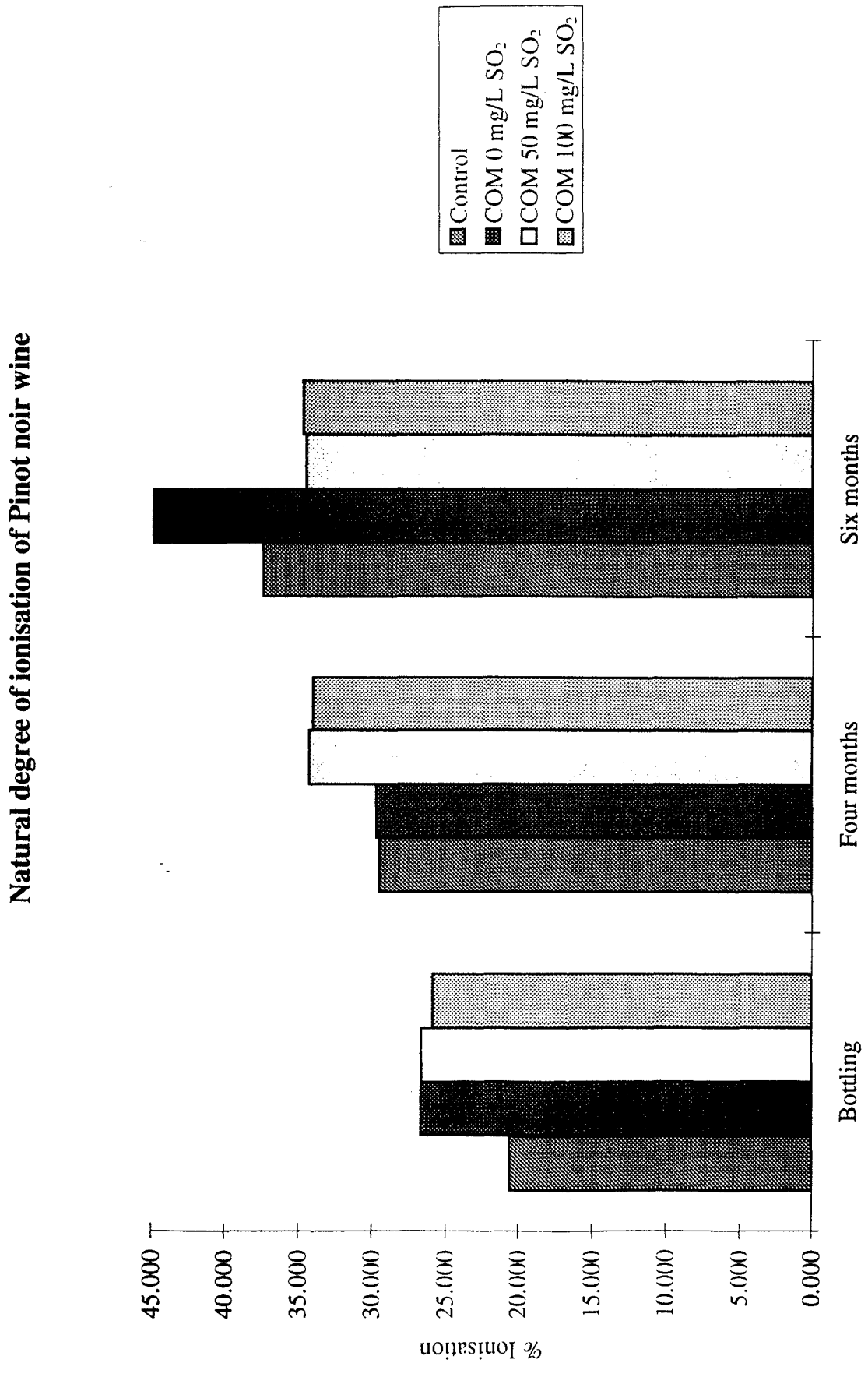
Appendix 4.4


Total Anthocyanin content of Pinot noir wine
Control
COM 0 mg/L SO2
COM 50 mg/L SO2
COM 100 mg/L SO2
Total anthocyanins (mg/L)
300.000
250.000
200.000
150.000
100.000
50.000
0.000
Bottling
Four months
Six months

Ionisation of Anthocyanins in Pinot noir wine

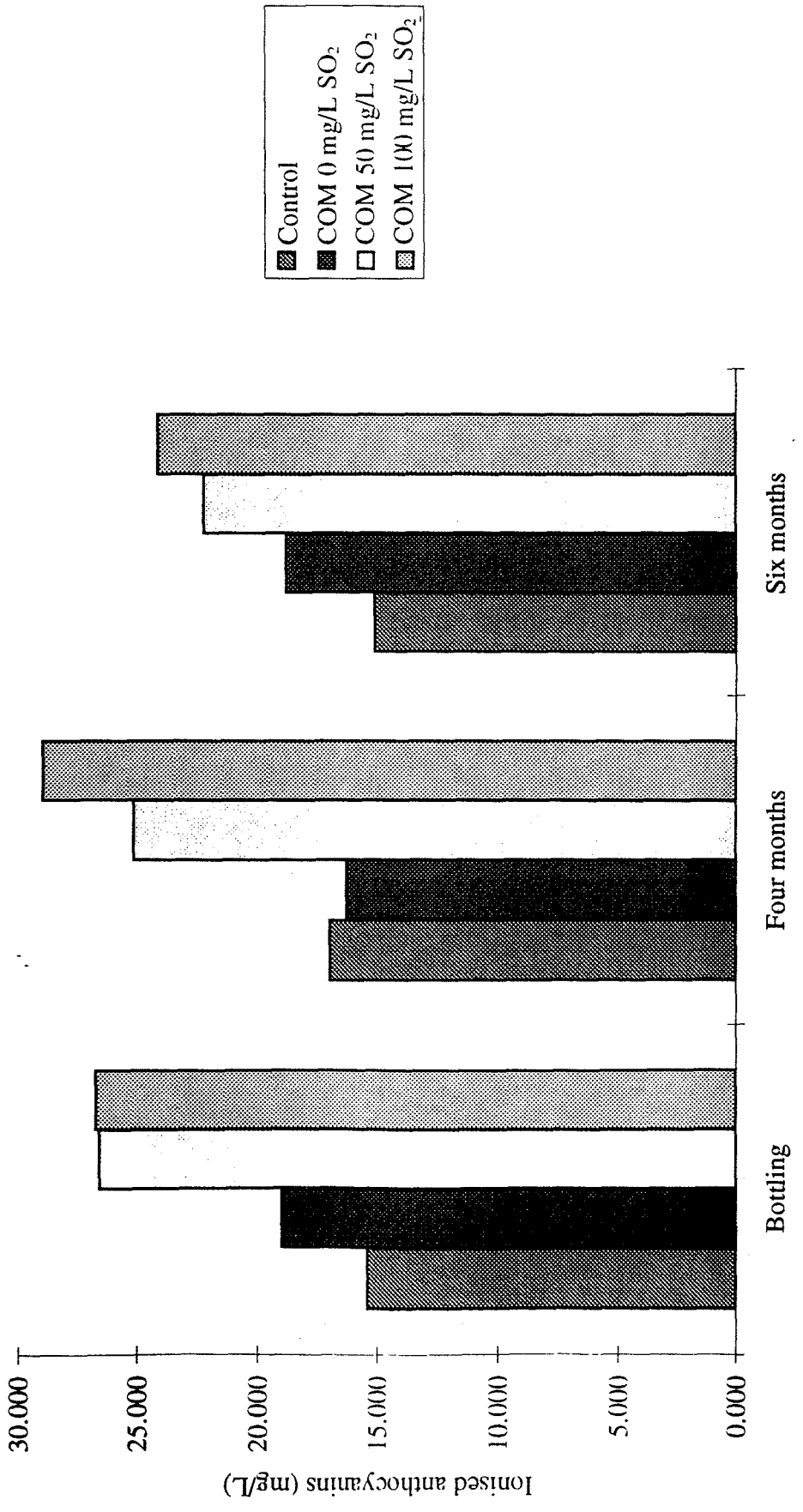


Appendix 4.6

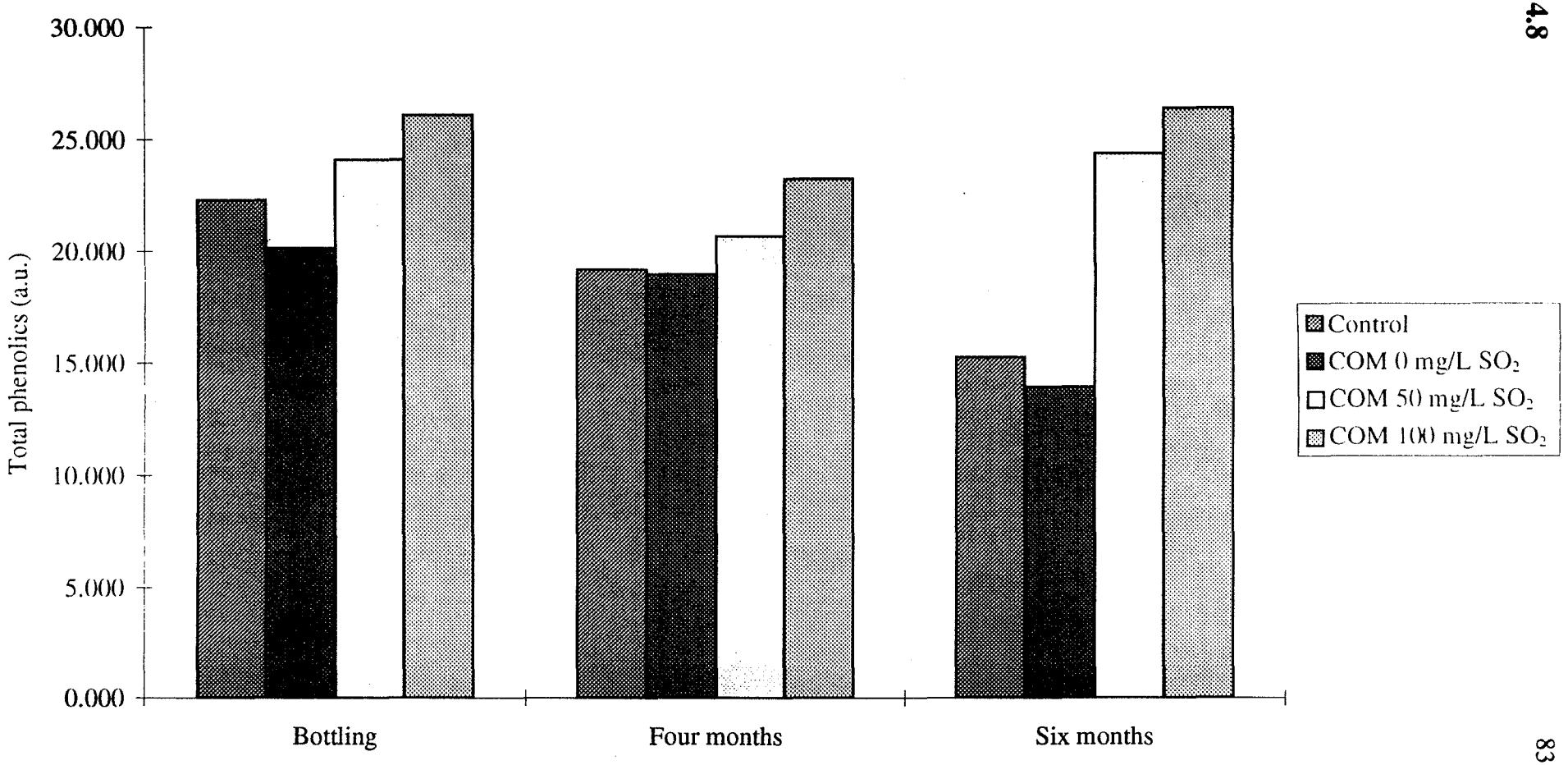

Natural degree of ionisation of Pinot noir wine
Control
COM 0 mg/L SO2
COM 50 mg/L SO2
COM 100 mg/L SO2
% Ionisation
45.000
40.000
35.000
30.000
25.000
20.000
15.000
10.000
5.000
0.000
Bottling
Four months
Six months

Appendix 4.7

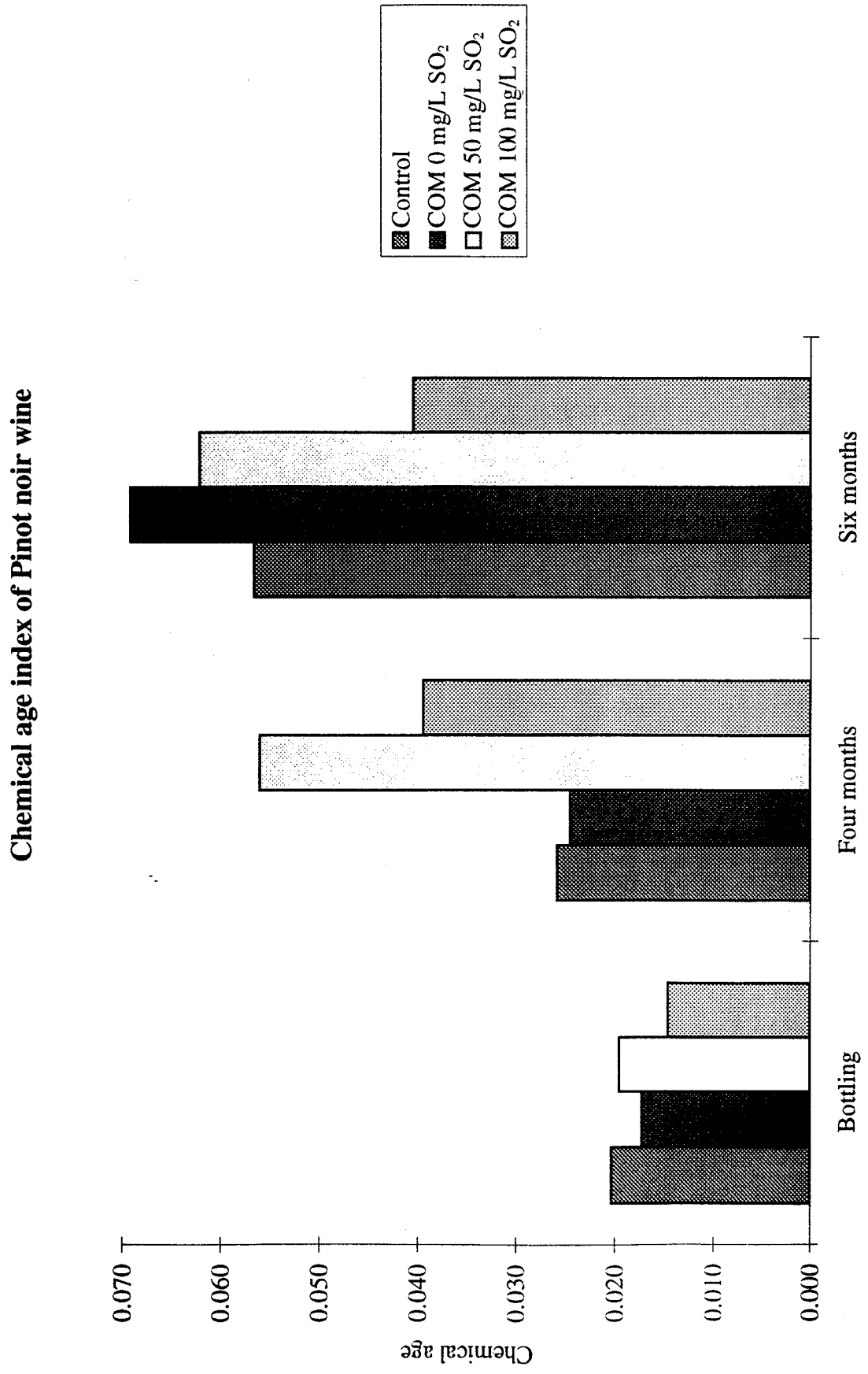
Ionised Anthocyanin content of Pinot noir wine



Total phenolic content of Pinot noir wine



Appendix 4.9


Chemical age index of Pinot noir wine
Control
COM 0 mg/L SO2
COM 50 mg/L SO2
COM 100 mg/L SO2
Chemical age
0.070
0.060
0.050
0.040
0.030
0.020
0.010
0.000
Bottling
Four months
Six months

Anthocyanin profile and content of Pinot noir wine by HPLC at bottling