Investigation of the effects of age, vintage and style on phenolic composition of Pinot Noir wines of a major New Zealand producer

---

A thesis

submitted in partial fulfilment

of the requirements for the Degree of

Doctor of Philosophy

at

Lincoln University

by

Meijing Zhao

Lincoln University

2022

Abstract of a thesis submitted in partial fulfilment of the
requirements for the Degree of Doctor of Philosophy

Investigation of the effects of age, vintage and style on phenolic composition of Pinot Noir wines of a major New Zealand producer

by
Meijing Zhao

Pinot Noir has become the most important red grape variety and built a solid reputation for the New Zealand wine industry. In terms of quality attributes, phenolic compounds are thought to be important for colour, basic tastes, and mouthfeel of red wine. This thesis investigates the phenolic composition of Pinot Noir wines and the potential effects of age, vintage, and style on phenolic compounds as well as wine colour.

The first stage included a general exploration of phenolic compounds of Pinot Noir wine where colour properties, total phenolics, and tannin concentrations of 22 Pinot Noir wines produced by Pernod Ricard Winemakers (New Zealand) Ltd. were determined using spectrophotometric measurements including the modified Somers assay and the methylcellulose precipitable (MCP) tannin assay. Generally, strong age-related trends were observed concerning colour properties and total phenolics, whilst tannin concentration fluctuated between vintages and styles.

The second aim was to focus on separating polymeric phenolics from monomeric phenolics using three different methods including isoamyl alcohol extraction (F-iso), solid phase extraction (SPE), and size exclusive chromatography (SEC). Five polymeric fractions were examined using spectrophotometric measurements. The red colour of polymeric phenolic material in old wines tended to be more resistant to changes in pH as indicated by a reduction in the factor A520 pH1/pH3.4 from 2.6 to 1.1 during ageing, with the degree of ionisation of these bleachable polymeric components increasing during ageing. This result for Pinot Noir wines is the first report of this age-related trend in polymeric material. In addition, colour and phenolic properties and tannin concentrations in extracted polymeric fractions were significantly influenced by wine age and style ($P < 0.05$). Tannin fractionation to some extent contributed to the discrimination of style, for example, MCP tannin in SEC fraction and total phenolics in SEC fractions contributed to the discrimination of super premium wines.

The final aim was to characterise the composition of monomeric and polymeric material using three chromatographic techniques. The monomeric material was analysed using RP-HPLC, while the phloroglucinol adducts of the total isolated polymeric material were determined by both HPLC and Orbitrap-UPLC-MS/MS to explore tannin composition as well as quantification markers of polymeric pigments.

Six monomeric phenolics, total monomeric red pigments and one type of polymeric pigment were significantly affected by vintage, age, and style ($P < 0.05$): i.e. protocatechuic acid, caftaric acid, *trans*-coutaric acid, grape reaction product (GRP), quercetin-3-glucoside, malvidin-3-glucoside, total red pigments, as well as the M3G-C/EC (A type)-F trimers and the corresponding T-A-F (A type)-F adducts (A, malvidin-3-glucoside; F, catechin/epicatechin; T, condensed tannin). General decreasing patterns with age were seen in *p*-hydroxybenzoic acid, caftaric acid, coutaric acid, flavan-3-ols, resveratrol, malvidin-3-glucoside, total red pigments, as well as yield of tannin, mDP (mean degree of polymerisation), average molecular weight, and catechin and epicatechin extension units, and coloured T-A and A-methlymethine-T adducts, whilst general increasing patterns were seen in *p*-coumaric acid, proportions of catechin and epicatechin terminal units, seed-derived epicatechin gallate subunits, and skin-derived epigallocatechin subunits, as well as proportions of A-type polymeric pigments.

Considering wine colour, the decreased proportion of malvidin-3-glucoside, T-A and A-methlymethine-T adducts, as well as the increased proportion of other red pigments measured by RP-HPLC contributed to the change of colour density and hue with age (3-19 years). In contrast, increased proportions of T-A-F (A type), A-F (A type)-T, T-F-A-F (A type) and T-A-F (A type)-F adducts resulted in colour reduction and compositional stability in old wines. The discrimination of one group of regionally distinct wines was driven by monomeric phenolics, whilst the discrimination of super premium wines was driven by the significantly higher colour density, SO_2-resistant pigments, total phenolics, and tannin concentrations, as well as higher proportions of malvidin-3-glucoside-(epi) catechin dimers and the corresponding T-A-F adducts.

With respect to wine age, vintage and style, this study has established three main associations with wine attributes: the first between wine age and colour properties, the second between style and colour and phenolic parameters, and the third between wine age and style, and monomeric and polymeric phenolics. High-quality wines have been found to be positively correlated with higher colour and phenolic parameters, and tannin concentrations, as well as higher proportions of malvidin-3-glucoside-(epi) catechin dimers. Thus, any modifications in winemaking techniques that could improve wine colour, total phenolics, tannin concentration, and malvidin-3-glucoside-(epi) catechin dimers are important to improve the perceived quality of the wine.

Keywords: A-methlymethine-T, age, style, colour, colour density, hue, M3G-C/EC (A type)-F, MCP tannin, monomeric phenolics, polymeric pigments, SO_2-resistant pigments, T-A, T-A-F (A type)-F, tannin composition, total phenolics.

Acknowledgements

I would like to thank Callaghan Innovation, Pernod Ricard Winemakers (New Zealand) Ltd., and Lincoln University for the funding and support.

An enormous thank you to my main supervisor, Associate Prof. Roland Harrison, for his support, guidance, commitments, and encouragement throughout my research. His patience and kindness meant more than he will ever know.

Another massive thank you to my associate supervisors Dr Bin Tian and Professor Paul Kilmartin, for their support, assistance in the laboratory work, and assistance with just about everything over the years.

I would like to thank Andrew Frost and the rest of the team at Pernod Ricard Winemakers (New Zealand) Ltd. for assisting with Pinot Noir wine sampling and the data collection of wine information.

I would like to thank Bruce Morris, Richard Malcolm, and the rest of the team at Hills Laboratories (Hamilton) for assisting with the Orbitrap-UPLC-MS/MS analysis.

A big thanks to the Wine, Food and Molecular Biosciences Department laboratory technicians and lecturers for helping me a lot throughout this project. Mr Karl Gately and Ms Letitia Stipkovits always kept the lab stocked and gave great advice. Mr Richard Hider, thank you for sharing all your knowledge on tannin separation. Ms Jenny Zhao, thanks for your help with HPLC analysis on the phenolic compounds. Dr Leandro Dias Araujo, thanks for all your help with the phloroglucinolysis and gel permeation chromatography analysis at University of Auckland.

I would like to thank all my friends for their tolerance and support on just about everything throughout the journey.

I owe my heartfelt gratitude to my dear parents and sisters, who have assisted me throughout my life. Thanks for your patience, kindness, knowledge, understanding and most of all for your love.

Table of Contents

List of Tables

List of Figures

Abbreviations

A-F	anthocyanin-(epi)catechin
A-F (A type)	anthocyanin-(epi)catechin (A type)
A-F (A type)-F	anthocyanin-(epi)catechin (A type)-(epi)catechin
A-F (A type)-Phloro	anthocyanin-(epi)catechin (A type) extension subunits
A-F (A type)-T	A-F (A type)-condensed tannin
AHC	Agglomerative Hierarchical Clustering
AM3G	malvidin-3-acetylglucoside
A-methylmethine-F-Phloro	A-methylmethine-(epi)catechin extension unit
A-methylmethine-T	A-methylmethine-condensed tannin
Aq	aqueous fraction from SEC
A-T	anthocyanin-condensed tannin
BC	Brancott Commercial wine style
BL	Brancott Letter
Bu	butanol fraction from SEC
C/EC-[M3G-C/EC (A type)]	catechin/epicatechin-malvidin-3-glucoside-catechin/epicatechin (A type)
C/EC-M3G	catechin/epicatechin-malvidin-3-glucoside
CAT	catechin terminal unit
ChemAge	chemical age
ColDen	colour density
COlDen corr.	colour density corrected for SO_2
DelofAn	degree of ionisation of anthocyanins
ECG	epicatechin gallate terminal unit
EPI/epi	epicatechin
F1	fraction 1 from SPE
F2	fraction 2 from SPE
F3	fraction 3 from SPE
F-A	(epi)catechin-anthocyanin
F-A-F (A type)	(epi)catechin-anthocyanin-(epi)catechin (A type)
F-iso	polymeric material retained after isoamyl alcohol extraction of anthocyanins
FS	full scan MS
Gallo	skin-derived epigallocatechin
Galloyl	seed-derived epicatechin gallate
GRP	great reaction product
GPC	gel permeation chromatography
HPLC	high-performance liquid chromatography
M3G	malvidin-3-glucoside
M3G-C/EC	malvidin-3-glucoside-catechin/epicatechin
M3G-C/EC (A type)	malvidin-3-glucoside-catechin/epicatechin (A type)
M3G-C/EC (A type)-C/EC	malvidin-3-glucoside-catechin/epicatechin (A type)-catechin/epicatechin
M3G-C/EC (A type)-phloroglucinol	malvidin-3-glucoside-catechin/epicatechin (A type) extension subunit
M3G-methylmethine-C/EC-phloroglucinol	malvidin-3-glucoside-methylmethine-catechin/epicatechin extension subunits
MCP	methylcellulose precipitable tannin assay
mDP	mean degree of polymerisation

MLF	malolactic fermentation
MOX	micro-oxygenation
MW	average molecular weight measured by phloroglucinol
OS	Central Otago wine style
P-CAT	catechin extension unit
P-ECG	epicatechin gallate extension unit
P-EPI	epicatechin extension unit
PI	product ion scan MS/MS
REML	Restricted Maximum Likelihood
SAXS	small angle X-ray scattering
SC	Stoneleigh Commercial wine style
SEC	size exclusion chromatography
SL	Stoneleigh Latitude wine style
SP	super premium wine style
SPE	solid phase extraction
SR	Stoneleigh Rapaura wine style
T-A	condensed tannin-anthocyanin
T-A-F	condensed tannin-anthocyanin-(epi)catechin
T-A-F (A type)	condensed tannin-A-F (A type)
T-A-F (A type)-F	condensed tannin-A-F (A type)-(epi)catechin
T-F-A-F (A type)	condensed tannin-(epi)catechin-A-F (A type)
TotAn	total anthocyanin
UPLC-MS/MS	ultraperformance liquid chromatography-tandem mass spectrometry

Chapter 1

Introduction

In the global wine market, Aotearoa New Zealand is a relatively small producing country which has nevertheless gained an international reputation, particularly for Sauvignon Blanc. Recently, however, there has been interest from the country's winegrowers in developing Pinot Noir as an additional offering to international wine consumers. In 2022 around 75% of the total red varieties producing area was in Pinot Noir, increasing from 5,488 hectares in 2013 to 5,807 hectares in 2022. Thus, Pinot Noir harvest has seen an 8.7% increase from 31,775 to 34,569 tonnes over the same period. Over the past ten years, Pinot Noir wine exports have also increased gradually to 10.47 million litres in 2022 (New Zealand Winegrower Inc., 2022). Noticeably, Pinot Noir has become the most important red grape variety and built a solid reputation for the New Zealand wine industry. Finally, in the last five years, research funded by Government and industry sources have focussed on improving the profitability of Pinot Noir production in Aotearoa New Zealand through improved understanding of the factors that control the relationships between yield and quality.

In terms of quality attributes, phenolic compounds significantly impact upon the mouthfeel and colour of red wine. These phenolic compounds are diverse and complex, and it is difficult to obtain compositional and structural information of monomeric and polymeric phenolics merely using one or two methods. Wine phenolics include anthocyanins, flavan-3-ols, and modified polymeric pigments. Anthocyanins can react with low molecular weight compounds such as acetaldehyde and pyruvic acid to create pyranoanthocyanins, as well as react with flavan-3-ols to form polymeric pigment. Furthermore , few studies have focused on characterising polymeric phenolics in Pinot Noir wines with different styles and quality levels. In addition, there is little information on different types of polymeric phenolic material between young Pinot Noir wines and older wines.

1.1 Project aims

Commencing in 2018, a PhD research project on characterising the phenolic compositions of New Zealand Pinot Noir was funded by R&D Student-Fellowship Grant between Callaghan Innovation, Pernod Ricard Winemakers New Zealand Limited, and Lincoln University. Commercial wines available to and selected by Pernod Ricard were used in undertaking this research. The ultimate goal of this project was to characterise polymeric phenolic material in Pinot Noir wines using multiple chemical and physico-chemical methods that might differentiate wines according to style and/or quality. It was intended that the results from this project would provide insights regarding complex phenolic

polymers present in Pinot Noir wines with respect to different vintages, styles, and grades of wine quality.

The emphasis and focus during the first stage of this project was to examine the whole unfractionated wine by spectrometric measurements including colour, total phenolics, and tannin concentrations (Chapter 4). This data would provide information on the change of wine colour and tannin and the potential impact of styles and quality. The second stage of this research project involved an attempt to separate polymeric phenolics from monomeric phenolics using three different methods including isoamyl alcohol extraction, solid phase extraction, and size exclusive chromatography. A total of six fractions were examined (Chapter 5). This data gave some insight into the potential importance of the nature of the polymeric material itself.

Finally, compositional information was obtained of both monomeric and (through chemical depolymerisation) polymeric material using chromatographic techniques (Chapter 6). Compounds were identified and quantified with methods developed specifically for monomeric and polymeric phenolics. These included: i) quantification of monomeric phenolics using RP-HPLC; ii) profile of tannin composition using phloroglucinolysis; iii) measurement of markers of polymeric pigments using combined phloroglucinolysis and UPLC-MS/MS methods. Overall, the data were assessed in terms of their potential importance and role in discrimination of wine age, vintage, and style.

1.2 Thesis structure

Including this introduction, this thesis contains seven chapters. Chapter 2 is a literature review providing insight on the thought process surrounding the sequencing of the project. This chapter includes relevant past and current studies completed on Pinot Noir wines. Chapter 3 describes the materials (in particular the wines) and methodologies used in the thesis. Chapters 4, 5 and 6 include introduction, results, and discussion for each aspect of the project described above. Chapter 7 provides the overall conclusions from the project and suggestions for future research. A complete list of references is at the end of Chapter 7 listed alphabetically.

Chapter 2

Review of Literature

Phenolic compounds are a determinant factor for red wine quality because they have an impact on important sensory attributes such as colour, bitterness, and astringency (Gutierrez-Escobar et al., 2021; McRae & Kennedy, 2011; Pittari et al., 2021). The focus of this chapter is to give some insight into the wine phenolic constituents typically present in both grapes and wines as well as the factors such as vineyard practices and winemaking management that give rise to them and their influence on sensory attributes.

2.1 Phenolic compounds in wines

In this section, a brief overview of phenolic compounds found in wine is given, subdivided into those found in grapes and those formed during winemaking and ageing (Figure 2.1).

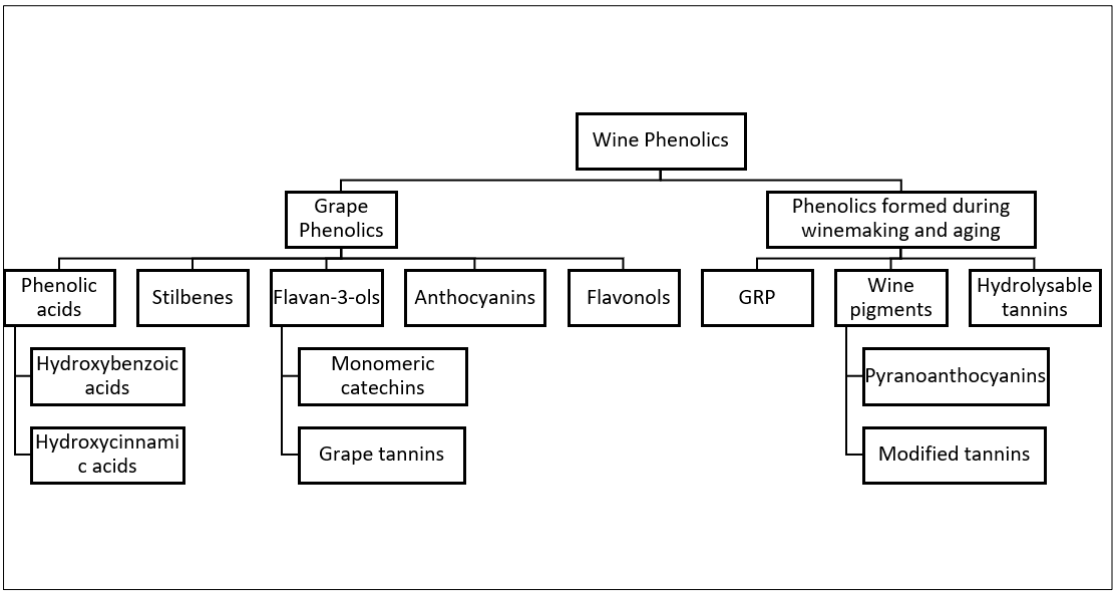


Figure 2.1 The principal phenolic compounds present in grapes and wines

2.1.1 Grape phenolics

Grape phenolics are mainly found in the skin and seed of the berry, while the juice and pulp have much lower concentrations (Kennedy, 2008). Grape-derived phenolics constitute a diverse group of secondary metabolites with several components: phenolic acids, stilbenes, flavan-3-ols, anthocyanins, and flavonols. The structure and composition of grape phenolics can be altered by fermentation reactions and chemical reactions during wine ageing.

2.1.1.1 Phenolic acids

Phenolic acids are usually divided into two main groups: hydroxycinnamates and hydroxybenzoic acids which may occur either in their free or conjugated forms. Coumaric acid, caffeic acid, and ferulic acid are the three common hydroxycinnamates. Their tartaric esters or diesters are preferentially formed in grapes, but these are susceptible to hydrolysis resulting in simple hydroxycinnamic acids being released in early finished wines. The hydroxycinnamates have been reported to produce bitterness and astringency in water (Somers & Vérette, 1988), but levels of these compounds were below the sensory threshold in wine. Perestrelo et al. (2020) observed that caftaric acid and coutaric acid were the most abundant phenolic compounds.

Hydroxybenzoic acids are found in grapes with small amounts of syringic, protocatechuic, and vanillic acids, while relatively high amounts of gallic acid are only found in wines which have been in contact with oak (Waterhouse et al., 2016).

2.1.1.2 Stilbenes

Resveratrol is the dominant stilbene and is generated by vines as glucosides in response to fungal infections. Resveratrol derivatives are found largely in the skin of the grape, which contributes to the higher amounts existing in red wine (Jeandet et al., 1995). Oligomers of resveratrol have been reported to be the actual antifungal compounds (Waterhouse, 2002). This work also showed that resveratrol and its O-methylated derivative pterostilbene displayed antimicrobial activity against wild yeasts and acetic acid bacteria (Vestergaard & Ingmer, 2019).

2.1.1.3 Flavonoids

Flavonoids are characterised by a three-ring system with a C6-C3-C6 structure. They are generally grouped into several classes: anthocyanins, flavonols, flavones, flavanes, flavan-3-ols, flavononols, flavanones, chalcones and dihydrochalcones. The main types of flavonoids in grapes and wines are flavonols, flavan-3-ols, anthocyanins and flavonols, thus they are discussed specifically.

2.1.1.3.1 Flavan-3-ols

Flavan-3-ols possess a saturated C ring and hydroxyl substitution group at the C3 position on the C-ring. Grape seed extracts contain three monomers (catechin, epicatechin and epicatechin gallate) and procyanidin oligomers, whereas grape skin extracts contain four monomers: catechin, epicatechin, gallocatechin and epigallocatechin (Mattivi et al., 2009). Flavan-3-ol molecules polymerise to form the grape oligomers and polymers, namely, condensed tannin or proanthocyanidins, sometimes quantified in terms of mean degree of polymerisation (mDP). The concentrations of condensed tannin ranged from 1.2 to 3.3 g/L, and grape tannins account for 25-50% of total phenolic compounds in a red wine (Singleton, 1992; Watrelot & Norton, 2020). The mDP of condensed tannins ranged from 4 to around 35, while skin tannin contains a higher mDP (20-35) and a smaller proportion of galloylated units (3-

6%) compared to that for the seed tannin (mDP, 4-11; galloylated units, 13-29%) (McRae & Kennedy, 2011). The low molecular weight of seed tannins may relate to the bitterness sensation in wine (Chira, et al., 2011). The increased number of monomeric units result in a decrease in the solubility of proanthocyanidins (Cheynier, 2000; Harbertson et al., 2002). *Vitis vinifera* grapes have a higher concentration of seed tannin (mg/g berry), compared to the skin tannin at harvest maturity (del Rio & Kennedy, 2006; Guaita & Bosso, 2019; Kyraleou et al., 2017). Watrelot and Norton (2020) summarised tannin concentration and composition in *Vitis vinifera* grape skins and seeds. It was found that Pinot Noir at harvest maturity had the lowest seed tannin concentrations (2-5 mg/g berry), followed by Syrah, Albarossa, Cabernet Sauvignon (33.5 mg/g berry), Barbera, and Nebbiolo (74 mg/g berry). Regarding the mDP in seed tannin, Cabernet Sauvignon showed the lowest mDP (3.8), followed by Barbera and Nebbiolo (4.1), Albarossa (5.2), Pinot Noir (6.9), and Syrah (8.0). With respect to skin tannin, Pinot Noir also showed the lowest skin tannin concentration (0.76 mg/g berry), followed by Syrah and Cabernet Sauvignon (1.0 mg/L berry), Barbera, Albarossa, and Nebbiolo (19.2 mg/g berry), whereas Albarossa had the lowest mDP (13.8), followed by Barbera (14.8), Nebbiolo (24.2), Pinot Noir (27), Cabernet Sauvignon (28), and Syrah (31).

The most common linkages between flavan-3-ols subunits are C4→C8 and C4→C6 positions (Monagas et al., 2005) while small amounts of A-type grape tannin, where an additional ester bond is found between C7 or C5 position from the lower subunit and C2 from the upper subunit. A-type grape tannins are produced in grape seeds (Herderich & Smith, 2008; Yilmaz & Toledo, 2004). However, wine oxidative ageing processes are likely to affect the formation of A-type tannins as demonstrated by oxidation under model wine conditions (He et al., 2008).

A number of methods for the analysis of naturally occurring grape tannins (proanthocyanidins) have been widely adopted (Duley et al., 2021; Kennedy & Jones, 2001). The HCl-butanol assay has been used and improved to estimate the total content of proanthocyanidins (Grabber et al., 2013). Phloroglucinolysis or thiolysis reveals the average subunit composition of proanthocyanidins and mean degree of depolymerisation (mDP) (Kennedy & Jones, 2001; Kyraleou et al., 2017; Wollmann & Hofmann, 2013). NMR enables more structure related quantification including the ratio of procyanidin to prodelphinidin (Duley et al., 2021). Engström et al. (2014) developed a rapid ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method for the qualitative and quantitative analyses of plant proanthocyanidins containing procyanidin and prodelphinidin without the depolymerisation step. Briefly, this method utilizes in-source collision induced dissociation to achieve the degradation of the proanthocyanidins followed by the single reaction monitoring to fragment the degradation products. The quantification markers for procyanidin and prodelphinidin extension and terminal units were used to achieve group-specified detection. The extension units had the fragmentations either at m/z 289 (terminal units) or m/z 287 (extension units), and prodelphinidin

at m/z 305 (terminal unts) or m/z 303 (extension units). In this method the original information about the composition of the proanthocyanidins is retained by the 2D chromatographic profiles (Salminen, 2018).

The structure and concentration of proanthocyanidins affect astringency attributes in red wine. The structure of proanthocyanidins vary in terms of flavan-3-ols subunits, the type of interflavan linkages connecting these subunits, mDP or size of tannins, and conformations of longer chain proanthocyanidins (Cheynier et al., 2006). Ju et al. (2021) noted that sensory evaluation and quantitative astringency levels were remarkably affected by the concentrations and compositions of condensed tannin in Spanish grapes. It was found that mDP had a positive correlation with astringency. Proanthocyanidins can also affect the stability of colour due to the reaction between grape tannins and anthocyanins, which results in the formation of stable polymeric pigments.

2.1.1.3.2 Anthocyanins

Anthocyanins are characterised by a fully aromatic, positively charged C-ring. Anthocyanins develop during véraison and are responsible for the change in colour of the skin from green through red to deep purple; they are found in the outer layers of skin cells (Kennedy, 2008). The major aglycones (anthocyanidins) of the anthocyanins in grape and wine are malvidin, cyanidin, delphinidin, petunidin, and peonidin (Cheynier et al., 2006). Pinot Noir is found to possess only these 5 anthocyanidins coupled with glucose at the C3 position. Malvidin-3-glucoside (M3G) and malvidin-3-acetylglucoside (AM3G) are the most abundant anthocyanins found in *Vitis vinifera* (de Freitas & Mateus, 2011a). Zhao et al. (2022) noted that M3G, peonidin-3-glucoside, and cyanidin-3-glucoside were more stable than delphinidin-3-glucoside and petunidin-3-glucoside in model wine. Other anthocyanins have also been reported concerning *V. vinifera* grapes such as malvidin-3-pentoside and malvidin-3-(6-feruloyl)-glucoside (Hermosín‐Gutiérrez et al., 2020).

In wine solutions, different structural forms of anthocyanins are in equilibria according to the pH, hydration, and the concentration of various co-factors (copigments) in the wine matrix (Bimpilas et al., 2016a; Morata et al., 2007; Trouillas et al., 2016). Copigments include catechin, epicatechin, phenolic acids, flavonols, and procyanidins (Asenstorfer et al., 2006).

The flavylium cation form (AH^+) that occurs dominantly at low pH (below 2) is red in colour, while the quinoidal base (A) and anionic quinoidal base (A^-) forms predominate as the pH rises and exhibit the colour of purple and blue, respectively. At pH 3.5, the quinoidal base is predominantly formed in wine rather than the flavylium ion (Asenstorfer et al., 2006). The hemiketal epimers (AOH) are formed by the hydration of flavylium cation at the C2 position and they are colourless, while the *trans*- or *cis*-chalcone isomers exhibit yellowish colour.

Bleaching occurs when the flavylium form reacts with the bisulphite nucleophile at the C4 position and disrupts the conjugation on the C-ring (Cheynier et al., 2006). In acidified solution (pH 1.0) all anthocyanins that are not bound in stable co-pigmented complexes are converted to the red cationic form and can be detected at 520 nm. This value is considered relative to the absorbance in model wine solution (pH 3.4) containing 0.375% sodium metabisulphite and consequently gives an estimate of the concentration of anthocyanin that has not been stabilised (Mercurio et al., 2007).

2.1.1.3.3 Flavonols

Flavonols contain a keto group at position C4 and an unsaturated bond between C2 and C3 and comprise a diverse range of glycosides and six flavonol aglycones. The principal aglycones include quercetin, myricetin, and laricitrin. Flavonols are found in the berry skin where they function to absorb UV radiation. High sunlight exposure increased the concentration of flavonols during pre-véraison period (Downey et al., 2004; Price et al., 1995a; Spayd et al., 2002).

2.1.2 Phenolics formed during winemaking and ageing

2.1.2.1 Grape reaction product (GRP)

Grape reaction product (GRP) is a quinone-thiol adduct formed during winemaking. During grape crushing and pressing, caftaric acid quinone is formed via enzymatic oxidation by polyphenol oxidase (PPO), which leads to browning in grape juice (Singleton, 1992). The subsequent chemical reaction between caftaric acid quinone and glutathione (GSH) leads to the formation of grape reaction product. High amounts of GSH in grapes decreased the browning in juice (Cheynier, 2012).

2.1.2.2 Hydrolysable tannins

Hydrolysable tannins are found in oak-treated wines and consist of gallic acid and ellagic acid esters of glucose or related sugars. The ester linkage of hydrolysable tannins is more susceptible to hydrolysis than the interflavan linkages of condensed tannins. Gallic acid and ellagic acid were formed on ageing. Large amounts of castalagin, vescalagin, and the roburins were extracted into wine from oak (Cadahia et al., 2001). The hydrolysable tannins are unlikely to affect the astringency sensation in wine (Li & Duan, 2019; Stark et al., 2010). Engström et al. (2015) developed a rapid ultraperformance liquid chromatography−triple-quadrupole mass spectrometry method (UPLC-QqQ-MS/MS) for ellagitannins and gallic acid derivatives.

2.1.2.3 Wine pigments

During the process of winemaking, anthocyanins form co-pigmented complexes including through self-association, as well as react with low molecular weight compounds such as acetaldehyde (Forino et al., 2020), pyruvic acid, cinnamic acids, monomeric flavan-3-ol (Fulcrand et al., 1998) and vinylphenol to create an anthocyanin-derived pigment family, called pyranoanthocyanins (Escott et al., 2016; Li &

Duan, 2019). These anthocyanins derivatives are thought to be important to the colour development and colour stability of red wines (He et al., 2010). In addition, anthocyanins can react with flavan-3-ols to form polymeric pigments in wines.

Self-association of anthocyanins occurs through hydrogen bonding (Hoshino et al., 1982). The number of hydroxyl and methoxyl groups on the B-ring had a positive effect on the self-association (Tsutomu, 1992). Lambert et al. (2011) suggested that malvidin-3-glucoside were more likely to undergo self-association rather than association with catechin, quercetin-3-glucoside, caffeic acid, and quercetin. The effect of self-association of anthocyanins on wine colour is controversial. The study by Boulton (2001) showed that self-association was not relevant to colour change in young red wines, while other studies concluded that it contributed to 8% to 60% of the absorbances increase at 520 nm (González-Manzano et al., 2009; Somers & Vérette, 1988).

Copigmentation also occurs when anthocyanins associate with non-coloured compounds (copigments) in wine. The anthocyanin-derived pigments such as pyranoanthocyanins also participate in the copigmentation to form noncovalent complexes. Malvidin-3-glucoside (M3G) was the most important anthocyanin involved. Both the flavylium cation and quinol base forms of anthocyanins are involved in the copigmentation. The copigments include a wide range of phenolic compounds, such as catechin, epicatechin, hydroxycinnamates, flavonols, and procyanidins (Asenstorfer et al., 2006).

It was concluded that binding constants (K) with flavones and flavonols were the highest ($K > 10^3\ M^{-1}$), followed by hydroxycinnamic acids ($K = 2\text{-}4 \times 10^2\ M^{-1}$), flavanols ($K = 1\text{-}2 \times 10^2\ M^{-1}$), and hydroxybenzoic acids ($K < 10^2\ M^{-1}$) (Ferreira da Silva et al., 2005; Galland et al., 2007; Leydet et al., 2012; Nave et al., 2012; Tsutomu, 1992). Zhao et al. (2022) suggested that the addition of quercetin-3-glucoside acting as a copigment in model wine had a positive effect on the stability of anthocyanins, regardless of its amount (0.1 mM or 0.4 mM). Trouillas et al. (2016) reviewed the relationships between structure-affinity and the stability of copigmentation. Copigmentation was affected by the extension of the π-conjugated system beyond the phenolic ring. The concentration of pigments and copigments in red wine affected the copigmentation. Cruz et al. (2010) noted that compared to procyanidin, vinylcatechin dimers showed higher affiliation with M3G and higher binding constants were observed. Surprisingly, Rustioni et al. (2012) reported that anti-copigmentation occurred with certain phenolic compounds and produced a colour loss. Anti-copigmentation thus was an unexpected hypochromic effect rather than the hyperchromic effect due to copigmentation.

Many studies had proved that copigmentation and phenolic composition influenced the wine colour (Bimpilas et al., 2016a; González-Manzano et al., 2009; Lambert et al., 2011). Bloomfield et al. (2003) noted that the addition of *p*-coumaric acid enhanced the colour in Pinot Noir and Cabernet Sauvignon (CS) wines. In young red wines, 30% to 50% of the colour are produced from copigmentation. Heras-

Roger et al. (2016) noted that copigmentation enhanced the wine colour hue. Copigmented wines had a higher mean value of CIELab units than non copigmented wines. It also suggested that there was a relationship between the phenolic compound and wine colour due to the copigmentation. The main relations were obtained with coumaric, coutaric acid, resveratrol, and flavonol. These compounds may react with the anthocyanins to form copigmented anthocyanins.

Pyranoanthocyanins are anthocyanin-derived pigments such as vitisin A, vitisin B, pinotins oxovitisins, and portisins (de Freitas & Mateus, 2011a; Oliveira et al., 2009). Vitisin A and B are the products of the reaction between anthocyanins and pyruvic acid and acetaldehyde, respectively (Fulcrand et al., 1998). Morata et al. (2007) noted that the addition of pyruvic acid and acetaldehyde to young red wine produced higher concentrations of vitisin A (around 2% of the total anthocyanin content) and vitisin B (1.35%). In contrast, the monomeric anthocyanins decreased by 81.5%. Separate additions of pyruvic acid and acetaldehyde also led to the formation of *p*-coumaroylvitisin A and *p*-coumaroylvitisin B. These pigments are more stable when subjected to pH changes or reactions with SO_2 than are anthocyanin monomers (Cheynier et al., 2006). Consequently, their formation has a significant effect on wine colour stability (de Freitas et al., 2004). In contrast, the concentrations of vitisin B diminished with wine storage time (Boido et al., 2006).

Pinotin is generated by the reaction between anthocyanins and hydroxycinnamates with a cyclization with the 5-OH group of the anthocyanin. They contribute to the red pigmentation of aged wine. He et al. (2010) first found oxovitisins in aged Port wine and these contributed to the yellow pigmentation of aged wine. Oxovitisins are the oxidation products of vitisin A. Pyranoanthocyanins are prone to react with phenols and flavanols to form new anthocyanin derivatives (de Freitas & Mateus, 2011b). Vinylpyranoanthocyanin-flavanol portisin also occurred in ageing wine and exhibited a bluish colour at acidic pH (Mateus et al., 2004).

When malvidin-3-glucoside reacts with acetaldehyde and catechin or epicatechin, ethyl-linked pigments are formed. Acetaldehyde can react as an electrophile to form ethyl bridges between anthocyanins and flavan-3-ols. These ethylene-bridged pigments show a bathochromic (red) shift and are less susceptible to colour loss by SO_2. They contribute to the purple colour present in young wines (Lee et al., 2004). Asenstorfer et al. (2006) investigated the structure of anthocyanin-derived pigments including vitisin A and diastereoisomers of 8,8-ethyl-linked pigments. It was suggested that their structures improved the stability of these pigments. Acetaldehyde may facilitate reactions between flavan-3-ols and anthocyanins to yield alkyl or aryl linked adducts (Es-Safi et al., 1999; Fulcrand et al., 1996). In the case of acetaldehyde, the formed adducts are referred to as A^+-ethyl-T adducts (Fulcrand et al., 2006). Zeng et al. (2016) identified the extension subunits of M3G-methylmethine-(epi) catechin-

phloroglucinol using UPLC-MS/MS and postulated the phloroglucinol mechanism for the polymeric pigments A-methylmethine-T.

Polymeric pigments refer to the production of polymers formed by the interaction between condensed tannins or proanthocyanidins (T) and anthocyanins (A). They influence the colour and astringency of red wine and are resistant to sulphur dioxide bleaching (Bimpilas et al., 2016a; Bimpilas et al., 2015; Cheynier et al., 2006). As these are the most prevalent form of stable pigment in Pinot Noir wines, they have been given more detailed consideration in this thesis than other forms of stable pigment. The mechanism of the polymerisation reaction between anthocyanins and flavan-3-ol monomers or tannin has been studied since the 1960s. Anthocyanins can react as either nucleophilic or electrophilic agents (Hayasaka & Kennedy, 2003). T-A^+ type pigments are produced when the C4 position of flavan-3-ols is bonded with the C8 or C6 position of the nucleophilic anthocyanins. Anthocyanins become the terminal unit in its flavylium form (A^+) and thus T-A^+ polymers are stable coloured adducts. They become predominant and improve the colour during wine storage in a modified environment and protect against oxygen. Anthocyanins also exhibit a strong electrophilic character at their C4 position in the flavylium form. This C4 can undergo a nucleophilic attack from the C8 or C6 of the final extension unit of grape tannins. This reaction leads to the formation of colourless polymers (A-T). As a result, anthocyanins are present as their flavene form. The A(flavene)-T type derivative can undergo oxidation (Cheynier et al., 2006; Fulcrand & Cheynier, 2004). This can regenerate the anthocyanin moiety in its flavylium form leading to the formation of a new coloured pigment (A^+-T). Alternatively, intramolecular binding can occur to produce an A-type linkage between the flavan-3-ols and anthocyanin unit and thus to produce a colourless polymeric compound, A-T (A type) derivative (Fulcrand et al., 2006; Remy-Tanneau et al., 2003; Salas et al., 2004; Salas et al., 2003).

2.2 Factors influencing phenolics compounds found in grapes and wines

As previously mentioned, phenolic acids, flavan-3-ols, stilbenes, anthocyanins, and flavonols originate from the grapes themselves, while GRP, pyranoanthocyanins, polymeric pigments, and hydrolysable tannins are generated through processes occurring during fermentation and ageing. As a result, the profile and concentration of phenolic compounds in wines are affected by many factors. Below, the effect of vineyards practices and winemaking management have been reviewed.

2.2.1 Vineyard factors

Genetic variation means that some varieties produce distinctive and higher concentrations of phenolic compounds than others. Other factors relate to vineyard sites and practices (climate conditions, harvest time, sun exposure, and canopy management) and also play an important role in the profile and concentrations of phenolic compounds in grapes.

2.2.1.1 Grape variety

Grape variety has proved to be one of the decisive factors to affect the phenolic compound profile and concentrations; for example, the red grape cultivar Tinto Fragoso had higher amounts of galloylated flavan-3-ols and stilbenes in seeds and myricetin 3,4'-diglucoside was also found first time in *V. vinifera* grapes by Pérez-Navarro et al. (2019). Many studies have proved that the content and phenolic composition of skins and seeds were crucial to differentiate the grape cultivars. Red grape varieties (Malbec, Cabernet Sauvignon, and Merlot) had higher amounts of procyanidin B1, procyanidin B2, kaempferol, myricetin, and *trans*-resveratrol than white grape varieties (Albariño, Chardonnay, and Gewürztraminer)(González-Manzano et al., 2009). Nikfardjam et al. (2015) had studied the differences of commercial red wines from Württemberg region for their phenolic composition. The result showed that Pinot Noir wines from this region contained the highest amounts of monomeric anthocyanins among 37 red wines, whereas Trollinger wines had the highest proportion of polymeric pigments. In addition, no significant differences were found in the mean degree of polymerisation.

Blanco-Vega et al. (2014) investigated over 280 red wines regarding grape cultivars and age by comparing the concentration and distribution of anthocyanin-related pigments. It was highlighted that pigment concentration varied considerably due to the diversity of grape varieties and wine age. The study found that Garnacha wines had the lowest concentration of anthocyanins (71 mg/L), while the highest was Petit Verdot (161 mg/L). Monomeric anthocyanins in Garnacha also contributed the lowest molar percentage of low molecular weight red pigments (77.5%), whereas Tempranillo contributed the highest percentage (90.3%). Regarding the anthocyanin derivatives, Cencibel wines had the highest contents and molar percentages of direct flavanol-anthocyanin adducts, whereas the lowest ones were found in Garnacha and Petit Verdot wines. Petit Verdot wines had the greatest content and molar percentages of the flavanol-ethyl-anthocyanin adducts. The content of vitisin A and B was not statistically different between wines from different cultivars, but the molar percentages of vitisin A were lower in Garnacha wines and higher in Merlot, Syrah, and Tempranillo wines. Syrah contained the highest concentrations of B-type vitisins. Garnacha were characterised by the greatest contents and molar proportions of 10-(3''',4'''-dihydroxyphenyl)-pyranoanthocyanins, and they were the important contributors to low molecular weight pigments in young wines. Garnacha grape had abundant hydroxycinnamic acid derivatives, which are the precursor reactants to form hydroxyphenyl-pyranoanthocyanins. In addition, Cabernet Sauvignon, Merlot, Petit Verdot, and Syrah wines had the greatest content of 10 flavanol-pyranoanthocyanins, whereas Merlot young wines were observed to have the greatest molar percentages.

Clonal selection could control the grape variability in the vineyard to achieve wine quality targets. Due to the genetic instability, there are various clones of Pinot Noir identified and the variation of clones may also affect the phenolic composition. For example, Casassa et al. (2021) found that Pinot Noir

wines from clones 777 and 2A had higher amounts of phenolic contents than those from clone 115. Farquhar (2001) evaluated the quality performance of the clones of Pinot Noir in Tasmania. Their mouthfeel attributes were found to be discriminant for the clones of Pinot Noir in the cool climate region.

2.2.1.2 Terroir

Terroir refers to the interactive ecosystem between environmental factors as well as human activity in the vineyard (Seguin, 1986). It involves climate conditions, soil type, and viticultural and oenological practices managed by winegrowers and winemakers. Grapes from a representative terroir are considered as the reflection of their origin in terms of their chemical compositions, including the phenolic profile, thus the corresponding wine is also the reflection of origin (Karaoğlan et al., 2015; Knight et al., 2015; Roullier-Gall et al., 2014). The profiles of monomeric anthocyanins, flavan-3-ols, stilbenes, and phenolic acids of grape are found to be directly influenced by terroir. The climate conditions, soil, and viticultural practices of those phenolic compounds in grape and wine are taken into consideration. For example, Jeandet et al. (1995) reported that the higher levels of *trans*-resveratrol related to the more pronounced fungal disease stress of *Botrytis cinerea* infection. The vineyard elevation is also positively related to the high stilbenes production in vine (Bavaresco, 2003).

Karaoğlan et al. (2015) characterised the relationship between terroir and the phenolic compounds of Muscat of Bornova wine from 3 wine regions in Turkey. The terroir of these grape growing sub-regions is unique in terms of climate (continentality), elevation level, and soil type. The fertility of soil was compared from the aspects of nitrogen, phosphorus, potassium, and organic material levels. Menderes region had the most fertile soil consisting of sand and loam, followed by Halilbeyli. Kemaliye has a distinct soil type consisting of 30% of lime, which provided more heat to grape and grapevine. In addition, the colour of soil also differed due to Fe, Na, Mg, and Ca elements, affecting the radiation gained from light reflection on the soil. Lower contents of caftaric acid and *p*-coutaric acid were observed in wines from Kemaliye compared to the other two regions, supporting the suggestion that limestone soils could inversely impact the concentration of those two acids (Lampir & Pavlousek, 2013). Wines from Halibeyli had the highest caftaric and *p*-coutaric acid, 80.01 mg/L, and 25.3 mg/L respective, and thus the red hue lasted for 4 months during storage. Wines from Halilbeyli also had the highest phenolic content. Sensory attributes were significantly impacted in terms of colour intensity, astringency, and bitterness due to the distinct terroir in each region.

Roullier-Gall et al. (2014) attempted to discriminate between different terroir by characterising the diversity of chemical composition of Pinot Noir from two vineyards in the Cote de Nuits across vintages 2010, 2011, and 2012. Two vineyards Flagey-Echezeaux (FE) and Vosne Romanee (VR) were recognised as two distinct terroirs. The grapes and related wines were processed by the same winemaker. The

Pinot Noir grape extracts and corresponding wines were analysed using Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR-MS) to obtain the spectrum of thousands of metabolites and thus fingerprints of chemical composition of each vineyard. Results revealed that geographical discrimination was achieved depending on difference of phenolic compounds for wines from the same vintage. VR wines were well separated from FE wines. Higher amounts of gallic acid, hydroxytyrosol, catechin, caffeic acid, malvidin, and resveratrol were seen in VR wines. In contrast, isoquercitrin, *cis*-piceid, quercitrin, and quercetin were higher in FE wines. For the 2012 vintage, these phenolics were found to be discriminant for the terroir-related separation, in other words, the different concentrations of phenolics were attributed to the terroir conditions. For example, VR wines had higher levels of *trans*-resveratrol and hydroxytyrosol. That suggested that the vine in the VR vineyard experienced more fungal disease pressure before harvesting and the indigenous microbiology in VR differed from FE vineyard (Piñeiro et al., 2011). Three clusters were formed from the individual samples according to the vintage, regardless of the geographical origin of the Pinot Noir grape. This discrimination was affected by elemental compositions characterised by FTICR-MS. More abundant free phenolic compounds were observed in grape samples from 2010, while samples from 2012 had more levels of glycoside. The discrimination of vintage was first seen with three vintage-related clusters, then for each vintage cluster, two sub-clusters were formed based on the class (must or skin samples). Lastly, must or skin samples in each sub-cluster were furtherly discriminated between the two terroir. This illustrates the effect of terroir on the chemical compounds is notable, regardless of the effect of vintage. The contribution of terroir to the phenolic compounds, glycosides, and other compounds appeared to be significant.

Urvieta et al. (2021) studied the influence of terroir and vintage on phenolic compounds. Malbec wines in this study were made from 23 regions across 12 GI (Geographical Indications) from Mendoza. The difference in regional climate was able to discriminate some of the GIs. According to the profile of phenolic composition, wines were clustered into 3 groups and this grouping was related to wine regions or GI. One group included wines from the small parcels from Gualtallary GI. The reason for the separation is that high elevation in the Gualtallary GI region influenced the microclimate such that it was cooler than the other GIs. The effect of vintage on phenolics, especially the monomeric phenolics correlated with some of the regions. Vintage 2016 was separated from 2017 and 2018. In wine from Uco Valley, compounds such as tyrosol and petunidin-3-glucoside were higher in 2016. In contrast, the contents of astilbin, malvidin-3-coumarylglucoside, and quercetin-3-glucoside were higher in 2017 and 2018. Thus, vintage separation in Uco valley was determined by these monomeric phenolics. Wines from Gualtallary were clustered into one group, and that was attributed to the higher concentrations of the major four monomeric anthocyanins as well as catechin. In some cooler regions, the amounts of cyanidin derivatives were higher. Variabilities in the concentrations of 5 phenolic compounds

contributed significantly to classifying the wine from different regions and vintages. These were caffeic acid, *p*-coumaric acid, delphinidin-3-glucoside, quercetin, and pennidin-3-glucoside. This study added some insights into the classification of wine from various regions and vintages based on monomeric anthocyanins, phenolic acid, flavonols, and flavan-3-ol.

Casassa et al. (2016) reviewed the influence of viticultural and winemaking practices on the phenolic compositions. Climate parameters included air temperature, rainfall, and radiation. The temperature has an impact on the phenology and grape maturity. Radiation impacts the photosynthesis. Research has proved that canopy management especially manipulating the leaf removal and cap removal contributes to the sugar accumulation and the phenolics profile in grape berries. Thus, sunshine exposure is considered the major factor before and during the grape ripening (Downey et al., 2006). Price et al. (1995b) suggested that sun exposure had a negative effect on caftaric acid in Pinot Noir wine. Pinot Noir skin had higher caftaric acid after grape cluster sun exposure, compared to the shaded treatment, but the hydrolysis rate of caftaric acid from sun-exposure was higher than that from shaded wine and thus produced more caffeic acid. Consequently, wine made from grapes with sun exposure had a lower amount of caftaric acid. Lemut et al. (2011) evaluated the influence of leaf removal on phenolic compounds in Pinot Noir grape from Vipava Valley. The timing of leaf removal affected three types of phenolics including hydroxycinnamic acids, anthocyanins, and flavonols. Flavonols and anthocyanins were significantly influenced by the treatment, while hydroxycinnamic acids were slightly influenced. Pinot Noir berry skin had the highest amounts of delphinidin-3-glucoside and petunidin 3-glucoside when leaf removal was carried out at berry set, while peonidin-3-Glu was higher with leaf removal at véraison. An increase of quercetin-3-glucoside also was observed with treatment at berry set compared to the untreated vineyard. The highest amount of flavonol was shown at the beginning of ripening and the increase of amounts the *trans*-caftaric and coutaric acids (both *trans*- and *cis*-) during the ripening in the vineyard treated at berry set. Flavonols had been more influenced by light rather than other factors, in agreement with the suggestion that canopy management could significantly modulate the production of flavonols (Cortell et al., 2005; Downey et al., 2006).

2.2.2 Winemaking factors

Anthocyanins, tannins, and their derivatives have structural similarities. These compounds affect the sensory components that characterise wine style. Consequently, the management of anthocyanins and tannins in the winemaking process is a key factor influencing red wine quality. As described above, during the winemaking process, some of the tannins extracted from the grapes bind with anthocyanins to form stable colour compounds while others exist as polymeric molecules of varying mass (Drinkine et al., 2007a; Kennedy, 2008). In addition, their structures of polymers change throughout winemaking process.

2.2.2.1 Maceration and winemaking techniques.

Maceration and winemaking techniques impact the composition of the colour and mouthfeel attributes of red wine by modulating the extraction of grape phenolics and formation of wine phenolic compounds (Alvarez et al., 2006; Gomez-Plaza et al., 2001). Maceration treatments could include extended maceration, pulsed electric field extraction, and microwave-assisted maceration. Winemaking techniques include cap management, micro-oxygenation, and the addition of grape pomace after fermentation.

Sener (2018) summarised the impact of maceration on red wine colour and sensory attributes. The two critical factors determining the extraction of phenolic compounds were considered to be maceration temperature and time (Gomez-Plaza et al., 2001; Sener, 2018). Numerous studies noted that higher maceration temperatures (15-30°C) increased the extraction of phenolic compounds and improve red wine colour (Casassa & Harbertson, 2014), but recent studies had more focused on low-temperature pre-fermentative maceration (5-10°C), such as cold soaking and must-freezing maceration (Alegria et al., 2014; Alvarez et al., 2006). Pre-fermentative cold maceration could reduce the oxidation of anthocyanin pigments and aroma compounds and inhibit undesirable enzymatic reactions and the growth of undesirable microbials (Lorenzo et al., 2005 662). Ruiz-Rodriguez et al. (2021) found that the total phenolics, total anthocyanins, and total tannins were mainly affected by the fermentation temperature, and less affected by the pre-fermentative temperature. They also found that the application of a temperature gradient from 10 to 20°C during fermentation enhanced the organoleptic properties of Cabernet Sauvignon wines. In addition, the colour stability was negatively affected by a constant high fermentation temperature. The duration of maceration also impacted red wine phenolic compounds, colour properties and the relevant sensory attributes. Studies show that extended maceration may result in a stable red colour, as well as greater tannin content, polymeric pigments, and astringency. Casassa et al. (2021) evaluated the impact of maceration length after pre-fermentative cold soak on phenolic and sensory compositions. Cold maceration at around 6°C did not improve the profiles of anthocyanins, polymeric pigments, and total phenolics compared to control wines (10 days maceration, 25.5°C), whereas cold soak maceration reduced the colour density. In the case of cold soak followed by a 5-day-maceration at 25.5°C, wine tannins were reduced by 71% (Cabernet Sauvignon) and by 29% (Merlot), corresponding with decreased astringency and bitterness. In contrast, cold soak followed by 10 days of maceration increased fruity notes, body, bitterness, and the astringent sensation of wines. Casassa et al. (2019) also evaluated the effect of extended maceration and post fermentation additions of grape pomace in Pinot Noir and Zinfandel wines. The results showed that extended maceration and the addition of pomace reduced the total anthocyanin content and anthocyanin-derived pigments, but total polymeric pigments were not affected by any of the winemaking treatments. Large polymeric pigments were higher in concentration

for extended maceration and the addition of pomace. In the case of tannins, only extended maceration for 6 months resulted in higher amounts of tannins, with 13-fold and 1.6-fold increases for Pinot and Zinfandel, respectively, while one-month extended maceration and the addition of pomace did not affect tannin contents. In conclusion, red wines with desirable colour and sensory attributes may be achieved by adjusting both maceration temperature and length to the desired style (Sener, 2018).

Muñoz García et al. (2021) evaluated the effect of microwave-assisted grape maceration. This enhanced the efficiency of fermentation kinetics, and shortened the lag phase, thus improving some volatile compounds as well as improving the sensory evaluation with increased aroma intensity. Moharrami and Hashempour (2021) noted that low-voltage electric field-assisted treatment considerably increased the amounts of phenolic acid and the antioxidant activity compared to ultrasound-assisted maceration and conventional maceration. In addition, Maza et al. (2020) evaluated the effect of pulsed electric field treatment on the reduction of maceration length. The moderate pulsed electric field required 4 days of maceration to achieve high anthocyanin content, colour density, and total phenolics, whereas intense pulsed electric field was more efficient with only 24 hours maceration required at the end of fermentation. In addition, Casassa et al. (2021) investigated the impact of different winemaking techniques on the phenolic compounds concerning three Pinot Noir clones 2A, 115, and 777. The addition of microwaved stems considerably increased wine tannins for all wines due to the extraction of stem-derived tannins. The use of saignée and extended 30-day-maceration also increased the tannin concentration, and the polymeric pigments content more than doubled. Thus, techniques such as saignée, extended maceration, and additions of microwaved stems enhanced the concentrations of tannin and polymeric pigments and increased the wine ageing potential. In addition, Gordillo et al. (2021) evaluated the effect of post-fermentative maceration with the addition of seeds on the phenolic composition and colour stability of Syrah wines. The addition of ripe seeds or overripe seeds into wines increased the total phenolics. Overripe-seeds vinification had higher contents of gallic acid, monomeric flavan-3-ols, and procyanidins than conventional vinification, whereas it also had higher catechin, procyanidin B2-3-O-gallate and the tetramer than ripe-seeds vinification. González-Neves et al. (2016) studied the effect of different winemaking processes and grape variety on the colour and composition of young red wines. They found that the addition of pectolytic enzymes and cold pre-fermentative maceration before traditional maceration increased colour density and total phenolics, anthocyanin in Merlot wines, but not in Tannat and Syrah wines. The grape variety and vintage had a more dramatically impact on the colour and phenolic compounds based on principal component analysis.

2.2.2.2 Micro-oxygenation (MOX)

In addition to maceration treatments, micro-oxygenation (MOX) has been widely utilised to improve the body, structure, and fruitfulness in red wines. MOX after fermentation facilitates colour stability

and decreases astringent tannins (Durner et al., 2015; Schmidtke et al., 2011). MOX with different oak alternatives positively impacted wine colour density by increasing polymeric phenolics and pigment content (Catania et al., 2021; Yang et al., 2021b). MOX combined with oak chips and staves had similar sensory characteristics to short-term barrel ageing in new American and French oak (Oberholster et al., 2015; Schmidtke et al., 2011). Geldenhuys et al. (2012) reviewed the crucial parameters for the effective performance of MOX. The study examined the methods for oxygen introduction to the wine, oxygen doses, and oxygen solubility. It also examined the chemistry of oxidation reactions between wine components and, changes in wine composition. Schmidtke et al. (2011) monitored the effect of micro-oxygenation by adding low levels of oxygen prior to malolactic fermentation in Pinotage red wine. MOX induced colour changes in Pinotage wine; decreases in anthocyanin content were strongly proportionally associated with increases in polymeric pigments. In contrast, tannin concentrations were not affected by the oxygen addition. Colour and phenolics showed small difference between the 16 mg/L and 32 mg/L oxygen treatments. A similar result was found by Durner et al. (2015). They evaluated the change of anthocyanin-derived compounds during MOX of red wines. Higher flavanol-to-anthocyanin ratio enhanced the formation of larger polymeric pigments. MOX wines had increased amounts of vitisin B, malvidin-3-glucoside-ethyl-(epi) catechin dimer, and double ethylidene-bridged (epi)catechin-malvidin-3-glucoside(epi)catechin trimer. Vitisin B and malvidin-3-glucoside-ethyl-(epi)catechin dimer were intermediates in polymerisation reactions, as their concentrations were constant and decreased after stopping MOX. Catania et al. (2021) had applied six MOX treatments by adjusting different times and oxygen rates prior to MLF and post-fermentation. After 6 months of bottle ageing, the formation of anthocyanins derived pigments were associated with the acetaldehyde levels. The increased acetaldehyde levels led to the increased vitisins B, whereas without acetaldehyde, vitisin A, pinotins, and direct adducts of flavan-3-ols and anthocyanins were produced. Wines from higher doses of oxygen 20 mg/L/month, showed a greater yellow colour and hue compared to other treatments, indicating that the related flavanyl-pyranoanthocyanins were formed and stabilised the colour. Baiano et al. (2016) studied the change of red wine by the micro-oxygenation and treatment with oak chips. Micro-oxygenated wines had the highest concentration of acids, esters, anthocyanins, flavan-3-ols, whereas micro-oxygenated wines showed less astringency and herbaceous character and intense spicy and fruity flavours compared to untreated and oak-treated Nero di Troia wines. Picariello et al. (2020) evaluated the impact of controlled oxygen supply during fermentation-maceration of Covina grapes. Oxygen supply during fermentation enhanced the formation of acetaldehyde-bridged pigments, thus improving the stability of colour. In contrast, MOX during fermentation-maceration reduced the fruity esters by yeasts.

In addition, Yang et al. (2021a) evaluated the effect of MOX before and after malolactic fermentation (MLF) on monomeric phenolics and tannin composition in young Pinot Noir wines. Post-MLF micro-

oxygenation resulted in the disappearance of *cis*- and *trans*-coutaric acid and in the conversion of *trans*-caftaric acid to caffeic acid due to MLF. MOX reduced the proportion of trihydroxylation but increased galloylation as well as increased astringent sensation. McRae et al. (2013) showed the galloyl group number was positively correlated with wine astringency. The higher oxygen dosage had more impact on tannin composition than the colour-related parameters. In conclusion, MOX during fermentation and after fermentation are regarded as winemaking techniques for the modification of red wine colour composition and stability, as well as astringency perception.

2.2.2.3 Yeast strain and bacteria

Different yeast starters and malolactic bacteria result in wines with different colour characteristics and tannin content (Swiegers et al., 2005). Wine yeast *Saccharomyces cerevisiae* have modified wine aroma, flavour, mouthfeel, colour, as well chemical composition, while *Oenococcus oeni* contributes to wines that undergo malolactic fermentation. Thus, wine yeasts and bacterial strains can produce desirable sensory attributes by facilitating the extraction of phenolic compounds, by modifying grape phenolics and by producing flavour-active metabolites (Zhang et al., 2021). Swiegers et al. (2005) summarised the relationships between wine microbials and their production of important flavour compounds in wine. Zhang et al. (2021) reviewed the impacts of *Saccharomyces* yeast and non-*Saccharomyces* yeasts on the phenolic compounds and highlighted the importance of mixed cultural fermentation on the phenolics composition in red wine. In addition to wine *Saccharomyces cerevisiae* and *Oenococcus oeni,* non-*Saccharomyces* yeast strains and lactic acid bacteria have been applied to the wine fermentation to improve the complexity of wine flavours and adaptability of the harsh wine environments (Berradre et al., 2020; Escribano-Viana et al., 2021).

The production of mannoproteins by yeast in wines has been reported to bring about diverse benefits, e.g. inhibition of tannin aggregation, reduction of tannin astringency, maintaining phenolic compounds solubilized in red wines, and enhancing colour stabilisation. Lai et al. (2020) found *S. cerevisiae* (BCRC 21685) mutant CM8 starter could significantly increase amounts of total tannins, anthocyanins, flavonols as well as higher colour and flavour attributes. These high-quality attributes were related to the interactions of high mannose mannoproteins with phenolic compounds. Caridi et al. (2017) evaluated the use of 6 different *Saccharomyces cerevisiae* strains in Calabrian Gaglioppo wine. Phenolics-related parameters were seen to be remarkably different from strain to strain including absorbances at 420, 520, 620, 280 nm, colour density, hue, total anthocyanins, total tannins, delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, and malvidin-3-glucoside. Jiang et al. (2020) compared three different yeast starters: *Saccharomyces cerevisiae* EC-1118, *Torulaspora delbrueckii Biodiva* and *Lachancea thermotolerans Concerto*. They had similar alcoholic fermentation rate. *L. thermotolerans* and *T. delbrueckii* fermented wines had greater antioxidant activity and colour stability compared to *S. cerevisiae.* In addition, Carew et al. (2013)

investigated five yeast treatments and their effect on tannin and colour composition. *Saccharomyces cerevisiae* RC212 wine possessed greater contents of total pigment, monomeric anthocyanin, stable pigment, and total tannins, and displayed high colour intensity. After 6 months of bottling, RC212 and *S. cerevisae* EC1118 wines had increased average stable pigment. The percentage of trihydroxylated subunits also differed from strain to strain. RC212 wine showed high mDP and average tannin size determined by GPC. Božič et al. (2020) evaluated the use of 95 yeast strains for their influence on colour-related phenolic compounds from synthetic grape must including Pinot Noir skin extract. The hydroxycinnamate decarboxylase activity (HCDC) of the selected strains was studied, as HCDC was associated with the formation of vinylphenolic pyranoanthocyanins. Results showed that all non-*Saccharomyces* strains except *P. manshurica* M49 produced higher concentrations of vinylphenolic pyranoanthocyanins than the *Saccharomyces* strains. The *Pichia guilliermondii* ZIM624 and *Wickerhamomyces anomalus* S138 strains produced the highest amounts of vinylphenolic pyranoanthocyanins. In addition, the authors observed the absorption of anthocyanins and pyranoanthocyanins by the yeast with the highest absorbing capability being 9%. This study also observed for the first time the formation of pyranoanthocyanin by the *S. paradoxus* strain. Escribano-Viana et al. (2021) selected the non-*Saccharomyces* yeast cultures from Rioja. A mixed inoculum of *Lachancea thermotolerans*-*Torulaspora delbrueckii* in a 30/70 ratio was finally selected due to the high production of glycerol and lactic acid as well as high implantation capacity. In addition, Uzkuc et al. (2021) researched the effects of spontaneous and inoculated fermentation on the concentration of anthocyanins and phenolic acid. Significantly higher contents of malvidin-3-glucoside, cyanidin-3-glucoside, peonidin-3-glucoside, gallic acid and catechin were observed in spontaneous fermentation. Thus, in spontaneously fermented wine, the loss of monomeric anthocyanin concentrations was 6% less than that in inoculated Cabernet Sauvignon wine.

In contrast, commercial *O. oeni* strains are prone to cause a reduction of colour in red wines. Burns and Osborne (2015) noted that *O. oeni* had degraded acetaldehyde and or pyruvic acid during MLF, which reduced the formation of pyranoanthocyanins. They also pointed out that the effect of MLF on the loss of colour was predominate in contrast with the colour increase by the addition of acetaldehyde. However, some lactic acid bacteria have been reported to produce acetaldehyde during MLF and facilitate the stability of anthocyanins, such as *Lactobacilli plantarum*, *Leaconostocs*, *streptococcus*, and *Pediococci* (Li et al., 2015).

Miyagusuku-Cruzado et al. (2020) evaluated that *Enterococcus mundtii*, *Lactobacillus plantarum*, *and Pediococcus pentosaceus* had the capability of producing 4-vinylphenols by decarboxylating hydroxycinnamic acid, such as *p*-coumaric acid, caffeic acid, and ferulic acid. Silva et al. (2011) also confirmed that *Lactobacillus plantarum*, *Lactobacillus collinoides* and *Pediococcus pentosaceu* could produce volatile phenolics including 4-vinylphenol and 4-ethylphenol. High tannin concentrations (1

g/L) inhibited the production of 4-vinylphenol. On the other hand, the phenolic acids could impact the growth of the yeast, *O.oeni*, and lactic acid bacteria (Campos et al., 2003; García-Ruiz et al., 2008), and phenolic extract such as eucalyptus extract could potentially be used as antimicrobial agents in winemaking to control bacteria growth and MLF (Sabel et al., 2017).

Minnaar et al. (2017) studied the sequential inoculation with *M.pulcherrima* yeasts and lactic acid bacteria in Syrah must compared to the Saccharomyces yeast. Syrah wine inoculated with lactic acid bacteria had greater flavonol compared to the wine without lactic acid bacteria. Wine inoculated with *S. cerevisiae* + *O. oeni* showed the greatest phenolic acid, while wine with *M. pulcherrima* + *S. cerevisiae* + *L. plantarum* had greater amounts of anthocyanins compared to wine without lactic acid bacteria. The sequential fermentation of yeast and lactic acid bacteria may enhance the red colour in Syrah wine and thus enhance the overall sensory quality. Minnaar et al. (2019) studied the application of co-inoculation of yeast/malolactic bacteria such as *Saccharomyces*/*O. oeni*/*Lb. plantarum* and *Saccharomyces*/non-*Saccharomyces + O.oeni/Lb. plantarum* in Syrah must during alcoholic fermentation. Co-inoculation of yeast/malolactic bacteria resulted in higher malvidin-3-glucoside and phenolic acids, while there were no differences in flavonols and flavan-3-ols. Co-inoculation of *S. cerevisiae* and malolactic bacteria modified the phenolic content of Syrah wines without any negative effects in the vinification process.

In conclusion, winemaking processes play an important role in increasing or decreasing the phenolic content thus impacting the colour and tannin parameters in wine.

2.3 Relationships between phenolic composition and wine age as well as wine style

2.3.1 Wine age and phenolic composition

During ageing, red wines undergo various chemical transformations, in particular redox reactions during oak maturation and bottle ageing. These produce significant changes in chemical composition, especially the formation of different types of modified tannins thus impacting the sensory attributes such as colour, astringency, and aroma.

Several studies had investigated the ageing effect on the red wine pigments composition and colour as well as the contribution of the main pigment families to wine colour (Bimpilas et al., 2016a; Bimpilas et al., 2015; Gambuti et al., 2018; Heras-Roger et al., 2016; Li et al., 2009). Red wine colour has been found to be determined by monomeric, copigmented anthocyanins, and low molecular weight and large polymeric anthocyanin. In young red wine, the content of monomeric anthocyanins and copigmentation play an important role. In old wines, due to the evolution of anthocyanins, pyranoanthocyanins and polymeric pigments mainly contribute to the colour stabilisation in old wines.

The percentages of monomeric anthocyanins, pyranoanthocyanins, copigmented and polymeric pigments over the total pigment content could be predictors to their contribution to wine colour hue and intensity (Bimpilas et al., 2016b; Heras-Roger et al., 2016).

Bimpilas et al. (2016a) evaluated the effect of polymeric and copigmented pigments on Merlot wine colour during storage for 6 months. Before ageing, monomeric anthocyanins accounted for 45% of total-coloured anthocyanins, followed by 26% of copigmented anthocyanins and 29% of polymeric pigments. Over 6 months of ageing, the polymeric pigments increased by the rate of 9.7% per month, and they reached 80% in wine with or without copigments additions by 6 months. The increased rates of polymeric pigments in copigments additive wines (treated wines) showed no significant difference from that of the untreated control wine. Consequently, regardless of the different concentrations of copigmented anthocyanins found in wine with or without copigments, the formation of polymeric pigments was not influenced. With regard to wine colour, the colour density and absorbance at 520 nm were affected by the addition of copigments. The colour density and absorbance difference at 520 nm decreased dramatically from around 35% to 0 during the three months of storage. The increase in colour intensity correlated with the evolution of polymeric pigments after 6 months of ageing. It was suggested that the colour contribution of copigmented anthocyanins declined and was attributed to the fact that copigmented anthocyanins dissociated and disappeared over the 3-month storage, while polymeric pigments were continuously formed. Heras-Roger et al. (2016) also investigated how wine age influenced the wine colour by the factors of copigmentation and polymeric pigments across 160 red wines from 2008 to 2012 vintages across 10 different varieties. The results indicated the copigmented anthocyanins increased the colour hue. The contribution of various components changed with age, such that the polymeric pigments factor increased from 0.36 to 0.63, while copigmentation factors decreased from 0.18 to 0.05, and the free anthocyanins factor decreased from 0.46 to 0.32. As a result, young vintage wines showed a dark red colour hue and had a higher of free anthocyanin and copigmented anthocyanins than the old wines. Conversely, old wines showed a significant increase in the polymeric pigment colour factor.

In addition, various studies focused on the evolution of anthocyanin derivatives either in commercial or in experimental wines, during winemaking and/or ageing as well as their impacts on colour intensity and hue (Del Fresno et al., 2020; Escott et al., 2018). Boido et al. (2006) determined the pigment composition and colour properties of the Tannat wines from 1998 to 2003. Anthocyanins, pyranoanthocyanins, direct condensation and acetaldehyde-mediated condensation of polymeric pigments were quantified using HPLC-MS. Only 2% of the initial anthocyanin area was observed in the oldest wine, and the contribution of anthocyanins to the total pigments decreased from 87% to 32% from 4-month wine to 64-month wine. The percentage of pyranoanthocyanins over the total pigment contents clearly increased with time, with 54% present in the oldest wines. The content of vitisin A

decreased with storage time but to a lesser extent than anthocyanins due to the lesser degradation rate and higher stability observed in vitisin A compared to anthocyanins. The contribution of vitisin A to the total pigments increased over ageing from 3.5% in the youngest wine to 17.4% in the oldest wine. Vitisin B decreased by 66% over the 16-month storage followed by a stable state. In the case of the 4-vinylphenol derivatives, their content remained almost constant during storage but their contribution to the total pigments increased from 1% to 21%. The contents of the vinylflavanol pigments were lower than other pigments, and they increased during 16-month ageing then decreased with storage time. In terms of direct condensation of anthocyanin and tannin products, their levels increased during the storage of 28-40 months mostly spent in the barrels followed by the diminish with time spent mostly in the bottle. The contributions of these polymeric pigments increased from 1% to 9%. Acetaldehyde-mediated condensation derivatives decreased significantly with time but their contribution to the total pigments remained constant at almost 0.8% during wine bottling. The correlation between their contributions to the total pigments and the CIELAB colour parameters indicated that the increase of colour hue observed in barrels might be a consequence of the increased proportions of the direct condensed polymeric pigments and pyranoanthocyanins, and to the decreased proportions of total anthocyanins, whereas the colour changes observed in the bottle ageing wines might be a consequence of the increased proportions of pyranoanthocyanins, and to the decreased proportions of total anthocyanins. Uzkuc et al. (2021) noted bottle ageing had increased the total phenolic contents both in Cabernet Sauvignon spontaneous and inoculated wines after 6 months of storage. Around 4% higher increase in total phenolic contents and an 8% lower decrease in anthocyanin content were seen in spontaneous fermentation compared to the inoculated wines. Bimpilas et al. (2015) noted that the phenolic compounds evolved for one-year ageing concerning Merlot and Syrah wines. The monomeric anthocyanins showed a tenfold decrease. The total phenolic content remained constant, while the decline of flavonol glycosides resulted in the increase of the flavonol aglycones after one year ageing.

Han et al. (2021) investigated the relationships between wine age and CIELab colour parameters and polymeric pigments in Cabernet Sauvignon and Cabernet Gernischt wines from 2003 to 2016. The polymeric pigments identified by HPLC-DAD was separated into five main chromatographic peaks by modifying the gradients. Results showed that the first and the third polymeric pigments showed a greater correlation with wine age. The concentration of the first eluted polymeric pigments was higher than the third ones in four wines from vintage 2013 to 2016 (4 years to 1-year old), while both increased significantly with age. The ratio of the first polymeric pigments to malvidin-3-glucoside, and CIELab colour parameters b* (relating to the tawny tonality) were positively correlated with age, while malvidin-3-glucoside (M3G), malvidin-3-acetylglucoside (AM3G), and a* (relating to the red tonality) were negatively correlated with wine age. However, the correlation coefficient was not high ($R^2 < 0.8$),

indicative of the fact that apart from age, variations in wine region and winemaking processes may also impact colour and polymeric pigments. K-means cluster analysis demonstrated the change of these parameters over 14 years of period. Compared to M3G and AM3G, the decreasing trend of a* was slightly slower. It may be attributed to the other pigmented compounds that are also responsible for the red tonality, such as vitisin B and ethyl-linked pigments. The first polymeric pigments showed an increase trend across wines aged 1-4 years old (vintages 2016 to 2013), then decreased rapidly for wines aged 5 and 6 years old, and then fluctuated around the initial level for older wines aged 7-14 years. In addition, as wine ageing low molecular wine pigments become more uniform in relation to their contents and molar percentages. Blanco-Vega et.al. (2014) identified 90 of low molecular weight pigments in diverse red wines. Disappearance of monomeric anthocyanins in most aged wines were expected. There was no significant difference observed in most of the anthocyanin-derived pigments including the direct flavanol-anthocyanins dimers, flavanol–ethyl-anthocyanin dimers, and vitisins A. Flavanol–ethyl-anthocyanin dimers were only found in young Tempranillo wine and disappeared in old Tempranillo wine maybe due to the instability of the ethyl linkage, whereas the increasing amounts of hydroxyphenyl-pyranoanthocyanins detected in aged wines indicated that the stability and continuous formation during ageing.

Different types of oligomeric pigments relative to red wine colour have been studied (Laitila, 2021; Laitila & Salminen, 2020; Laitila et al., 2019). Laitila and Salminen (2020) found that wine colour intensity was relevant to the oligomeric pigments for over three hundred red wines from different wine regions from 1 to 6 years old. Oligomeric pigments including T-Mg or T-methylmethine-Mg were semi quantitatively estimated (T, condensed tannin; Mg, malvidin glycoside). Carboxypyranomalvidins was found to be the most important monomeric pigments for the colour intensity. Pyranoanthocyanins, T-Mg and T-methylmethine-Mg were the primary contributors to the colour intensity in the whole wine set. High concentrations of T-A and T-methylmethine-Mg and the high ratios of large oligomeric pigments over the total pigments were correlated with the high colour density in wine. In young wines (1-year old), colour intensity was mainly contributed by the M3G derivatives. Malvidin glycosides were less impactful on the colour compared to other pigments even in the youngest wines, while B type vitisins were more impactful on the colour intensity than the ratio of large oligomeric pigments as well as T-A type pigments. Laitila (2021) also investigated the relations between wine age and the composition of polymeric pigments. Concentrations of T-Mg, and T-methylmethine-Mg decreased notably, whereas concentrations of T-Mg-(epi) catechin (A type) remained unchanged. The biggest impact of age was seen on the concentrations of T-methylmethine-Mg adducts, as their concentrations decreased significantly from 90% to 21.7% over 6 years. The concentration of T-Mg adducts decreased from 80.5% to 41.5%. Size distribution of T-Mg, and T-methylmethine-Mg were also affected by ageing, while size distribution parameters of T-Mg-(epi) catechin (A type) remained unchanged. Sizes of T-

methylmethine-Mg adducts were the most significantly affected by ageing. Small and medium-sized oligomeric adducts of T-methylmethine-Mg decreased by around 7% and 3%, separately, whereas large oligomeric adducts increased by 10%. In the 6-year-old wines, large oligomeric adducts of T-methylmethine-Mg accounted for nearly a half of the areas of the total fingerprints. In contrast, small and medium-sized oligomeric adducts of T-Mg pigments decreased by 4% and 2%, separately, whereas large oligomeric adducts of T-Mg increased from 37% to 42%. The average sizes of T-Mg adduct were less than the average sizes of T-methylmethine-Mg adducts. In the 6-year-old wines, their main individual compounds in the fingerprints of T-Mg and T-Mg-(epi) catechin (A type) adducts appeared to be the dimeric adducts regardless of their increased average sizes. Laitila (2021) concluded that T-methylmethine-Mg adducts were the most unstable compounds, as both their concentration and composition changed notably over ageing. The second most unstable polymeric adducts were T-Mg. T-Mg-(epi) catechin (A type) were the most stable compounds based on concentration but there were some compositional changes based on the 2D fingerprints. This study also confirmed that the evolutionary trends for T-methylmethine-Mg and T-Mg adducts agreed with the evolutions of the individual dimeric adduct (Alcalde-Eon et al., 2006; Blanco-Vega et al., 2014).

Additionally, several reports evaluated the ageing effect on the tannin composition as well as the perceived mouthfeel attributes. As red wines age, red wines are often perceived as decreasing in astringency. Some reports suggested that this was related to a decrease in tannin concentration with wine age (Cheynier et al., 2006), although other reports showed that this occurs irrespective of tannin concentration, suggesting that other changes, including the gradual modification of wine tannin structures, influence the perceived wine astringency (Vidal et al., 2003). Chira et. al. (2011) found the reduction of astringency over ageing was due to the structural modifications of tannins in both Cabernet Sauvignon and Merlot wines, while the bitterness was not significantly different during ageing.

McRae et al. (2012) focused on the effect of wine age on the sensory profiles of different phenolic fractions across the 30 and 50 age series. This study separated the polymeric pigments into two fractions. The more hydrophilic fraction had higher amounts of epigallocatechin subunits. In younger wine tannins, the percent of epigallocatechin subunits was around 30% compared with around 20% in older wine tannins, which is likely to be a consequence of gradual oxidation. The percent of epicatechin gallate subunits was slightly higher in aged wine tannins than in younger wine. McRae et al. (2013) also investigated the sensory properties of wine tannin fractions isolated from 3- and 7-year-old Cabernet Sauvignon wines from Coonawarra, Australia. The result showed the aqueous subfractions had a greater mean degree of polymerisation (mDP) and contained a higher proportion of epigallocatechin subunits than the butanol-soluble subfractions, while the older wine tannin fractions showed fewer epicatechin gallate subunits than the younger tannin fractions. The tannin subfractions in model wine

at equimolar concentrations revealed that the larger, more water-soluble wine tannin subfractions from both wines were perceived as more astringent than the smaller, more hydrophobic, and more highly pigmented butanol-soluble subfractions, which were perceived as hotter and more bitter. It was indicated that their greater hydrophobicity and colour incorporation in the butanol fractions was negatively associated with astringency, and these characteristics are also associated with aged wine tannins. As those larger, water-soluble tannins had a greater impact on the overall wine astringency, winemaking processes that modulate concentrations of these were likely to influence astringency most significantly.

In conclusion, wine age affects composition and structure of the tannin and pigments in red wine. Changes in tannin concentrations and structure influence the perceived mouthfeel attributes, and the evolutions of different types of wine pigments influence wine colour hue and intensity. The contribution of monomeric anthocyanins, co-pigmented anthocyanins, and polymeric anthocyanins to the total pigments may explain the colour change in red wine from purple to orange hue during winemaking, barrel maturity and bottling ageing process. In aged wine, the decreasing concentration of red pigmented compounds and the increasing percentages of orange compounds are partially associated with the colour evolution. However, the detection and quantification of large molecules polymeric pigments and tannin remains as an issue.

2.3.2 Wine quality and phenolic composition

Wine styles are diverse in terms of vintage, series, and quality levels. For example, readily drinkable, inexpensive red wines are the most prevalent wine styles, and tend to display less colour, lighter and more astringent tannins, and less intense and complex aromas and flavours in contrast with high quality wines (Grainger, 2009). It has been considered that different styles of wines display a range of unique characteristics regarding sensory properties such as colour, aromas, and mouthfeel. Producing different styles of wines depends on a range of natural and human factors. The principal factors in the vineyard and winery include the growth of vine, terroir, vine management, and winemaking process. As reviewed in 2.2, these factors have great impacts on the chemical transformations of phenolic compounds including the monomeric phenolics as well as modified tannins, which impact the overall sensory evaluation in red wine. For example, the high volume, inexpensive wines such as Cabernet Sauvignon and Merlot wines, are widely produced in southern France. This growing region tends to be warm, sunny, and dry and is suitable for the growth and maturity of tonnages of grape berries. Despite the high yield, the contents of phenolic composition in grape skin appear to be adequate for producing these fruity wines with adequate colour. In contrast, Pinot Noir grapes are rarely processed into high-volume, inexpensive wines due to the varietal characteristics and the cool climate growing conditions.

Pinot Noir grows best in cool and moderate climate regions. These Pinot Noir wines display stable colour and greater intensity and complexity in terms of aroma and mouthfeel (Robinson, 2016).

Colour stability and astringent sensation are the important traits of high-quality red wines. These sensory attributes have a relationship with the composition and structure of the phenolic compounds in red wine (Saenz-Navajas et al., 2011; Somers & Evans, 1974). Somers and Evans (1974) reported the correlations between wine quality and colour density and anthocyanin equilibria in young red wines. There was a positive linear correlation between wine quality rating and wine colour density in young Cabernet Sauvignon and Syrah, whereas no apparent correlation between colour density and anthocyanin concentrations as a consequence of the variations observed in degree of ionisation of anthocyanins as well as the related variation in colour contribution from the polymeric pigments fraction. The polymeric pigments were strongly correlated with the content of ionised anthocyanins. Saenz-Navajas et al. (2011) established the correlations between the perceived wine quality and pigment composition as well as colour parameters in Spanish red wines. The wine with higher quality scores presented more red nuances and lower yellow nuances as well as darker colour regardless of the ageing time. High lightness and yellow nuances for low quality score wines were likely to do with the long ageing time or the wine oxidation, indicating a decrease in wine sensory quality. Pyranoanthocyanin pigments made major contribution to red colour of the aged wines rather than the increased yellow nuances. The study by Valentin et al. (2016) showed that the major factors of Pinot Noir wine quality judgement were the mouthfeel balance, while the perceived colour also had minor effect on sensory assessment. Gomez-Plaza et al. (2016) investigated the relationship between wine quality and tannin composition as well as tannin concentrations in Monastrell wines from the same region in Spain. The result indicated that premium wines with high market price were seen with higher phenolic content and tannin contents compared to the low market priced commercial wines. The chromatic colour result showed a clear relation with the projected market price. The increases of colour intensity and total anthocyanins were also observed with high priced wines, indicating the increased extraction of phenolic compounds during the premium wine making process, potentially due to the long skin maceration times or oenological practices that improved phenolics extractability. Increased polymeric anthocyanins and the absorbance at 620 nm (associated with blue colour due to co-pigmentation and ethyl-bridged pigments) in the premium wines reflected enhanced condensation reactions between anthocyanins and tannins and co-pigmentation processes with other phenolics. The mDP had a positive correlation with wine price, as the proportion of the polymeric phenolics measured using size exclusive chromatography were greater in expensive wines. Studies on wine quality gradings of young red wines have indicated that larger proportions of skin-derived tannin subunits in the wine were associated with a higher quality grading (Vidal et al., 2004).

Astringency is an important mouthfeel attribute, and it affects the perceived quality of wines. Astringency refers to the drying and puckering sensation in the mouth producing by the interaction between wine tannin and salivary proteins. The sensation of astringency includes a range of perceptions from a velvety smooth texture to a harsh drying sensation (González-Muñoz et al., 2022). The binding of tannin with salivary protein is affected not only by tannins and proteins, but also by anthocyanins, polysaccharides as well as other wine components. As opposed to astringency, bitterness is a taste induced by many components, such as peptides, acids, and salts. Monomeric (epi)catechin and low molecular weight condensed tannins are more bitter than astringent, whereas polymeric phenolics of large molecular weight are more astringent than bitter. Pavez et al. (2022) recently studied red wines in terms of wine style, origins, and varieties. This study suggested that the tannin content influenced wine astringency and dryness most. The secondary most important chemical parameters were found to be total phenolic content and colour index. Finally, alcohol level and pH also correlated to the overall astringent attributes. In addition, Araujo et al. (2021) investigated the relationship between wine quality and the perception of mouthfeel as well as chemical compositions in 18 Pinot Noir wines. The wine quality was judged by 17 wine professionals. Those sensory results indicated that low-judged quality wines were attributed to the bitterness and tannin harshness, while high-judged quality wines were attributed to good mouthfeel characters including the smoothness/silky, fullness, overall body, and viscosity. Perceived astringency and tannin expression (soft, harsh, fine) were the major drivers for the wine quality judgement. The results indicated that the tannin concentration and structure were the determinant factors regarding the astringency attributes. Parr et al. (2020) noted that the perceived quality and complexity of New Zealand Pinot Noir wines were associated with the varietal typicality. The key drivers of perceived quality were the sensory attributes including varietal typicality, tannin expression, overall structure, and fruity aromas, while colour was not a major driver but also influenced the wine quality perception. Manso-Martinez et al. (2021) noted that the perceived wine quality was correlated with the anthocyanin and phenolic content. High quality wines had the higher proportions of phenolic compounds, greater astringency, and deeper colour regarding Tempranillo Tinto progenies wines from 2017 and 2018 vintages. Parpinello et al. (2009) also found highly rated quality wines were associated to the high colour density concerning the consumer preference for Italian Novello red wines.

To understand the importance of wine astringency, various studies have focused on the causes of astringency and how tannin composition and structure influenced the perceived sensory quality. Many authors had proven that wine astringency correlated with phenolic parameters (Robinson, 2016; Saenz-Navajas et al., 2011). Merkyte et al. (2020) reviewed the main phenolic compounds used as the chemical indicator for the assessment of wine quality. Choi et al. (2020) noted that total phenolic content and the polymeric tannin content showed strong positive correlations with perceived

astringency. Therefore, total phenolic content and the polymeric tannin content could be used as an index for predicting the astringency of wines These results were in accordance with the observations by Preys et al. (2006) who found that astringency was strongly positively correlated with the total concentration of tannins, the percent galloylation (i.e. epicatechin gallate subunits) and the mean degree of polymerisation. To determine the key factors driving the mouthfeel of red wine on a molecular level, Hufnagel and Hofmann (2008) applied the sensomics approach to red wines and identified the astringent mouthfeel and bitter-tasting compounds in wines. Velvety astringent was imparted by three flavan-3-ol glucosides and dihydroflavon-3-ol rhamnosides, while the puckering astringent lingering sensation was caused by a polymeric fraction exhibiting molecular weights > 5 kDa. Sensomics analysis of the key odour and taste compounds in a Dornfelder red wine, followed by full flavour re-engineering and omission experiments demonstrated that polymeric fraction not only impacted the astringent perception but also affected the perception of volatile aroma compounds. Piombino et al. (2020) gave some insights to the diversity of astringency in Italian red wines. The astringency sub-qualities were found to be discriminant for the wines with different cultivars, but the total phenolics and total tannin concentrations were not predictive of all the astringent perceptions including the drying intensities, harsh, unripe, dynamic, complex, and velvet.

Wollmann and Hofmann (2013) has demonstrated that eight different phenolic polymers of GPC fractions were not significantly different in the astringent threshold concentrations, and that the mean degree of polymerisation as well as the galloylation degree had no significant influence on the astringency of the polysaccharides-rich fraction, as previous studies reported that wine polysaccharides did not evoke any astringent sensation and can even decrease the overall astringency of wines. In contrast, some studies reported tannins size or mDP was positively correlated with protein precipitation, with molecules of higher mDP having an increased ability to precipitate proteins (McRae & Kennedy, 2011). This was due to their increase in the number of functional groups available on the tannin to interact with a protein. For example, the tannin-protein reactivity of procyanidin dimer B3 with commercial BSA doubled that of the monomer (+)-catechin (Ferrer-Gallego et al., 2012). In addition, increasing the degree of galloylation of tannins has been shown to increase protein precipitation. By enhancing the hydrophobic interactions with increased π-π interactions, the tannin-protein complexes can interact more strongly. Examples of this include experiments where (−)-epicatechin or (−)-epigallocatechin do not precipitate with salivary proteins or gelatin, however, (−)-epicatechin-3-O-gallate or (−) epigallocatechin-3-O-gallate will cause precipitation with the proteins (Cheynier et al., 2006; González-Muñoz et al., 2022). It has also been shown that increasing the number of hydroxyl groups on the flavan-3-ol backbone (i.e. from epicatechin to epigallocatechin) could increase the interactions and overall binding to proteins. McRae and Kennedy (2011) reviewed the relationship between astringency and wine tannin. Although there was a positive correlation between

the polymeric pigments in the presence of anthocyanins and the puckering sensation, the reduction in the protein-binding capability was also found in polymeric pigments. Tannins with larger molecular size and structural flexibility, as well as a higher proportion of catechin subunits to epigallocatechin or epicatechin, and more common linkages, had a higher protein -binding capability (Arts et al., 2002; Cheynier et al., 2006). Overall, changes in tannin composition and structure during winemaking and ageing process are likely to impact on the protein-tannin binding capability.

In conclusion, red wine quality is associated with perceived sensory characteristics, such as colour, astringency, and bitterness. These sensory characters have been correlated with different types and concentrations of phenolic compounds, such as tannin, polymeric pigments, and anthocyanins. However, how wine quality is affected by the tannin composition and structure is still uncertain.

2.4 Recent analytical techniques for the determiniation of phenolic compounds

It is an analytical challenge to characterise the large number and variety of phenolics. Many studies of tannins from grapes or oak barrels have been published, whereas only a few publications deal with the characterisation of polymeric pigments from red wine. The methods for the quantification of individual anthocyanins, monomeric anthocyanin derivatives, and small dimers of anthocyanins-(epi) catechin are well developed using liquid chromatography mass spectrometry (LC-MS/MS) in numerous studies (Alcalde-Eon et al., 2006; Blanco-Vega et al., 2014; Delgado-Povedano et al., 2021; Willemse et al., 2015). The gel permeation chromatography method enables the elution of different sizes of large polymeric pigments, and thus the determination of the distribution and size of large polymers (Kennedy & Taylor, 2003). Capillary zone electrophoresis (CZE) method was used for the separation of polymeric pigments from monomeric anthocyanins and anthocyanin derivatives due to their different charge/size ratios. Polymeric pigments had higher charge/size ratio than the monomeric phenolics. Compared to HPLC, CZE proved to have better separation efficiency, reduced analysis time, and reduced solvent consumption for the analysis of polymeric pigments (Sáenz-López et al., 2004). However, the different types of polymeric phenolic compounds cannot be detected individually using GPC and CZE methods, such as the T-Mg, T-Mg-(epi) catechin (A type), and T-methylmethine-Mg (T, condensed tannins; Mg, malvidin glycoside). As a result, the characterisation of red wine pigments appears to be still an analytical issue as it involves the fractionation of different types of pigments and the quantification of the separated fractions using HPLC coupled with mass spectrometry.

A large number of studies have published the most widely used spectrophotometric and chromatographical methods for the analysis of grape and wine phenolics (Aleixandre-Tudo et al., 2017; Escott et al., 2018; Escott et al., 2016; Lee et al., 2004). Aleixandre-Tudo et al. (2017) noted that modified Somers assay (Mercurio et al., 2007) was a simple and robust way to estimate anthocyanins

while good correlations were also found between the other two methods: methyl cellulose precipitable assay, and bovine serum albumin for total tannin measurement. In addition, CIELAB parameters were utilised for colour measurements in wine from the visible spectra (380-770 nm). The results noted that colour change with age was more qualitative rather than quantitative due to the increased hue value (h*ab) from purple-red hues to more orange-red ones, yet no clear trend observed for the chroma (C*ab) and lightness (L*) with time. Lorrain et al. (2013) gave some insights regarding the development of the analytical techniques of phenolics from grapes and wines. It noted the method of solid-phase extraction offer good separation of phenolic compounds in wines. Reverse-phase LC (RPLC) and normal-phase LC (NPLC) could separate different phenolic compounds. In terms of polymers with high-molecular mass, NPLC analysis could be better than RPLC. Zeller (2019) reviewed the analytical methods for condensed tannins in past studies. Gutierrez-Escobar et al. (2021) reviewed the analytical techniques employed to quantify the phenolic compounds in must and or wine including NIRs and UV absorbance (phenolic index, Folin-Ciocalteu index), as well as chromatographic techniques for the quantification of phenolic compounds. The GC-MS technique coupled with a solid phase microextraction were applied to quantify the phenolic compounds including *trans*-resveratrol and *cis*-resveratrol, catechin, epicatechin, and piceatannol in wine as well as the total phenolic content (Viñas et al., 2009). High performance liquid chromatography (HPLC) coupled with a diode array detector (DAD) has been utilised by various studies to determine the concentrations for individual monomeric phenolic compounds including the hydroxycinnamic acid (320 nm), benzoic acid (280 nm) stilbenes (320 nm), epicatechin and catechin (280 nm), flavonols (360 nm), as well as malvidin-3-glucoside (520 nm) (Baiano et al., 2016; Suprun et al., 2021). In addition, HPLC analysis of red wine pigments enables the separation of anthocyanins and low molecular weight anthocyanin-derived pigments (Blanco-Vega et al., 2011), whereas large molecular weight polymeric pigments remain as an unresolved broad peak on HPLC chromatograms. Mass spectrometry (MS) was introduced to fragment and quantify the fragmented fractions of polymeric pigments. Frey (2015) had separated anthocyanins and anthocyanin-derived pigments in red wines using LC/MS. Blanco-Vega et al. (2014) utilised the HPLC-DAD-ESI-MS/MS method to identify and quantify the low molecular weight red wine pigments. Up to 68 red wine pigments were quantified in most wines including grape anthocyanins, flavanol-anthocyanins adducts, and pyranoanthocyanins from wines regarding different vintages and varieties. Guadalupe et al. (2006) developed a multiple-step analytical method for quantification of different types of phenolic compounds including monomeric phenolics, polymeric pigments, and condensed tannins. The method was based on the phenolic fractionation by gel permeation chromatography, and it confirmed that the HPLC-DAD and HPLC-MS were able to quantify the monomeric phenolics, dimeric anthocyanins. It also confirmed the overall negative charge of polymeric pigments by performing the precipitation test with gelatin. The GPC method improved the quantifications of condensed tannin by

the traditional vanillin assay. CZE method has separated the polymeric pigments into seven peaks based on the GPC fractionation.

Recently, ultra-high performance liquid chromatography (UPLC) has become more widely used combined with mass spectrometry for the quantification of the complex phenolic polymers of large molecular weight in must and wine (Engström et al., 2014; Engström et al., 2015; Mouls & Fulcrand, 2012a; Stoj et al., 2020). Mouls and Fulcrand (2015) investigated the oxidation markers of flavan-3-ol monomers, dimers, and polymeric tannins after chemical depolymerisation using UPLC-MS. The oxidation markers of condensed tannins were quantified in model solutions and confirmed in wine (Mouls & Fulcrand, 2012a). The oxidation marker for epigallocatechin dimers were the fragments at m/z 611. The mechanism of intermolecular or/and intramolecular reactions resulted from oxidation were revealed and the quantification marker ions were clearly obtained for the terminal and extension subunits of the tannin fractions. Engström et al. (2015) developed the rapid UPLC-tripe-quadrupole mass spectrometry. The method enables measurement of group-specific fingerprints for polymeric phenolics from plant extracts without the step of depolymerisation. The phenolic groups included ellagitannins, gallic acid derivatives, quinic acid derivatives quercetin-based flavonol glycosides, kaempferol-based flavonol glycosides, and myricetin-based flavonol glycosides. Additionally, UPLC performed well for wine phenolic compounds derived from different grape cultivars.

Salminen (2018) reviewed modern liquid chromatography tandem mass spectrometry method to give some insights into the two-dimensional tannin (MS-MS) fingerprints including the ellagitannins, gallic acid derivatives, gallotannins, and proanthocyanins groups. The depolymerisation step was done by the collision-induced dissociation (CID) in the electrospray ionization (ESI) by increasing the cone voltage. CID enables fragmentation of polymeric tannins into small functional group fragments, which could be specifically detected by the multiple reaction monitoring (MRM) techniques as in a routine method. More recently, an increasing number of studies have focused on the techniques of UPLC coupled with tandem mass spectrometry (MS/MS) to obtain more compositional and structural information on anthocyanin-derived polymeric pigments. Zeng et al. (2016) utilised high-resolution mass spectrometry (ESI-Q-TOF) coupled with UPLC to successfully identify the quantification markers released from polymeric pigments after depolymerisation reactions. Four types of primary polymeric pigments were quantified including the coloured T-A type, colourless T-A-(epi)catechin (A type), colourless A-(epi)catechin (A type)-T, and coloured A-methylmethine-T polymeric pigments.

Currently, there are only a few methods available for the structural quantification of polymeric pigments (Laitila, 2021; Laitila & Salminen, 2020; Laitila et al., 2019). MS-MS fragments are potentially utilized as the quantification marker ions for the group specific quantifications of different type polymeric pigments. In the positive mode, T-Mg type polymeric pigments are fragmented to m/z 373,

and T-Mg-(epi) catechin (A type) pigments are fragmented to m/z 343, and (M3G-(epi) catechin) (A type)-T to m/z 577, and T-methylmethine-Mg to m/z 357 (Pati et al., 2006; Zeng et al., 2016). By utilising these quantification marker ions quantified in the literature, Laitila et al. (2019) developed a rapid group specific multiple reaction monitoring mode of UPLC-MS/MS method for the detection and semi-quantification of the three types of polymeric pigments. The method utilised a wide range of cone voltage to achieve the depolymerisation step for T-Mg, T-Mg-(epi) catechin (A type), and T-methylmethine-Mg type polymeric pigments. The phloroglucinolysis or thiolysis method was not necessary to depolymerise the polymeric phenolics. As a result, the 2D chromatographic fingerprints of the targeted polymeric phenolic compounds were produced by utilising a single UPLC-MS/MS method. However, the actual concentrations and the average chain lengths of these three types of pigments were not able to be reported using the group specific UPLC-MS/MS.

Laitila (2021) utilised the group specific multiple reaction monitoring mode of UPLC-MS/MS method to investigate the composition and the evolution of three types of polymeric pigments including T-Mg, T-Mg-(epi) catechin (A type), and T-methylmethine-Mg in wine. This study semi-quantified the concentrations of the three types of polymeric pigments by reporting the percentage value in comparison with the reference wine. The calculation based on 2D fingerprints qualified the composition of those polymeric pigments. In terms of young wines, the result showed that Pinot Noir had the lowest concentrations of T-Mg, T-Mg-(epi) catechin (A type), and T-methylmethine-Mg, while Cabernet Sauvignon and Syrah had the highest of all three types of polymeric adducts. In addition, Pinot Noir wines from Pfalz region had the lowest average sizes of T-Mg, T-Mg-(epi) catechin (A type), and T-methylmethine-Mg compared to the Pinot Noir wines from the Beaune region and other Cabernet Sauvignon, Merlot, and Syrah wines. Pinot Noir wines had significantly different compositions compared to other wines based on 2D fingerprints. Pinot Noir wines lacked the peaks of many individual compounds from the 2D fingerprints of T-Mg adducts due to the difference in malvidin glycoside composition. T-Mg-(epi) catechin (A type) adducts in Pinot Noir wines were less abundant than the wines from other varieties. Pinot Noir wines 2D fingerprints also showed more variations within the group of T-methylmethine-Mg. In addition, the major individual compounds of T-Mg, T-Mg-(epi) catechin (A type), and T-methylmethine-Mg were the dimeric adducts in Pinot Noir one-year-old wines.

In addition, many studies have shown that analytical results may differ between different methods. For example, McRae et al. (2014) found that average tannin molecular weight could be determined by three methods, i.e. gel permeation chromatography (GPC), depolymerisation reactions and small angle X-ray scattering (SAXS), and different values were obtained, although a strong correlation was detected between GPC and SAXS results. Depolymerised reaction result was not strongly correlate with SAXS

results. SAXS results suggested that older wine tannin was larger than young wine tannin and tannin composition did not have great impact on tannin molecular size.

Current studies have involved a more diverse range of analytical techniques, such as UPLC-MS/MS, small angle X-ray scattering (SAXS), and nuclear magnetic resonance spectroscopy (NMR) to gain more structural and compositional information of individual polymeric phenolics in wine (Escott et al., 2016; Laitila et al., 2019; Pinasseau et al., 2016; Wollmann & Hofmann, 2013). To characterise polymeric pigments and proanthocyanins, Escott et al. (2016) has combined three analytical methods, HPLC-DAD-ESI/MS, GC-FID and Fourier-transform infrared spectroscopy (FTIR). The primary types of pigments in red wine including vitisin A and pyranoanthocyanins were observed. Similarly, UPLC/time-of-flight mass spectrometry (UPLC/TOF-MS), matrix-assisted laser desorption (MALDI) and NMR were used to obtain the chemical structure of the complex polymers (> 5 kD) by Wollmann and Hofmann (2013). They noted that the procyanidin chain was revealed as the structural backbone and different linkages such as acetaldehyde and ethyl bridge had been detected. This study also found that NMR experiments could identify the (R)- and (S)- configuration of the ethyl bridge for the specific thiolysis product and MALDI-TOF-MS analysis could reveal the epicatechin units (m/z 288) for the oligomer fraction. Laitila (2021) created multiple reaction monitoring methods combined with UPLC-MS/MS to build up the high-efficient 2D chromatographic fingerprints for the different types of polymeric pigments. This method also revealed their compositional information including the concentration and size distributions as well as the evolutionary trends of polymeric pigments (Laitila, 2021; Laitila & Salminen, 2020a). However, their characteristics remain an issue because of the compositional and structural complexity of polymeric phenolic compounds in red wine, even though many analytical techniques have been employed for the detection and quantification of wine phenolics.

Chapter 3

Materials and Methods

3.1 Wine samples

Twenty-two commercial Pinot Noir wines produced by Pernod Ricard Winemakers (New Zealand) Ltd. from 2002 to 2018 vintages (Table 3.1) were selected for this study. One bottle of each wine was used for analysis. Pernod Ricard produces Pinot Noir wines from Marlborough and Central Otago regions with various stylistic characteristics and branding. These include wines made from grapes sourced from different subregions in Marlborough as well as from Central Otago. These wines are made in general styles for each of the Stoneleigh, Brancott and Last Shepherd brands. The wine code in Table 3.1 displays information regarding series as well as vintage. The initial two letters refer to the style, and the following two digits refer to the vintage. The chosen wines include ten individual vintages (2002, 2003, 2007, 2010, 2011, 2014, 2015, 2016, 2017, and 2018). In addition, there were eight different wine series involved, including four from Brancott (BC, Brancott Classic; BD, Brancott Dror; BL, Brancott Letter; BR, Chosen Rows), three from Stoneleigh (SC, Stoneleigh Classic; SL, Stoneleigh Latitude; SR, Stoneleigh Rapaura), and one from Central Otago (OS, Last Shepherd). BD15 and BR15 were designated as super premium wines, while the remaining wine series were at premium grade except for the classic series (BC and SC) that were considered at commercial grade. A total of six wine styles were designated based on wine series and quality grades including BL, C, OS, SL, SP, and SR.

Additional information was also obtained from Pernod Ricard Winemakers (New Zealand) Ltd. This included: proportions of clones contributing to the final wine, parameters relating to winemaking, and wine chemical analysis at bottling. In order to determine whether there were any patterns relating to wine age and/or styles, relationships in these provided data were explored using Agglomerative Hierarchical Clustering analysis (AHC) and type III ANOVA analysis (Langsrud, 2003; Roullier-Gall et al., 2014). The details of statistical analysis are described later in this chapter.

Table 3.1 Commercial wines used in the study

Wine code	Vintage	Series	Styles
BC17	2017	Brancott Classic	C
BD15	2015	Brancott Dror	SP
BL02	2002	Brancott Letter	BL
BL11	2011	Brancott Letter	BL
BL14	2014	Brancott Letter	BL
BL15	2015	Brancott Letter	BL
BL17	2017	Brancott Letter	BL
BR15	2015	Brancott Chosen Rows	SP
OS17	2017	Central Otago Last Shepherd	OS
OS18	2018	Central Otago Last Shepherd	OS
SC03	2003	Stoneleigh Classic	C
SC07	2007	Stoneleigh Classic	C
SC17	2017	Stoneleigh Classic	C
SL11	2011	Stoneleigh Latitude	SL
SL15	2015	Stoneleigh Latitude	SL
SL16	2016	Stoneleigh Latitude	SL
SL17	2017	Stoneleigh Latitude	SL
SR02	2002	Stoneleigh Rapaura	SR
SR10	2010	Stoneleigh Rapaura	SR
SR14	2014	Stoneleigh Rapaura	SR
SR16	2016	Stoneleigh Rapaura	SR
SR17	2017	Stoneleigh Rapaura	SR

3.1.1 Basic wine chemical parameters at bottling

Table 3.2 shows the results of the basic chemical parameters for the wines. Specifically, the wines were dry: titratable acidity ranged from 4.79 to 6.60 g/L (tartaric acid equivalent), volatile acid ranged from 0.49 g/L to 0.94 g/L (acetic acid equivalent) and pH levels ranged from 3.47 to 3.67. The wines from the Letter series had a higher alcohol level with an average of 14.04 % (v/v) compared to other series. BD15 and BR15 had the same pH (3.53) but differed in alcohol with BD15 having 12.94 % (v/v) compared to BR15 at 14.50 % (v/v). Free SO_2 amounts ranged from 20 to 47 mg/L, while molecular SO_2 amounts ranged from 0.24 to 0.47 and total SO_2 ranged from 77 to 180 mg/L. The clustering analysis was performed based on the data in Table 3.2.

Table 3.2 Chemical analysis of wines at bottling

Wine code	Alcohol (ABV)	Titratable acidity (g/L as tartaric acid)	pH	Volatile acidity (g/L as acetic acid)	Free SO_2 (mg/L)	Molecular SO_2 (mg/L)	Total SO_2 (mg/L)	Residual sugar (g/L)
BC17	12.51	5.26	3.65	0.94	47	0.41	NA	NA
BD15	12.94	5.45	3.53	0.89	33	0.53	150	2.21
BL02	15.08	5.65	3.67	0.71	25	0.31	78	3.70
BL11	14.10	6.34	3.51	0.74	31	0.59	106	2.92
BL14	14.04	5.86	3.60	0.68	34	0.56	124	1.99
BL15	14.00	5.92	3.54	0.61	37	0.67	95	2.33
BL17	12.98	5.03	3.60	0.79	36	0.52	132	1.71
BR15	14.50	5.70	3.53	0.80	31	0.57	141	2.90
OS17	13.35	5.30	3.65	0.49	43	0.38	101	2.02
OS18	13.94	5.86	3.60	0.55	25	0.50	102	2.43
SC03	13.10	5.77	3.65	0.62	21	0.29	77	2.90
SC07	12.86	6.20	3.46	0.62	20	0.48	94	3.65
SC17	13.20	4.79	3.57	0.75	35	0.44	85	3.51
SL11	13.88	5.76	3.56	0.71	25	0.49	104	2.69
SL15	14.00	5.87	3.54	0.71	40	0.44	122	2.35
SL16	13.69	5.76	3.54	0.93	32	0.53	150	2.22
SL17	12.74	5.65	3.60	0.86	37	0.24	155	1.99
SR02	14.00	5.90	3.64	0.64	29	0.25	80	2.30
SR10	13.97	6.60	3.47	0.68	25	0.55	91	3.11
SR14	13.98	5.97	3.52	0.73	31	0.54	115	2.62
SR16	12.93	5.90	3.49	0.91	37	0.33	180	2.18
SR17	12.94	5.45	3.53	0.89	33	0.53	150	2.21

*NA, the data not available or found in the winery dataset.

Agglomerative Hierarchical Clustering (AHC) analysis was performed based on these basic wine parameters (see Appendix, Figure A.1). There were 3 clusters identified and the separation of the clusters was essentially age-related. The first cluster included the three wines, BL02, SR02 and SC03, while wines from vintages 2016-2017 plus BD15 were clustered into the second group. The remaining wines aged from 2007-2015 plus OS18 were grouped. Table A.1 shows the ANOVA type III SS analysis for the basic chemical parameters (excluding residual sugars and total SO_2 as some data were missing) based on the above clustering result. Statistically, the alcohol level, titratable acidity, pH, volatile acid, free SO_2, and molecular SO_2 were significantly different among three age-related clusters. Increasing values with cluster vintage range were seen in alcohol and pH levels, while generally acetic acid and free SO_2 decreased. In contrast, the titratable acidity and molecular SO_2 had distinct patterns, as wines from had the highest averaged titratable acidity and molecular SO_2, at 6.01 g/L and 0.54 g/L, respectively, compared to the old and young wines. However, old vintage wines had a greater amount of titratable acidity but a smaller amount of molecular SO_2 than the mid-aged wines despite not being statistically different.

Young vintage wines from 2016 and 2017 plus BD15 had significantly lower average levels of alcohol (13.03%) compared to the highest alcohol levels in old vintage wines from 2002 and 2003 (14.06%). Old vintage wines also had the highest pH (3.65), but young vintage wines had a slightly higher pH than mid-aged wines, despite not being statistically different. In contrast, young wines had the highest average acetic acid and free SO_2 (at 0.83 g/L and 37 g/L, respectively), followed by the mid-age wines and old wines. BD15 appeared similar to young wines due to the lower levels of alcohol and titratable acidity but a higher amount of volatile acidity was observed in BD15, compared with the other wines from 2015. In contrast, OS18 clustered in the mid-aged rather than the young group due to the higher alcohol levels, titratable acidity, and molecular SO_2, yet the lower levels of free SO_2 compared to 2017 wines (Table 3.2).

Thus, vintage had an impact on these chemical parameters. Old vintage wines BL02, SR02, and SC03 were characterised by the highest levels of alcohol and pH, while the lowest amounts of volatile acidity and free and molecular SO_2. These parameters contributed to the separation of this group from others. In contrast, the inverse trend was seen in the young vintage wines from 2015-2017. Wines from 2007 to 2015 plus OS18 were seen with the highest amounts of titratable acidity and molecular SO_2. This analysis revealed that general chemical parameters could distinguish most of the wine samples between vintages. However, the difference between wine styles was not able to be discriminated simply depending on these basic chemical parameters.

3.1.2 Clonal composition

It is well-recognised that clones can significantly influence wine composition and style. Table 3.3 shows the relative percentage of clones selected for 22 individual wines. There were seven major clones selected including clones 828, 923, 667, 777, 10/5, 115, UCD5, and UCD6.

Table 3.3 The relative percentage of clones selected for 22 individual wines

Wine code	828, 923	clone 667	clone 777	10/5	clone 115	UCD 5	UCD 6	Not recorded
BC17		5	18	9	31	34	3	
BD15	2		40		58			
BL02			3	1		47	48	
BL11			40		32	25		2
BL14			63			37		
BL15	36		44		9	11		
BL17			46			40	14	
BR15	2		73		25			
OS17			9	11	2	11	15	52
OS18		2	2			1	9	86
SC03			14	9	18	40	10	10
SC07			6	22	9	42	21	
SC17	2	1	32	8	11	34	9	2
SL11		46	38	9	4	2		
SL15		14	46	1	10	28		

SL16	34	29		3	34	
SL17	25		45	17	12	
SR02		22	24	1	27	26
SR10		60			40	
SR14		82		18		
SR16	1	99				
SR17	14	38	1	13	33	

The average proportions of the clones contributed to the eight wine series (Table A.2).BD15 and BR15 included two predominant clones, 777 and 115, and small percentages of clones 828 and 923. The percentage of the dominant clone in each was 58% of clone 115 in the BD wine and 73% of clone 777 in the BR wine. In contrast, other wine series had 5 to 6 clone selections. Regarding OS17 and OS18, a large proportion (52% and 86%, respectively) of the clonal information for this series was not recorded, thus the seven recorded clones accounted for an exceedingly small percentage. Brancott Classic was sourced from clones UCD5 (34%), 115 (31%), 777 (18%), and 10/5 (9%), while the Stoneleigh Classic series was dominated by clones UCD5 (39%), 777 (17%), 10/5 (13%), 115 (13%), and UCD6 (13%). Regarding the Letter series, three main clones were found including clones 777, UCD5, and UCD6 with the average percentages being 39%, 32%, and 13%, respectively. The Stoneleigh Rapaura series was dominated by two clones 777 (60%) and UCD5 (20%), while the Latitude series was dominated by three clones 667 (30%), 777 (28%), UCD5 (20%), and 10/5 (14%). In summary, different wines had their typical clone combinations that may influence the sensory quality as well as other constituents.

The AHC analysis of the wine samples was performed according to the proportion of the various clones included (Figure A.2). This clustering was not related to vintage. Instead, the wine region may play a key role in discrimination involving wines. OS17 and OS18 were clustered together (C4). Closely, SL11 and SL17 were clustered into one group (C5). BL15, BR15, BD15, SR16, and SR14 were separated from the others (C2). BL02, SR02, and SC07 formed another cluster (C3). The remaining wines from 2003 to 2017 vintages were grouped (C1). Table A.3 shows the ANOVA type III SS analysis for the different wines according to the five clusters. Statistically, these five clusters had significantly different proportions of clones 667, 777, 10/5, UCD5, UCD6, and the proportions of unrecorded clones. In contrast, the proportions of clones 828 & 923, as well as 115, were not seen as statistically different. The discrimination of wines OS17 and OS18 was attributed to the lowest mean proportions of clones 777 and the highest mean proportions of clones not recorded. SL11 and SL17 were seen with the highest mean proportion of clones 667 and 10/5, as well as the absence of clone UCD6. Wines BL15, BR15, BD15, SR16, and SR14 were characterised by the highest proportion of clones 828, 923, 777, and 115. BL02, SR02, and SC07 were characterised by the greatest percentages of clones UCD5 and UCD6, the second greatest of clone 10/5, yet the second smallest percentages of clone 777 selected and the absence of clone 667. The remaining ten wines from 2003 to 2017 were characterised by the second largest mean proportions of clones 667, 777, 115, and UCD5.

3.1.3 Winemaking treatments

According to the records of winemaking activities, there were similarities and differences in the vinification and ageing treatments between wines (Table 3.4). Thus, the proportion of machine-harvested grapes varied from 0 to 100%. Five wines had a very low proportion (<5%) of machine-harvested fruit (BD15, BR15, SR02, BL02 and SR14), and four wines had a very high proportion (>90%) of machine-harvested fruit (BC17, SC17, SL17 and SR16). Data on maceration were not available for all the wines, but the wines with available data seemed to follow a similar pattern. Over 70% of the wine went through post-fermentation maceration from more than 12 days. As shown in Table 3.7, the average maceration days for half of the wine samples were not available. SR14 had the longest average maceration of 17.0 days based on data for 96% of the wine, followed by BL11 with 85% of the wine macerated for 16.1 days, while the remaining samples went through 12 to 15 days of maceration. SL15 had the shortest average maceration days for 12.8 days based on 85% of the wine. The average maceration time for SR10 was 13 days. Three wines had macerated for around 14 days, BL14, BD15, and BR15, based on comparable percentages, from 80% to 84%. SL16, BL15, and SR16 had been macerated for around 15 days with respective 73.7%, 84.19%, and 82.9% maceration information.

With the exception of BC17 and SC17, a high proportion of the wines were matured in barrels as is normal for this style, with the average age of the barrels used being in the range of 0.4 to 1.3 years (leaving aside SC17 for which only 7% was barrel-aged). Whilst BC17 had not undergone any barrel ageing, oak chips were employed. SC17 had a similar chip treatment to BC17. In terms of the time spent in barrels, this varied considerably from 99 to 559 days. BD15 and BR15, considered the highest quality levels of wines in this study, had spent the longest ageing periods in barrels, for 549 and 559 days, respectively. In contrast, the barrel ageing periods for SC07, OS17 and OS18 were relatively short, being 99, 112 and 136 days, respectively. Wines from the Letter, Latitude, and Rapaura series had around 180 to 350 days of barrel maturation.

Table 3.4 Harvest, maceration, and maturation information of wines

Wine code	Machine harvest (%)	Hand harvested (%)	Average maceration (d)	Recorded proportion of wine with maceration data (%)	Proportion matured in barrel (%)	Time in barrel (d)	Average wood age (y)
BC17	90.7	9.3	NA	0.5	0	0	NA
BD15	3.7	96.3	14.8	81.0	95	549	1.20
BL02	1.6	98.3	NA	NA	100	326	0.90
BL11	22.1	77.9	16.1	96.3	100	264	1.11
BL14	27.8	72.2	14.4	80.2	100	357	0.41
BL15	59.3	40.7	15.4	84.2	100	182	1.29
BL17	36.5	63.5	NA	NA	100	281	0.86
BR15	3.4	96.6	14.9	84.8	100	559	0.59
OS17	84.0	16.0	NA	1.0	2	112	NA
OS18	59.3	40.7	NA	NA	4	136	NA
SC03	66.4	23.2	NA	2.5	90	NA	1.27
SC07	85.3	11.6	13.9	80.8	96	99	1.26
SC17	97.0	2.8	NA	0.3	7	226	0.08
SL11	46.9	53.1	NA	15.1	100	290	1.01
SL15	70.8	29.2	12.8	84.6	100	329	1.07
SL16	82.1	17.9	15.3	73.7	100	290	0.51
SL17	100	0.0	NA	NA	100	258	0.76
SR02	2.1	96.7	NA	NA	100	267	1.01
SR10	22.4	77.6	13.0	100	100	250	1.00
SR14	0.0	100	17.0	85.2	100	325	0.86
SR16	96.5	3.5	15.8	82.9	100	281	1.17
SR17	64.4	35.6	NA	NA	100	265	1.00

*NA, the data is not available or missing

3.1.4 Sensory evaluations by Michael Cooper

Eleven wines in this study have been reviewed by Mr Michael Cooper, one of New Zealand's most acclaimed wine writers (Table 3.8). These reviews are published in "New Zealand Wines: Michael Cooper's Buyer's Guide" and are consulted in New Zealand and around the world by highly engaged consumers and professional wine producers, distributors, sommeliers and retailers (Cooper, 2022). Table 3.5 reproduces the information concerning wine price, quality classifications, age of the wine at tasting review, and the specific tasting notes. SC17 as the only wine reviewed from the Classic styles had the lowest quality rating, only 2.5 out of 5. The wine was described as simple textured, red berry dominant compared to other premium Pinot Noir wines. This Classic wine was considered as an inexpensive and easy drinking wine and priced at NZ$16. OS18 from Central Otago 2018 was rated as 3.5 and described as rich aromas of ripe red berry and gentle tannins. Stoneleigh Latitude wines SL15, SL16, and SL17 from Rapaura Road vineyards shared similar quality ratings (3.0 to 3.5). The aroma and flavour of these wines also displayed high consistency and some extent of complexity, with red plum, and spice flavours. Rapaura wines SR10, SR14, and SR16 had higher quality ratings, 4 stars out of 5 compared to the Latitude series, with correspondingly higher prices. In particular, SR10 was rated with 5 stars. These wines were deep ruby in colour with concentrated aromas of ripe cherry, plum, and spice flavours and they revealed good complexity and excellent long ageing potential. Letter series wines BL11, BL15, and BL17 had been rated with 4 to 4.5 stars. They were described as having silky tannins, strong ripe red berries flavour and a persistent finish.

In summary, these Pinot Noir wine quality classifications were consistent with their price points as well as the sensory quality in terms of colour, balance and complexity of aromas and flavour in wines. According to Mr Michael Cooper's assessment, Classic wines were sourced from machine-harvested grapes and used oak chips additives rather than barrel maturation. Therefore, they obtained the lowest quality ratings, with their market price also being the lowest. Compared to Classic wine, Stoneleigh Latitude and Central Otago wine series (OS18) spent different times in new or different-year-old oak barrels and received higher quality ratings. Correspondently, these premium wines displayed a higher extent of complexity in terms of sensory attributes and were priced higher. The Stoneleigh Rapaura and Brancott Letter series had been rated the highest and showed excellent sensory complexity and ageing potential, and thus their prices were also greater.

Table 3.5 Part of wine assessments according to Michael Cooper's wine guidebooks (Cooper, 2022)

Wine code	Vintage	Price (NZD)	Quality	Age at review	Tasting note
BL11	2011	45	4/5	3	The 2011 vintage (4*), grown in 'key Marlborough vineyards', is ruby-hued and mouth filling, with fresh, youthful cherry, strawberry, and spice flavours, showing good complexity, and silky tannins. A savoury, age-worthy wine, it is best opened in mid-2014+.
BL15	2015	33	4.5/5	2 & 3	The 2015 vintage (4.5*), still very youthful, is a classy, vibrantly fruity red, well worth cellaring to 2018+. Bright ruby, with a fragrant, ripe, cherryish bouquet, it is full-bodied and supple, with strong, ripe cherry and plum flavours, savoury notes adding complexity, and a persistent finish.
BL17	2017	25	4/5	4	The 2017 vintage (4*) is a savoury red, barrel-aged for nine months. Ruby-hued, it is fragrant and full-bodied, with ripe, plummy, spicy flavours, showing good complexity, and a firm finish. Best drinking 2021+.
SR10	2010	36	5/5	3 & 4	The outstanding 2010 vintage (5*) is fragrant, rich, and silky, with deep colour, sweet-fruit delights and plum, cherry and spice flavours, showing lovely texture, complexity, and depth.
SR14	2014	28	4/5	3	The 2014 vintage (4*) is a deep ruby, sturdy, savoury wine, named after the Rapaura series of soils, rather than the district in the Wairau Valley. A single-vineyard red, it is full-bodied, with ripe plum and spice flavours, revealing good complexity, and a moderately firm finish. It is still youthful; open 2017+.
SR16	2016	27	4/5	3	The 2016 vintage (4*) is a deep ruby, fragrant red, named after the Rapaura series of soils, rather than the district in the Wairau Valley. Mouthfilling and supple, with exceptionally good depth of ripe, cherryish, plummy, slightly nutty flavours, oak complexity, and a textured finish, it is still youthful; open 2019+.
SC17	2017	16	2.5/5	3	From Pernod Ricard NZ, this red is grown on the warm north side of the Wairau Valley. The 2017 vintage (2.5*) is ruby-hued, with slightly earthy aromas. Medium to full-bodied, it has a decent depth of ripe plum and spice flavours and a smooth finish. Enjoyable young.
OS18	2018	25	3.5/5	3	Fresh and supple, the 2018 vintage (3.5*) is enjoyable now. Bright, light ruby, it is a skillfully balanced red, with moderately concentrated, ripe cherry, plum and spice flavours, gentle tannins, and an easy-drinking charm. (From Pernod Ricard NZ.)
SL15	2015	23	3.5/5	2 & 3	Celebrating the 'Golden Mile' along Rapaura Road, on the stony north side of the Wairau Valley, the 2015 vintage (3.5*) is deep ruby, mouthfilling and well-rounded, with generous, ripe plum and spice flavours, showing some savoury complexity.
SL16	2016	22	3/5	1	Celebrating the 'Golden Mile' along Rapaura Road, on the stony north side of the Wairau Valley, the 2016 vintage (3*) is bright ruby, mouthfilling and smooth, with a decent depth of youthful red berry, plum and spice flavours, showing some savoury complexity.
SL17	2017	20	3.5/5	3	Celebrating the 'Golden Mile' along Rapaura Road, on the stony north side of the Wairau Valley, the 2017 vintage (3.5*) is a deep ruby, medium to full-bodied wine. It is moderately concentrated, with plummy, spicy flavours, showing a touch of complexity, and good harmony. Drink from now onwards.

3.2 Methods

3.2.1 Chemicals

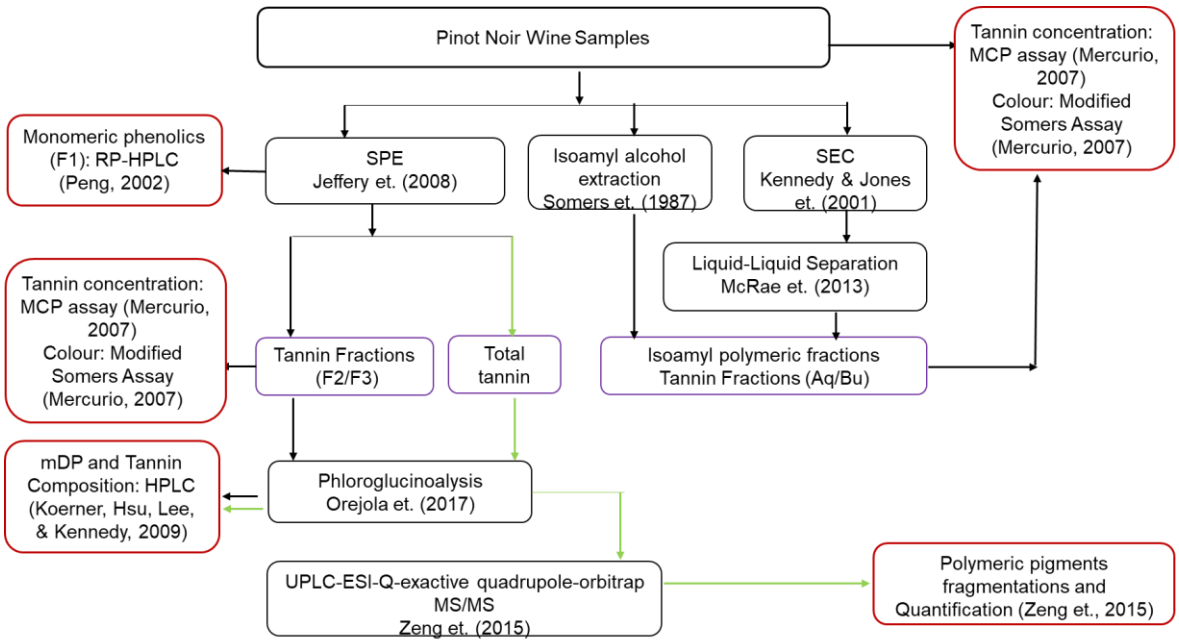
Chemicals used in the various procedures described below were purchased from Sigma-Aldrich (Sydney, Australia). These were: absolute ethanol, acetaldehyde (99%), acetic acid, acetone, acetonitrile, ammonium dihydrogen phosphate, ammonium sulphate, ascorbic acid, butanol, ethyl acetate, formic acid (98-100%), hydrochloric acid (32%), isoamyl alcohol, lithium chloride, methanol, methylcellulose, N, N-dimethylformamide, phloroglucinol, phosphoric acid, potassium hydrogen tartrate, sodium acetate, sodium chloride, sodium metabisulfite, tartaric acid.

Phenolic compounds (caffeic acid, catechin, epicatechin, epicatechin-o-gallate (98%), epigallocatechin (95%), malvidin-3-glucoside, *p*-coumaric acid (98%), quercetin, rutin, syringic acid, *trans*-resveratrol (99%)) for use as external standards were also purchased from Sigma-Aldrich (Sydney, Australia).

Solvents used were HPLC or LC-MS grade, all other chemicals were analytical reagent grade, and water was purified from a Milli-Q purification system (IQ 7003, Merck KGaA, Germany).

3.2.2 Wine fractionation and characterisation

In this study, polymeric phenolics were isolated from the wines by three different means described in detail below: solid phase extraction (SPE), size exclusion chromatography (SEC), and isoamyl alcohol extraction. After isolation, the various tannin fractions and subfractions were quantified and characterised (Figure 3.1). Phloroglucinolysis was undertaken to determine the tannin composition of fractions using HPLC analysis. In addition, the phloroglucinol adducts were further characterised using UPLC-MS/MS. All analyses were carried out in duplicate.



*SEC, solid exclusive chromatography; Aq, the aqueous fraction; Bu, the butanol fraction; SPE, solid phase extraction; MCP, methyl cellulose precipitable.

Figure 3.1 Schematic diagram showing procedures for separation and analytical methods performed in this study

3.2.2.1 Solid Phase Extraction (SPE)

Separation of wine fractions by solid phase extraction was carried out using the method described by Jeffery et al. (2008). Briefly, an HLB SPE cartridge (3 mL, 60 mg, Waters, Australia) was activated with 2 mL of methanol and equilibrated with 2 mL of H_2O before loading 1 mL of wine sample. The cartridge was dried with a gentle stream of nitrogen gas for around 10 min. Three fractions were eluted. Fraction 1 (F1) was eluted with 40 mL of 95% acetonitrile/5% 0.01 M HCl (v/v). Then two tannin fractions were collected, the first (F2) eluted with 5 mL of methanol/0.1% (v/v) formic acid, and the second (F3) eluted with 300 µL of formic acid followed by 2.7 mL of 95% (v/v) methanol. After F1 elution, the total tannin fractions were eluted with 300 µL of formic acid followed by 3 mL of 95% (v/v) methanol/H_2O. The fractions and total tannins were dried at 35 °C by a vacuum rotary evaporator. Finally, F1 and F2 were dissolved in 1 mL of 10% ethanol/0.1% formic acid while F3 was dissolved with 10 µL of formic acid and followed by 990 µL of 10% ethanol/0.1% formic acid. Fraction 1 consisted predominantly of monomeric phenolic material, while F2 and F3 were polymeric phenolic fractions with different hydrophilicity (McRae et al., 2012).

In terms of phloroglucinol analysis, the total tannin fractions were isolated from 3 mL of wine samples and reconstituted with 640 µL of pure methanol, while 4 mL of wines were prepared for F2 and F3 and reconstituted with respective 100 µL and 500 µL of methanol.

3.2.2.2 Size Exclusion Chromatography (SEC)

Wine tannins were prepared using SEC on an Aldrich chromatography column as reported by Kennedy and Jones (2001). Briefly, a glass low-pressure chromatography column was packed with Toyopearl HW-40F size-exclusion medium (Optigen Scientific Pty Ltd., Port Adelaide, SA, Australia). Firstly, the column was equilibrated with 20 mL H_2O/0.1% v/v formic acid and then a volume of 10 mL wine sample was loaded, followed by 20 mL of H_2O /0.1% v/v formic acid added to remove the residual organic acids and sugars. Subsequently, 1:1 methanol/H_2O with 70 mL of 0.1% v/v formic acid was used to remove smaller molecules including monomeric flavan-3-ols, anthocyanins, and small oligomers. Finally, 20 mL of 2:1 acetone/H_2O/0.1% v/v formic acid was used to elute the wine tannin.

The wine tannin was purified by liquid-liquid fractionation. Firstly, acetone was removed using a rotary evaporator at 30 °C, with water added to bring the total tannin solution to 6.5 mL. Secondly, ethyl acetate (2 mL) was added to the aqueous tannin solution in a separation funnel, shaken vigorously, and the layers allowed to separate. The aqueous fraction was removed from the funnel and re-extracted with a further 2 mL of ethyl acetate. Any remaining organic solvent was removed from the tannin aqueous phase using a vacuum rotary evaporator. The total tannin samples were stored at −18 °C until further use.

3.2.2.3 Liquid-liquid fractionation of SEC isolates

SEC isolates were then further fractionated using the liquid−liquid separation (McRae et al., 2013). Butanol (6 mL) was added to the 6 mL of tannin aqueous solution in a separation funnel, shaken vigorously, and the layers allowed to separate. Both phases were collected and dried in a vacuum using a rotary evaporator (30 °C) followed by freeze-drying to remove any residual water. For each wine, the total tannin sample, the aqueous fraction (Aq) and the butanol-soluble fraction (Bu) were obtained and further analysed, respectively.

3.2.2.4 Isoamyl alcohol extraction

According to a previous protocol established by Somers (1971), the polymeric phenolic material was separated from monomeric anthocyanins by extracting the latter using isoamyl alcohol. Specifically, isoamyl alcohol (500 mL) was equilibrated with a saturated solution (pH 3.7) of potassium hydrogen tartrate in 10% (v/v) aqueous ethanol by shaking with 4 x 50 mL volumes of this solution. The wine (or pigment solution) (2.0 mL) was then extracted with 4 x 10 mL aliquots of this mixture in a small centrifuge tube. Phases were separated by brief centrifugation and the lower layer was removed between each extraction. The absorbance of the aqueous phase (polymeric phenolic material) was then measured at 420 and 520 nm at pH 1.0 and 3.4, respectively.

3.2.2.5 Tannin concentration

The tannin concentrations of the fractions and the whole wines were quantified in duplicate using the methylcellulose precipitation (MCP) tannin assay (Mercurio et al., 2007). Briefly, sample (25 µL) was mixed with 300 µL of polymer solution (0.04% MCP in H_2O) for the treatment mixture while H_2O was used for the control mixture in the separate microtube. Samples were shaken gently for 1 min and allowed to rest for 2 min. A saturated ammonium sulphate solution (200 µL) was added to each tube and the total volume was made up to 1 mL in H_2O. Tubes were shaken for a further 1 min and allowed to stand for 10 min prior to centrifuging at 10,000 g for 5 min. The supernatant was transferred to a cuvette, and the absorbance was measured at 280 nm (A280 nm) with a UV/VIS spectrophotometer. Tannin concentration was calculated as the difference between the A280 nm of the control and treatment tubes compared with a standard curve of epicatechin concentrations and hence reported as epicatechin equivalent (mg/L).

3.2.2.6 Colour measurements

The colour in the whole wines and fractions was determined by the modified Somers assay (Mercurio et al., 2007). There were four different treatments involved and eight colour related parameters and total phenolics were determined.

1) A sample was diluted 1:9 with a model wine solution (0.5% tartaric acid in 12% ethanol) and the absorbance was measured at 420 and 520 nm (A420 and A520, respectively). The combined absorbance units of both wavelengths gave the wine colour density, and the hue was given by A420/A520 (Colour density = A420 + A520; Hue = A420/A520).

2) The SO_2 non-bleachable pigment measurement was given by the absorbance at 520 nm of the samples after reaction (1:9) with the second solution (0.375% sodium metabisulfite, 0.5% tartaric acid in 12% ethanol) for 1 h (SO_2 non-bleachable pigment = A520sulfite).

3) Another portion was treated with 0.1% acetaldehyde in 0.5% tartaric acid/12% ethanol and the absorbance were read at 420 and 520 nm after 45 minutes. The combined absorbance at both wavelengths gave the corrected wine colour density (Corrected colour density = A420acetal + A520acetal).

4) The total anthocyanin concentration of the samples was calculated based on the absorbance at 520 nm after reaction (1:49) with 1 M HCl solution for 3 h (A520/HCl) in the dark and the A520/sulfite and the known absorption coefficient for malvsidin-3-glucoside (M3G). Hence, the total anthocyanin concentration was expressed in mg/L M3G equivalent (Mercurio et al., 2007) (Total anthocyanins = 20 * (A520/HCl-1.667*A520sulfite)).

In addition, the degree of ionised anthocyanins was the ratio of the anthocyanin colour at wine pH to anthocyanin colour at pH < 1.0 (The degree of ionised anthocyanins = (A520 – A520sulfite)/(A520/HCl-A520sulfite)). Chemical age 1 was given by the ratio of A520sulfite to A520acetal, while chemical age 2 was given by the ratio of A520sulfite to A520/HCl. Total phenolics was given by A280/HCl.

3.2.2.7 HPLC analysis of fraction F1

The analysis of fraction F1 was conducted using HPLC. Samples were injected onto an Agilent 2 series instrument according to the method described by Gómez-Alonso et al. (2007). The column used was an EXL-1110-1546U C18 (3 µm particle size; 150 x 4.6 mm; Advanced Chromatography Technologies; Scotland). The column temperature was set at 20 °C. The gradient solvents used were solvent A with 0.05 M NH4H2PO4 (pH 2.6, consuming 20 mL/sample), solvent B with 100% acetonitrile (consuming 17 mL/sample) and solvent C with 0.2 M H3PO4 (pH 1.5, consuming 35 mL/sample). The injection volume was 10 µl at a flow rate of 0.8 mL/min. Wine phenolics were monitored at four wavelengths, namely 280, 320, 360 and 520 nm.

3.2.2.8 Phloroglucinolysis

The composition of tannin fractions isolated from selected wine samples was characterised using acid-catalysed depolymerisation in the presence of excess phloroglucinol as previously described (Kennedy & Jones, 2001). The isolated tannin methanolic extracts of total tannin, F2 and F3 fractions were prepared as above and 50 µL was reacted with 50 µL of phloroglucinol solution (100 g/L in methanol with 20 g/L ascorbic acid and 0.2 M HCl) at 50 °C for 25 min, and then cooled in ice for one minute followed by sodium acetate solution (70 mM, 150µL) addition. The reaction products were filtered using a 0.22 µm RC (Phenomenex, Torrance, USA) syringe filter before HPLC analysis. Samples (20 µL) were injected and analysed using HPLC with a C18 column (Kinetex C18, 100 mm x 4.6 mm, 100 Å, 2.6 µm, Phenomenex, Torrance, USA) at 30 °C. The mobile phase was 0.1% formic acid in water for solvent A and 0.1% formic acid in acetonitrile for solvent B with a gradient as follows: 5% B from 0 to 3.1 min; 5% to 20% B from 3.1 to 9.2 min; 20% to 40% B from 9.2 to 16.9 min; 40% to 90% B from 16.9 to 18.5 min and maintained until 21.5 min; 90% to 5% from 21.5 min to 23 min. The flow rate was 1.3 mL/min, and the post-run was 7 min. The elution of peaks was monitored at 280 nm.

3.2.2.9 UPLC-ESI- Q-Exactive quadrupole-orbitrap MS/MS analysis for phloroglucinol adducts

Total tannin fractions from 3 mL aliquots of each wine were separated as described above and reconstituted with 0.64 mL pure methanol. These samples underwent acid-catalysed depolymerisation in the presence of excess phloroglucinol as described above. Once the reaction was completed in the Lincoln University laboratory, they were frozen at -80 °C and sent by overnight courier to Hills

Laboratories at Hamilton where the UPLC-ESI-Q-Exactive quadrupole-orbitrap MS analysis was performed according to the published method (Zeng et al., 2016).

Thus, the reaction mixture was separated using UPLC chromatography (Hypersil GOLD C18 column, 100 mm x 2.1 mm, 1.9 µm particle size, Thermo Fisher Scientific, CA, USA) with a gradient of.1% formic acid in Type-1 water (Solvent A) and 0.1% formic acid in methanol (Optima grade) (solvent B) at a flow rate of 0.3 mL/min: 6% solvent B starting from 0 to 0.5 min, then the content of B was increased to 95% within 14 min, and then kept constant for 4 min, then decreased to 6% from 18 to 18.5 min, and maintained until 22 min. The oven was kept at 40 °C.

The targeted MS and MS/MS analyses were performed on a Q-Exactive quadrupole-orbitrap mass spectrometer (Thermo Fisher Scientific, CA, USA). This mass spectrometer was tuned weekly using LTQ ESI Positive Ion Calibration Solution. Full MS-SIM scan mode (single ion monitoring) was performed from 2 to 16 min. The resolution power was 70,000 FWHM at m/z 200. The mass spectra were acquired over a mass range of m/z 750 to m/z 1150 in the positive ESI mode according to the literature (Zeng et al., 2016). The identified compounds were quantified by calculating the corresponding peak areas.

The product ion mode or the parallel reaction monitoring mode (PRM) acquired MS/MS scans were based on the specified inclusion list. The product ion PRM mode provided better sensitivity or selectivity compared to the full MS-SIM mode. This targeted MS/MS mode was performed from 4 to 10 min with positive ESI mode. The MS/MS resolution power was 35,000 FWHM, with the isolation window being m/z 1.0 and CE being 30, using the following five parent ion masses: m/z 933.24, 907.20, 1071.27, 783.21, 781.19 (Zeng et al., 2016).

3.2.3 Statistical analysis

Descriptive statistics were performed first to describe and visualise the data for different vintages and styles. Then, data were subjected to Agglomerative Hierarchical Clustering analysis (AHC, Euclidean distance proximity type and Ward's agglomeration method) to assess overall differences between both the measured parameters and the wine samples (Addinsoft, 2022).

Statistical significance was investigated using type III Sum of Squares (SS) ANOVA analysis. Type III Sum of Squares (SS) analysis was preferable to test the difference of variations with the unbalanced design observed from the involving data (Langsrud, 2003). There were unequal sample sizes present across different vintages and styles. The multiple pairwise comparisons were performed by Turkey's test to analyse the differences between paired clusters with a confidence interval of 95%. All the statistical analysis above were performed using XLSTAT (2022.2.1 version, Addinsoft Ltd).

In addition, the Restricted Maximum Likelihood (REML) procedure was performed using (Genstat 19th Edition, VSN international Ltd), considering the statistical analysis in Chapter 6. Fixed model REML

analysis was chosen to examine the effect of two factors as well as their interaction effect (vintage*style).

Chapter 4

Spectrophotometric Measures and Tannin Concentrations of Whole Wines

4.1 Introduction

Published reports have revealed substantial information about phenolics including anthocyanins and tannin in Pinot Noir wines (see Chapter 2). Several studies have connected chemical data with mouthfeel attributes collected through additional sensory studies and many of them focused on the evolution of colour and their influencing factors such as age and region (Bindon et al., 2014; Cliff et al., 2007; de Beer et al., 2017; Huang & Xu, 2021; McRae et al., 2012; McRae et al., 2013). Some studies have examined the evolution of colour and phenolic compounds in young wines over a few years and have focused on the transformation of the different pigments (Berrueta et al., 2020; Bimpilas et al., 2015; González-Neves et al., 2016). Further, a report from McRae et al. (2012) investigated the effect of wine age by examining wine colour-related parameters and tannin concentrations for up to 30-year-old wines and different trends in colour-related parameters were noted. Several studies (Longo et al., 2020; Roullier-Gall et al., 2014) have investigated the effect of wine region by identifying the colour and tannin attributes in Pinot Noir wines from the same vintage across different regions, and results showed that anthocyanins, as well as tannin concentrations, contributed to the discrimination of wines from different regions. While informative, these studies typically considered only a few wines from several vintages and different regions, leaving open the question of how the colour and tannin concentrations of Pinot Noir wines change between different vintages and wine styles. Furthermore, there is little information on the impact of wine styles on colour and tannin concentrations.

In this chapter, the effects of vintage, age, and styles on wine colour properties, total phenolics, and tannin concentrations were examined in Pinot Noir wines, using the modified Somers assay to determine the colour properties and total phenolics, and the MCP method to determine the tannin concentration. The data from this comparative analysis could provide a better understanding of the colour and tannin changes of Pinot Noir wines, and the potential to differentiate between wine vintages and styles.

4.2 Results

4.2.1 Colour-related parameters and total phenolics and tannin according to vintage

A total of twenty-two different Pinot Noir wines from ten vintages from 2002 to 2018, belonging to three subregions, three different quality levels, and eight different wine series (see Chapter 3), were analysed using spectral measurements. As previously described, eight colour-related parameters and total phenolic and tannin concentrations were determined using standard methods.

The individual scattergrams for these ten chemical parameters plotted against vintage are shown in Figures 4.1 and 4.2. Clear patterns were observed for most of the colour parameters, but there were also variations within vintages.

Total anthocyanins of young wines were higher than those of old wines from 2002 to 2018, except for wines from 2014 and 2015. Compared to vintage 2016, the median content in 2015 was higher, while that in 2014 was almost the same. SR16 and SL16 showed nearly the same amounts of anthocyanins, around 43 mg/L, whereas BL14 contained 47 mg/L of total anthocyanins, higher than SR14 (38 mg/L). For the 2015 vintage, total anthocyanins also showed some variations between wines, ranging from 34 to 55 mg/L. BR15 had the greatest total anthocyanins, followed by BL15, BD15, and SL15. Variations in total anthocyanins were also observed in vintage 2017 from 70 to 106 mg/L, with OS17 having the highest amounts, followed by BL17, BC17, SL17, SR17, and SC17. Despite these variations in 2017, the median anthocyanins appeared to be higher than that of 2016, but lower than OS18 from 2018. In addition, almost the same amounts of anthocyanins were determined from OS17 and OS18. In addition, small variations were also found in 2002 and 2011 wines. Regardless of differences within vintages, the age-related pattern of decline in total anthocyanins was clear.

Regarding the degree of ionisation of anthocyanins, a distinct pattern was detected. In theory, the degree of ionisation of anthocyanins (%) should be < 100, but the values of the older wines including SC03, BL02, SR02, and SR10 were > 100. This is probably caused by slightly inaccurate measurements when lower amounts of anthocyanins are present in older wines. According to the calculations from Mercurio et al. (2007), the degree of ionisation of anthocyanins was estimated by the ratio of bleachable coloured forms of anthocyanins to total anthocyanin content. As older wines had quite low amounts of total anthocyanins ranging from 2 to 9 mg/L, this led to the great variability in the measurements and thus the abnormality occurred in the result of the degrees of ionisation of anthocyanins.

Other colour parameters showed the opposite trend with age, including chemical age (1 and 2), hue, SO_2-resistant pigments, and (to a lesser extent) colour density and density corrected for SO_2. Younger

wines had lower median chemical age (1 and 2) as well as hue than older wines across vintages from 2002 to 2018 regardless of the individual differences examined within these vintages. The exceptions were also examined within and between vintages. BL11 and SL11 showed higher values of chemical age 1 than wines from 2003, 2007, and 2010, while BL14 and SR14 had lower values of chemical age 1 and 2 than wines from 2015. Hue showed greater variation within vintages compared to chemical age. The greatest hue was measured in SC03 and SC07, and the median hue in 2015 was lower than that in 2016.

Similarly, an overall increasing trend was seen with increasing age for SO_2-resistant pigments despite there being some variations both between and within vintages. BR15 had the greatest concentration of SO_2-resistant pigments (1.91 AU), followed by BD15, BL02 and SR10, while the lowest value of 0.80 AU was found in OS17 and OS18. The median values of younger wines were lower than the older wines with some exceptions found in 2007, 2010, 2015 and 2017. Thus, SR10 had higher amounts than SR02 and SR03. In addition, because the highest amounts were measured in BR15 and BD15 and relatively low amounts were observed in BL15 and SL15, the median values for 2015 were higher than the mean values, resulting in a higher median of SO_2-resistant pigments than that in 2010 and 2011. Similarly, 2017 wines showed a higher median of SO_2-resistant pigments than 2016 due to the great difference found among six individual wines. Regardless of the variations determined within the vintages, a pattern of increasing concentration of SO_2-resistant pigments with age was perceived.

Furthermore, although colour density and colour density SO_2-corrected showed significant variations between and within vintages especially from 2010 to 2017, the broadly increasing pattern with wine age was also noted. Colour density corrected for SO_2 was slightly higher than colour density, as expected. The younger wines generally had lower colour density than older wines. BD15, BR15, BL02, and SR10 showed greater colour density and SO_2-corrected colour density than BL15, SR02, and SL15. The median colour density of OS17 and OS18 was lower than the median in vintages 2016, while the median colour density SO_2-corrected was higher than that in 2016. The greater variations in vintages 2015 and 2017 were also noted.

In contrast, to the colour parameters, MCP tannin concentrations showed no vintage-related pattern. Tannin concentrations varied from 900 to 1440 mg/L (epicatechin equivalent). The greatest amount of tannin was measured in SR02. There were large fluctuations in tannin concentrations between vintages (Figure 4.2). For example, the amounts of tannin in BL02 and SC07 were lower than SC03, while SC03 was lower than SR10, and SR10 was higher than OS17. In addition, large variations within the vintage were also seen in 2002, 2011, and 2014 to 2018. For example, in 2017 the lowest tannin concentration was found in SR17, followed by SC17, BL17, OS17, BC17, and SL17. In terms of vintage 2015, the tannin concentration of BR15 was the highest, followed by BD15, BL15, and SL15. From 2010 to 2016, it

appeared that the median tannin concentrations decreased. However, that trend may also be related to the variations within the vintage rather than age. SL11, BL14, BR15, and SR16 had higher amounts of tannin than BL11, SR14, SL15, and SL16. In conclusion, tannin concentrations fluctuated between vintages and within vintages.

Although total phenolics showed a general decreasing trend with wine age, variations in total phenolics were also seen between and within vintages, similar to MCP tannin. The median of total phenolics in younger wines was generally higher than that in older wines. However, OS18 had lower total phenolics than SL17, BC17, and SR17, but higher than SC17 and BL17. The highest amount of total phenolics was seen in SL17, while the lowest was observed in SC07 rather than in the older wines from 2002 to 2016. The median total phenolics in 2014 were higher than that in 2015 but lower than in 2011, and 2010. SR10, BL11 and SL11 had higher total phenolics than SR14 and BL14, and SC03 had higher amounts than OS17. Large variations in total phenolics were observed especially among vintages 2017, 2016, 2015, 2014, and 2011.

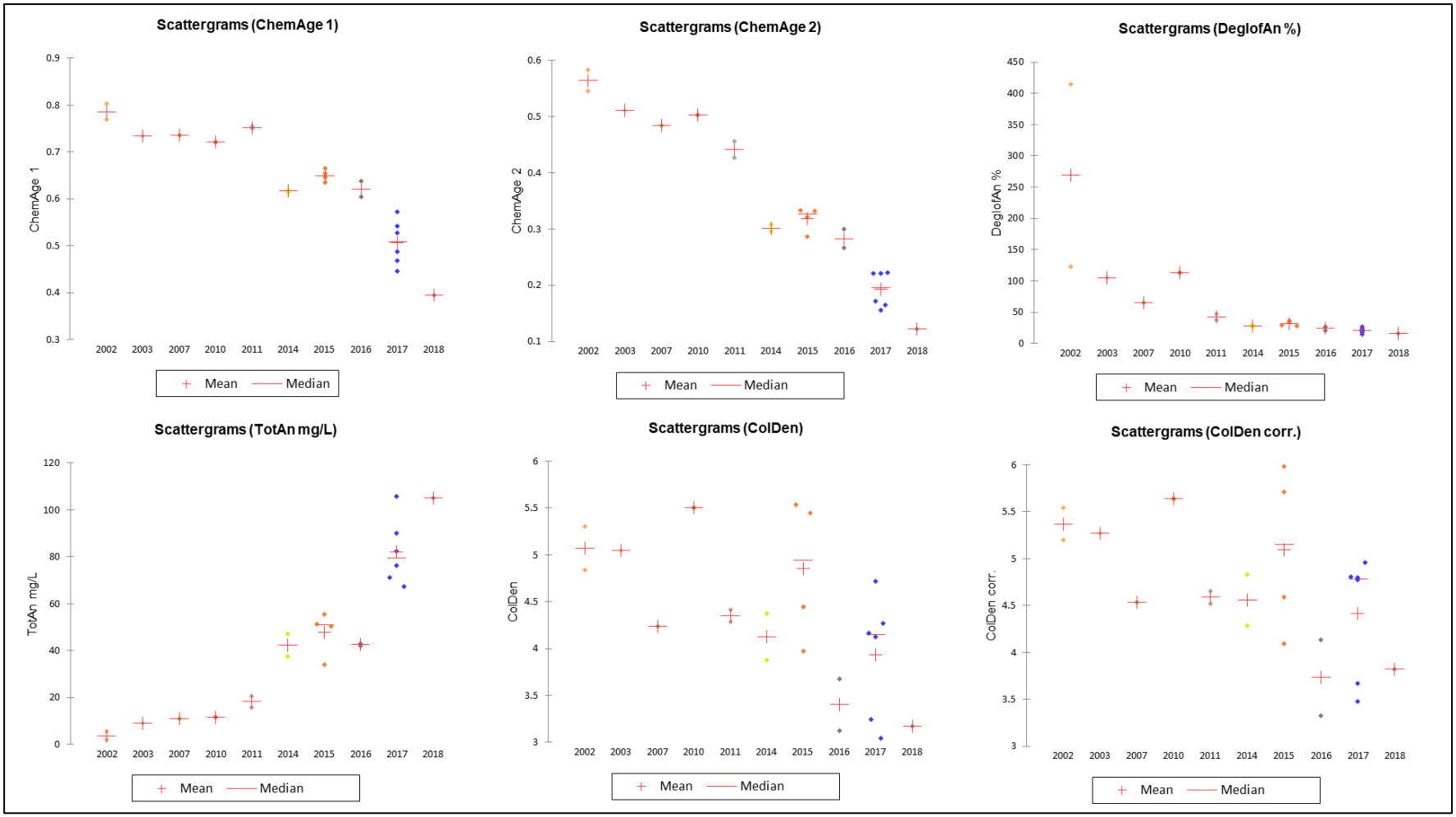


Figure 4.1 Scattergrams of Chemical age (1 and 2), degree of ionisation of anthocyanins (DeglofAn%), total anthocyanins (TotAn), colour density (ColDen), and colour density corrected for SO_2 (ColDen corr.) according to vintages from 2002 to 2018

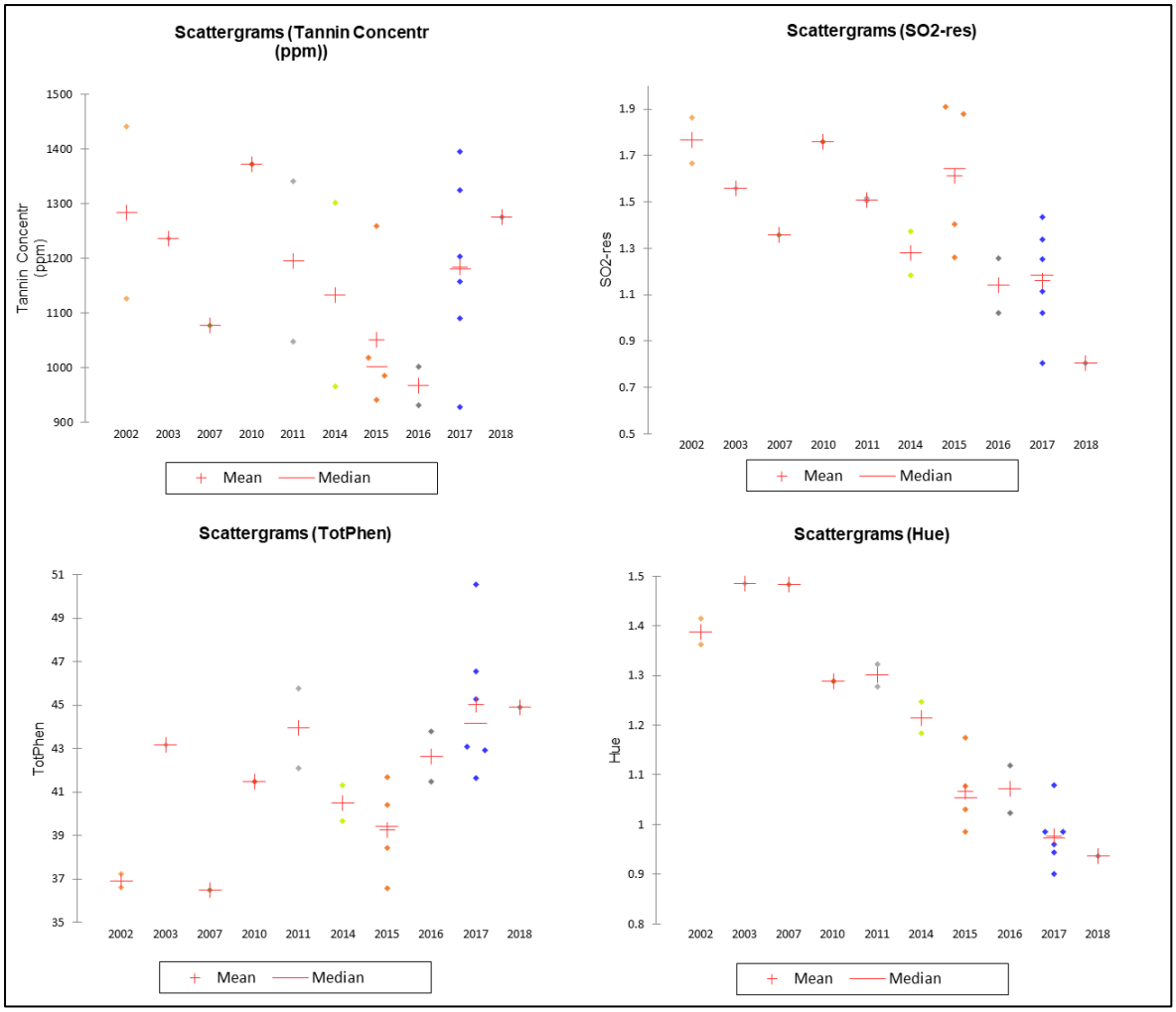


Figure 4.2 Scattergrams of tannin concentration, SO_2 resistant pigments (SO_2-res), total phenolics (TotPhen), and hue according to vintages from 2002 to 2018

Table 4.1 shows the analysis of variance (ANOVA) for the measurements in the whole wines according to 10 vintages. The Type III Sum of Squares (SS) ANOVA analysis was conducted to manage the unbalanced design rather than the normal ANOVA. Statistically, significant differences between 10 vintages were seen among most of the chemical parameters ($P < 0.05$), except for MCP tannin concentration ($P = 0.572$), colour density ($P = 0.065$), and colour density SO_2-corrected ($P = 0.237$). Multiple pairwise comparisons analysis showed that the differences between young vintages and old vintages were statistically significant, while the differences between two consecutive or close vintages were not statistically significant. For example, between vintages 2002 and 2017, most of the parameters were significantly different except for MCP tannin, colour density, and colour density corrected for SO_2. In contrast, between 2016 and 2017, and between 2002 and 2003, no statistical differences were seen in all parameters. Vintage 2015 only showed statistically significantly higher SO_2-resistant pigments than vintage 2016, while the other parameters were not significantly different.

In summary, three different trends were seen, namely, not age-related, positively age-related, and negatively age-related. Tannin concentrations, colour density, and colour density SO_2-corrected were not significantly different between vintages, whereas great fluctuations within vintage were observed. Positive significant relationships with age were seen in chemical age 1 and 2 as well as hue, while negative significant relationships with age were observed in total anthocyanins and total phenolics. Despite the general positive and negative trends observed in these parameters, the distinctions within the vintage were also noticeable.

Table 4.1 Analysis of variance (ANOVA) concerning individual vintages for chemical measurements in the whole wines

Vintage	ChemAge 1	ChemAge 2	DegIofAn (%)	TotAn (mg/L)	ColDen AU	ColDen corr. AU	Hue AU	SO_2-res AU	TotPhen AU	MCP Tannin (mg/L)
2002	0.79 a	0.56 a	269 a	3.7 c	5.07	5.37	1.39 ab	1.77 a	37 bc	1284
2003	0.73 a	0.51 ab	104 b	9.1 c	5.05	5.27	1.48 a	1.56 ab	43 ab	1236
2007	0.74 a	0.48 b	65 b	10.8 c	4.23	4.54	1.48 a	1.36 abc	36 c	1077
2010	0.72 ab	0.50 ab	114 ab	11.4 c	5.50	5.64	1.29 bc	1.76 a	41 abc	1371
2011	0.75 a	0.44 b	43 b	18.2 c	4.35	4.59	1.30 bc	1.51 ab	44 a	1195
2014	0.62 c	0.30 c	28 b	42.4 b	4.12	4.56	1.22 cd	1.28 abc	41 abc	1133
2015	0.65 bc	0.32 c	32 b	47.7 b	4.84	5.09	1.07 de	1.61 a	39 abc	1051
2016	0.62 c	0.28 c	24 b	42.5 b	3.40	3.73	1.07 de	1.14 bc	43 abc	966
2017	0.51 d	0.19 d	21 b	82.1 a	3.93	4.42	0.98 e	1.16 bc	45 a	1183
2018	0.40 e	0.12 e	16 b	105.2 a	3.18	3.82	0.94 e	0.81 c	45 a	1276
Pr > F(Vintage)	<0.0001	<0.0001	0.024	<0.0001	0.065	0.237	<0.0001	0.040	0.032	0.572
Significant	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	No

For each chemical parameter, means followed by a different letter were significantly different using Tukey's test ($P < 0.05$). ChemAge1, chemical age 1; ChemAge2, chemical age 2; DegIofAn, degree of ionisation of anthocyanins; TotAn, total anthocyanins; ColDen, colour density; COlDen corr., SO_2 corrected colour density; TotPhen, total phenolics; SO_2-res, SO_2-resistant pigments.

4.2.2 Colour-related parameters and total phenolics and tannin according to wine styles

Based on wine regions and anticipated quality, a total of six wine styles were categorised, including four wines of Commercial (C), two wines of Super Premium (SP), five wines of Brancott Letter (BL), four wines of Stoneleigh Latitude (SL), five wines of Stoneleigh Rapaura (SR), and two wines of Central Otago (OS). Apart from C and SP wines, wines from the other four wine styles were grouped by corresponding wine series. C wines include Stoneleigh (SC03, SC07, and SC17) and Brancott (BC17) wine, and SP wines include BR15 from Brancott Chosen Rows and BD15 from Brancott Dror.

The individual scattergrams for the 10 chemical parameters were plotted against wine style (Figures 4. 3 and 4.4). Differences were revealed between and within the styles regarding the colour and phenolic measurements. The age-related patterns were also noted and affected the deviations between wine styles and individuals. Thus, the median chemical age 1 and 2 were similar except for Central Otago wines, which had the lowest chemical age 1 and 2 because only two young wines OS17 and OS18 were included. Similarly, total anthocyanins were the highest in OS for the same reason. In the case of other colour parameters, it was difficult to observe any differences between styles because of large variability within each, except that somewhat higher colour density and SO_2-resistant pigments were seen in super premium wines (BR15 and BD15) in spite of only two wines at an intermediate age. Similarly, no clear differences between styles for total phenolics and MCP tannins were discerned. Tannin concentrations scattered broadly between and within the styles and no trend was discerned. OS possessed the greatest median tannin concentration, followed by C, SL, SP, BL, and SR. The median tannin concentration of SR was much lower than the mean values due to the substantially higher amounts of tannins found in SR02 and SR10, at 1441 and 1372 mg/L, respectively. In contrast, the BL series had a more symmetrical distribution. The tannin concentrations of the BL series from the highest to lowest, were BL14, BL17, BL02, BL11, and BL15, ranging from 1302 to 985 mg/L. The variations within the C series were smaller than BL, with the highest amount found in BC17, followed by SC03, SC17, and SC07. As for SP, BR15 had nearly 100 mg/L higher amount of tannin than BD15. In terms of SL, there were two higher points of tannin found in SL17 and SL11 as well as two lower points observed in SL15 and Sl16. OS17 and OS18 were distributed between 1200 to 1300 mg/L, lower than SL17 and SL11, but the median tannin of OS18 turned out to be the highest. Concentrations of total phenolics varied between wine styles, from the highest to the lowest median in the order of OS, C, SR, SL, BL, and SP. Colour density, colour density SO_2-corrected, and hue had similar scattered results as total phenolics. OS had the lowest median values of colour density and hue, SP had the highest values of colour density, and C showed the highest hue. Large fluctuations were determined in series BL, SL, and SR, and did not find any clear pattern over age. Thus, no significant differences were found between

the wine styles among all the chemical parameters (Table 4.2), indicating greater variation within rather than between wine styles.

Overall, the OS was characterised by the highest total anthocyanins, phenolics, and tannin concentrations as well as the lowest median values of colour density, hue, and SO_2-resistant pigments. Commercial wines from 2003 to 2017 with the greatest median chemical age, possessed the highest hue, and the second highest SO_2-resistant pigment, total phenolics and tannin concentrations and colour density, but lower anthocyanins than OS, and SP. These were in accord with the trends observed across vintages. Super premium wines from 2015 revealed a distinct pattern, with the second oldest chemical age, but possessed the highest median SO_2-resistant pigments, colour density as well as the second highest anthocyanins and hue. The remaining three styles, SL, SR, and BL, from 2002 to 2017, showed big fluctuations between and within the styles. Within the styles, the role of age was also more noticeable.

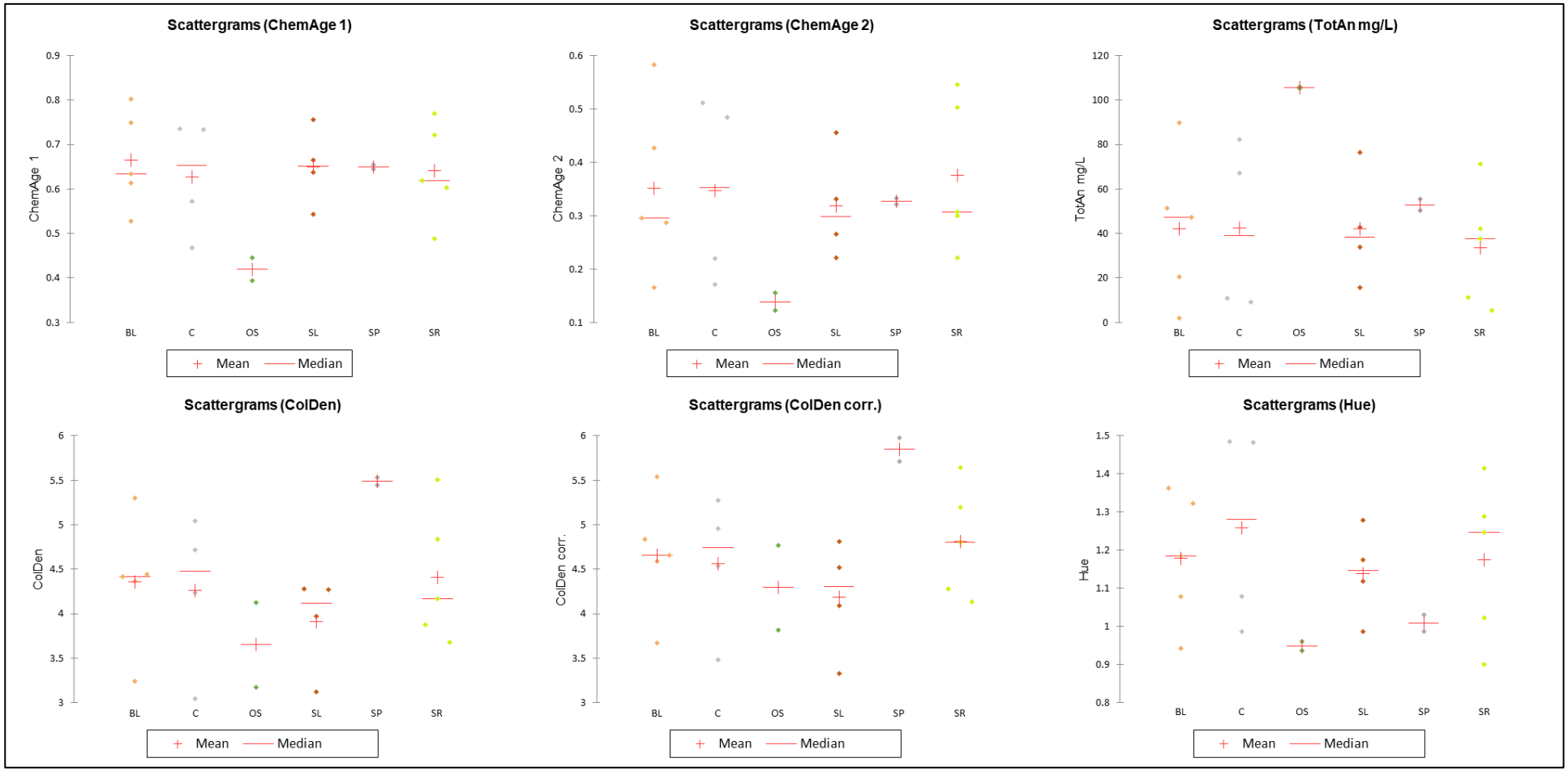


Figure 4.3 Scattergrams of 6 colour-related parameters in terms of 6 wine styles

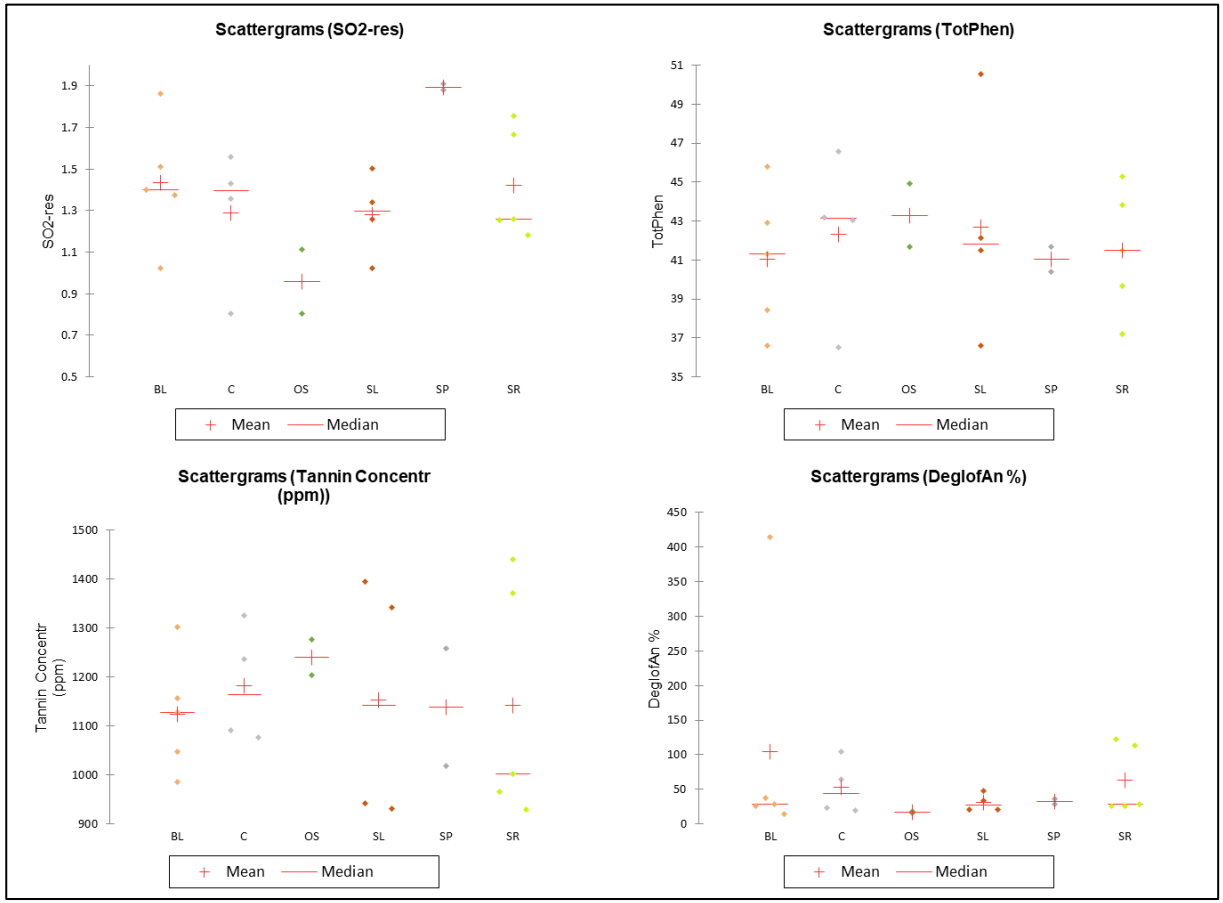


Figure 4.4 Scattergrams of 3 colour-related parameters and tannin concentration in terms of 6 wine styles

Table 4.2 Analysis of variance (ANOVA) with respect to wine styles for chemical measurements in the whole wines

Style	ChemAge 1	ChemAge 2	DegIofAn (%)	TotAn (mg/L)	ColDen AU	ColDen corr./AU	Hue AU	SO_2-res AU	TotPhen AU	MCP Tannin (mg/L)
BL	0.67	0.35	104	42	4.35	4.66	1.18	1.44	41	1124
SP	0.65	0.33	32	52	5.49	5.85	1.01	1.90	41	1138
SR	0.64	0.38	64	34	4.41	4.81	1.18	1.42	41	1142
C	0.63	0.35	53	42	4.26	4.56	1.26	1.30	42	1183
SL	0.65	0.32	31	42	3.91	4.19	1.14	1.28	43	1153
OS	0.42	0.14	17	106	3.65	4.30	0.95	1.00	43	1240
Pr > F(Styles)	0.170	0.497	0.815	0.147	0.184	0.150	0.436	0.058	0.971	0.982
Significant	No	No	No	No	No	No	No	No	No	No

BL, Brancott Letter; SP, Super Premium; SR, Stoneleigh Rapaura; C, Commercial; SL, Stoneleigh Latitude; OS, Central Otago Last Shepherd.

4.2.3 Relationships between chemical parameters

Table 4.3 shows the Pearson correlation coefficients between the 10 chemical parameters determined with statistically significant values ($P < 0.05$) shown in bold text. Chemical age 1 and chemical age 2 were strongly correlated ($r = 0.946$) with each other as expected, and they were significantly correlated with all the other chemical parameters with the exception of MCP tannin. In fact, MCP tannin was not significantly correlated with any of the other nine chemical parameters determined in this study. In contrast, total anthocyanin concentration was negatively correlated with the other colour-related parameters, in particular with hue and SO_2-resistant pigments. In fact, SO_2-resistant pigments whilst not correlated to hue were strongly correlated to both measures of colour density. Overall, therefore, the most significant relationships amongst the 10 chemical parameters measured for this set of 22 wines demonstrated a lack of correlation between MCP tannin and any colour-related parameter, whilst total anthocyanin was negatively correlated with hue and SO_2-resistant pigments were correlated with colour density.

Table 4.3 Pearson correlation coefficients between 10 chemical parameters of the 22 wines in the study

Variable	ChemAge 1	ChemAge 2	DegIofAn (%)	TotAn (mg/L)	ColDen	ColDen corr.	Hue	SO_2-res	TotPhen	MCP tannin (mg/L)
ChemAge 1	**1**	**0.946**	**0.560**	**-0.942**	**0.597**	**0.462**	**0.828**	**0.734**	**-0.504**	0.081
ChemAge 2	**0.946**	**1**	**0.677**	**-0.949**	**0.638**	**0.538**	**0.885**	**0.724**	**-0.508**	0.184
DegIofAn	**0.560**	**0.677**	**1**	**-0.553**	**0.465**	**0.426**	**0.513**	**0.507**	**-0.429**	0.120
TotAn	**-0.942**	**-0.949**	**-0.553**	**1**	**-0.471**	-0.344	**-0.892**	**-0.593**	**0.531**	0.005
ColDen	**0.597**	**0.638**	**0.465**	**-0.471**	**1**	**0.975**	0.345	**0.956**	-0.207	0.337
ColDen corr.	**0.462**	**0.538**	**0.426**	-0.344	**0.975**	**1**	0.242	**0.912**	-0.116	0.398
Hue	**0.828**	**0.885**	**0.513**	**-0.892**	0.345	0.242	**1**	0.400	**-0.510**	0.146
SO_2-res	**0.734**	**0.724**	**0.507**	**-0.593**	**0.956**	**0.912**	0.400	**1**	-0.267	0.269
TotPhen	**-0.504**	**-0.508**	**-0.429**	**0.531**	-0.207	-0.116	**-0.510**	-0.267	**1**	0.263
MCP tannin	0.081	0.184	0.120	0.005	0.337	0.398	0.146	0.269	0.263	**1**

*Values in bold are different from 0 with a significance level alpha=0.05.

4.2.4 Agglomerative Hierarchical Clustering (AHC) analysis

Figure 4.1 is the dendrogram of Agglomerative Hierarchical Clustering (AHC) analysis of twenty-two Pinot Noir wine samples with regards to colour measurements, MCP tannin and total phenolics concentrations. The dissimilarity at which two observations are combined into a cluster is called the cophenetic distance. Thus, this distance on the vertical axis, rather than the horizontal spacing, indicates the dissimilarity (or similarity) between individual samples. By specifying the desired number of groups (2-5), the AHC analysis partitioned the wines into three groups according to these measurements, generally corresponding to wine age.

The first group (Group 1) was the least homogeneous and consisted of seven wines from vintages from 2002 to 2011, 2014, and 2015. The second group (Group 2) includes wines from vintages of 2014, 2015, and 2016, and the third group (Group 3) includes wines from vintages 2017 and 2018. It appeared that AHC resulted in groups of wines differing primarily in age Thus, the central objects (SC03, SR14 and OS17) were in accord with three different age groups. The central object is determined as the closest to the centroid of the relevant group.

Wines from vintage 2014 and 2015 were partitioned between Groups 1 and 2. This agreed with the descriptive results in Figures 4.1 and 4.2. Compared to SR14, BL14 had a slightly lower hue, but greater values of the other colour parameters as well as tannin concentration. BD15 and BR15 were in a different cluster from BL15 and SL15. Compared to BL15 and SL15, BD15 and BR15 had higher values of SO_2-resistant pigments, colour density, and hue and thus they appeared more similar to older wines in Group 1 despite higher values of total anthocyanins, as shown in Figure 4.3 and 4.4.

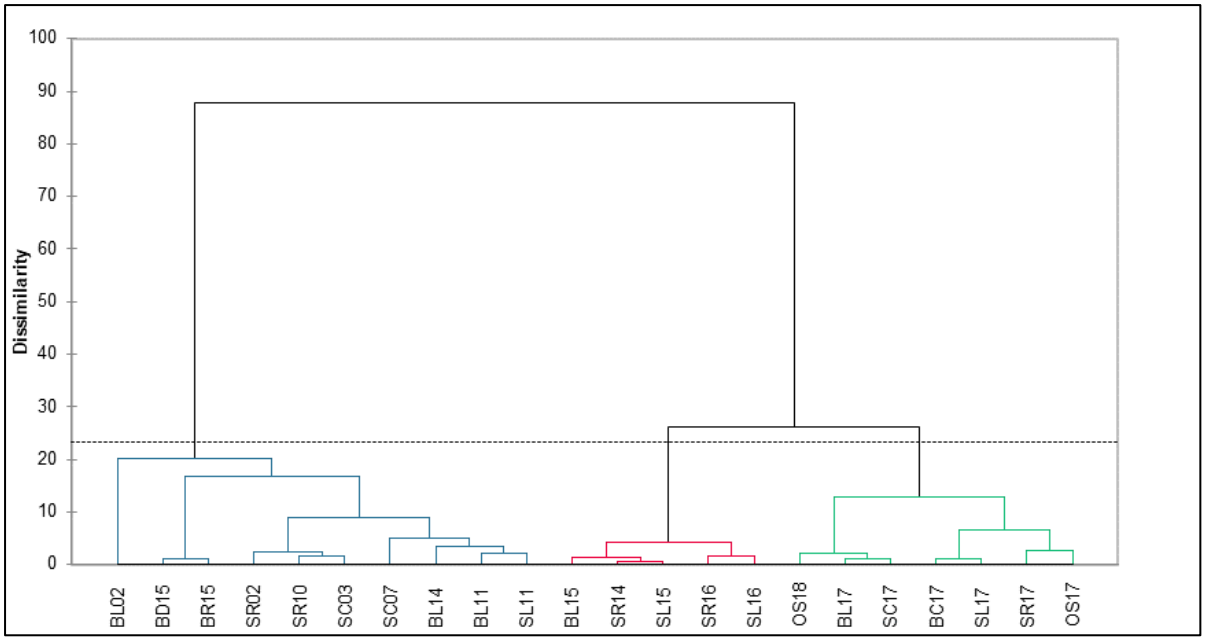


Figure 4.5 Dendrogram of Agglomerative Hierarchical Clustering (AHC) analysis of colour and phenolic measurements from 22 wine samples (Cophenetic correlation coefficient = 0.575)

Table 4.4 shows the analysis of variance (ANOVA) for the chemical measurements according to the clustering results. Statistically significant differences were seen among 9 out of 10 parameters ($P < 0.05$), except for the estimated ionisation of anthocyanins ($P = 0.110$). The ANOVA applied to AHC groups, roughly corresponding to wines increasing in age from Group 3 to Group 1, showed consistent increases in chemical age, hue, SO_2-resistant pigments and colour density, and a decrease in total anthocyanins, and despite the lack of statistical significance, an increase in the degree of ionisation of anthocyanins. However, statistically significant differences between groups for SO_2-corrected colour density, total phenolics and MCP tannin concentration did not follow the same pattern. Group 2 showed the lowest values of these parameters, whereas Group 1 showed the highest values for SO_2-corrected colour density and MCP tannin and Group 3 showed the highest total phenolics.

Table 4.4 Analysis of variance (ANOVA) with respect to AHC groups for chemical measurements in the whole wines

Cluster	ChemAge 1	ChemAge 2	DegIofAn (%)	TotAn (mg/L)	ColDen	ColDen corr.	Hue	SO_2-res	TotPhen	MCP Tannin (mg/L)
Group 1 (Old)	0.72 a	0.45 a	100	23 b	4.90 a	5.12 a	1.28 a	1.64 a	41 b	1222 a
Group 2 (Mid-aged)	0.63 b	0.30 b	28	42 b	3.82 b	4.09 b	1.13 b	1.23 b	40 b	965 b
Group 3 (Young)	0.49 c	0.18 c	20	85 a	3.82 b	4.33 b	0.97 b	1.11 b	45 a	1197 a
Pr > F(Clusters)	<0.0001	<0.0001	0.110	<0.0001	0.001	0.002	0.000	0.000	0.011	0.007
Significant	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes

For each chemical parameter, only means followed by a different letter were significantly different using Tukey's test ($P < 0.05$). *ChemAge1, chemical age 1; ChemAge2, chemical age 2; DegIofAn, degree of ionisation of anthocyanins; TotAn, total anthocyanins; ColDen, colour density; COlDen corr., SO_2 corrected colour density; TotPhen, total phenolics; SO_2-res, SO_2-resistant pigments.

Figure 4.2 illustrates the result of Agglomerative Hierarchical Clustering (AHC) in terms of the dissimilarity between the chemical measurements, akin to the correlation between these parameters (Table 4.3). On this basis, it can be observed that MCP tannin concentration, colour density and SO_2-resistant pigments clustered together. This was due to their moderately weak correlation with wine chemical age parameters, while total phenolics and total anthocyanins were grouped together, which showed a significantly negative correlation with chemical age. Parameters having a positive correlation with chemical age were clustered together, including chemical age, hue, and degree of ionisation of anthocyanins. Colour density and SO_2-resistant pigments were also strongly correlated. These agreed with the correlation matrix results reported in Table 4.3.

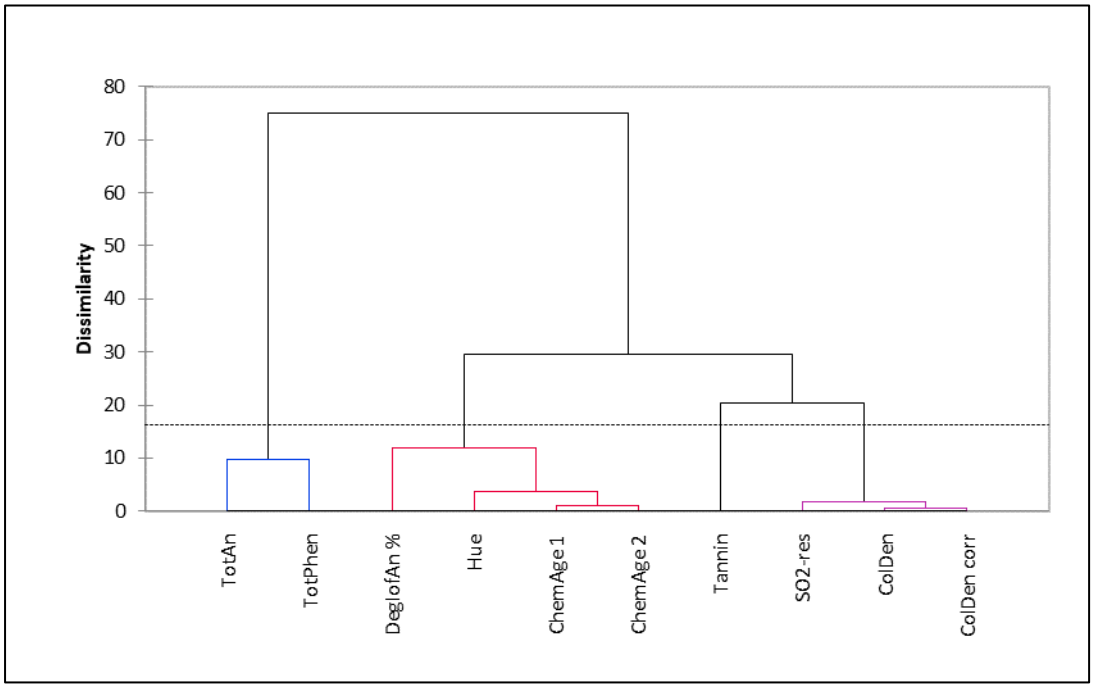


Figure 4.6 Dendrogram resulting from AHC analysis for 10 chemical parameters measured in the whole wines (Cophenetic correlation coefficient = 0.892)

4.3 Discussion

Wine age had a noticeable effect on wine colour parameters. Thus, chemical age 1 and 2 showed an expected increasing trend with wine age, as previously reported ((Longo et al., 2020). Chemical age relates to the increase in wine colour from oligomeric and polymeric pigments (Mercurio et al., 2007).

Statistically, significant differences between three age-related clusters were discerned. However, the effects of vintage and wine styles in other parameters were also discerned which modified general trends with age. Observed total anthocyanins reduced significantly with increasing wine age, indicating the instability of anthocyanins was in accord with previous observations (Alcalde-Eon et al., 2006). Monagas et al. (2006) found that the decrease of total anthocyanins along with wine age may be due to the degradation and formation of polymeric pigments, rather than the formation of pyranoanthocyanins. In terms of vintage and wine styles, large individual variations were also seen clearly but the variations were not statistically significant. Wines of OS17 and OS18 were observed to have higher concentrations of anthocyanins compared to the other five wine styles, likely because these wines had the lowest median chemical age, and spent less time (112 and 136 days, respectively) in barrel compared to the other wine styles. Thus, the higher anthocyanin concentrations may relate to the age, wine region as well as oak ageing time (Petrozziello et al., 2018; Wei, 2014). For the wines used in this study, the impact of wine age on total anthocyanins was dominant rather than vintage and wine style.

Hue and colour density showed an expected trend of gradually increasing over time in Pinot Noir wines (Heras-Roger et al., 2016; Perez-Prieto et al., 2003), although previous studies also reported the decline of colour density and increase of colour hue in Cabernet and Shiraz wines over 30 to 50 years old. Hue showed a significantly strong positive correlation with wine age and a strong negative correlation with total anthocyanins (0.828, -0.892), while colour density had a high correlation with non-bleachable pigments (0.956), and a less strong correlation with wine age as well as total anthocyanins (0.597, -0.471, respectively). This agreed with the previous reports (Bimpilas et al., 2016a; Laitila & Salminen, 2020). It indicated that different forms of coloured pigments may also influence colour density. Laitila and Salminen (2020) demonstrated that wine colour intensity was related to the oligomeric pigments, especially carboxypyranomalvidins and the oligomeric pigments (F-A or F-methylmethine-A). The concentration and size of those two types of pigments were found to influence the colour intensity. In young wines (1-year-old), colour intensity was significantly influenced by the malvidin-3-O-glucoside (M3G) oligomeric adducts. Bimpilas et al. (2016a) also confirmed that the colour density was increasingly correlated with the evolution of polymeric pigments.

Correspondingly, SO_2-resistant pigments increased significantly with wine age, which agreed with previous studies (Bimpilas et al., 2016a; Fu et al., 2015). The formation of pyranoanthocyanins and polymeric pigments, both resistant to SO_2 bleaching, was responsible for the increasing trend over time (Rivas et al., 2006). In this study, significant differences between vintages and wine styles were also noted. In 2015, the mean of SO_2-resistant pigments was significantly higher than vintages in 2016, 2017, and 2018 due to the higher amounts found in SP wines, BR15 and BD15 (Table 4.2 and Figure 4.2). Moreover, SP wines had significantly higher amounts of SO_2-resistant pigments than C, SL, and OS (Table 4.2). This was also confirmed by the AHC results in that BD15 and BR15 were clustered into the same subgroup, and well separated from the other two 2015 wines, BL15 and SL15. The concentrations of SO_2-resistant pigments are influenced by several factors, such as variety, storage time, bottle ageing, and co-pigmentation (Blanco-Vega et al., 2014; Wei, 2014). The two SP wines, BD15 and BR15, had high alcohol levels and distinct clonal selection with two dominant clones, 115 and 777. Different winemaking and maturation practices may also affect concentrations of non-bleachable pigments (Berrueta et al., 2020). As shown in Chapter 3, these wines spent more time in the barrel, around 550 days, compared to the other wine styles. Moreover, BR15 also had a lower average wood age (0.59) than BD15 (1.20) which might have contributed to higher amounts of SO_2-resistant pigments observed in this wine. Thus, the SP wines were distinguished by their distinct wine style regardless of wine age and both wine age and wine style contributed to SO_2-resistant concentrations. This suggested that the modifications of winemaking techniques for the SP wines were optimised to improve the perceived quality of the wine, highlighting the contribution of winemaking especially oak ageing to the management of wine colour and mouthfeel and thus the consequent wine quality.

Apart from the effect of wine age, vintage and wine style also influenced colour density as well as hue. Two commercial wines, SC03 and SC07, had a greater hue compared to the wines from 2002, indicating more rapid chemical oxidation and the development of brick/red-brown colours compared to other older wines from different wine styles (Canas et al., 2022). In contrast, SP wines had a higher colour density than SL and OS wines (Tables 4.1 and 4.2).

There was a general declining trend with wine age for total phenolics, with some differences between vintages being significant, while the variations between wine styles were not significant. There are several factors influencing the total phenolic content such as sun exposure, fining agents, duration and temperature for macerations (Carrasco-Sanchez et al., 2018; Sener, 2018; Song et al., 2015). This demonstrates how winemaking can affect wine styles. Overall, total phenolic content was negatively significantly affected by wine age but also varied between wine styles.

The average wine MCP tannin concentration was statistically different among the age clusters, but the trend of the tannin concentration was not consistent, as the medium-aged wines had a significantly lower mean tannin concentration than old and young wines. However, the trends based on the mean values of the three clusters were not able to describe the complete scattering patterns of tannin concentrations between vintages and styles, as no age-related trends were seen indicated by the extremely low correlation coefficients found between chemical age and tannin concentration (Table 4.1 and 4.2). Although there were no significant differences present between and within vintages and styles, great fluctuations of tannin concentrations were still noticeable (Figures 4.2 and 4.4). Wine tannin concentrations were affected by viticultural practices as well as winemaking interventions, as reviewed in chapter 2, such as grape variety, sun exposure, maceration, yeast selection, oxygen exposure, and barrel treatment (Carew et al., 2013; Casassa et al., 2013; Casassa et al., 2015; Gambacorta et al., 2011; Smith et al., 2015).

Although AHC analysis showed a clear pattern in relation to wine age, three wines (BD15, BL14 and BR15) clustered together with older wines. The separation was potentially due to the greater total SO_2-resistant pigments. Winemaking effects, such as different times and temperatures for maceration may explain these results (Casassa & Harbertson, 2014; Cojocaru & Antoce, 2017; Setford et al., 2017). Compared to the AHC analysis of the general chemical parameters (Figure 3.1, Chapter 3), the age-related pattern was more clearly revealed in the AHC result on the basis of spectrophotometric and tannin results, indicating that colour parameters, total phenolics and tannin were more discriminatory than the general chemical parameters.

4.4 Conclusion

Overall, these results demonstrated a strong age-related pattern for the phenolic parameters measured, with the exception of tannin concentration. These parameters (except tannin concentration) were strongly correlated with measures of chemical age. This was confirmed by cluster analysis using AHC, which also revealed that wines in the SP group were more similar to older wines than anticipated based on their actual age. However, other than this, there was no clear differentiation between styles. In the case of MCP tannin, concentrations were not related to wine age; instead, it was potentially associated with vintage and region. The SP wines were distinguished by higher amounts of SO_2-resistant pigments and greater colour density compared to the other wine styles. Therefore, SO_2-resistant pigments play an important role in the discrimination of wines according to age and style. Thus, the characterisation of SO_2-resistant pigments (fractionation and structure) is the primary subject of the following chapters.

Chapter 5

Fractionation of Wine Polymeric Phenolic Material and Spectrophotometric Characterisation

5.1 Introduction

Wine phenolics include both monomeric and polymeric materials, and their impacts on wine colour and wine quality have been investigated by many authors (Cheynier et al., 2006; Gutierrez-Escobar et al., 2021; Harrison, 2018; Kassara & Kennedy, 2011). Polymeric pigments were found to be the main contributors to colour stability and mouthfeel attributes in red wine (Bimpilas et al., 2015; Heras-Roger et al., 2016; Vidal et al., 2003). To separate the polymeric from monomeric material, a number of methods had been established and used widely in wine studies. These include: isoamyl alcohol extraction (Somers, 1971), solid phase extraction (SPE) (Jeffery et al., 2008; McRae et al., 2012) and size exclusive chromatography combined with liquid-liquid separation (McRae et al., 2014; McRae et al., 2013; Weber et al., 2013; Wollmann & Hofmann, 2013).

Studies have also noted that polymeric pigments were less sensitive to the pH changes compared to anthocyanins and tend to be non-bleachable for SO_2 (Bloomfield, 2006; Cheynier et al., 2006; Somers, 1971; Somers & Evans, 1974). Somers (1971) established a correction factor of 1.667 for polymeric pigments corresponding to a pH change from pH 3.4 to < 1.0 in young Shiraz wines. Thus, the absorbance at 520 nm of non-bleachable pigments increased by the factor of 1.667 from that at pH 3.4 to pH 1.0. Later, Somers and Evans (1977) suggested that that factor ranged from 1.5 to 2.0 for the wines studied. However, there is little information on the relevance of this factor for Pinot Noir wines from different vintages and styles (Wimalasiri, 2020). More recently, Jeffery et al. (2008) used SPE to separate monomeric phenolics (F1) from two polymeric fractions (F2 and F3). The physicochemical properties of the two polymeric fractions differed, probably attributable to the oxidative transformation of those polymers (del Alamo et al., 2004; Jeffery et al., 2008). Many other studies have focused on SEC to characterise the composition and sensory properties of wine polymers (McRae et al., 2013; Weber et al., 2013; Wollmann & Hofmann, 2013).

However, despite a large body of information on polymeric phenolic fractions, their exact nature remains unclear. The objective of this chapter was to examine the spectrophotometric properties of a range of polymeric fractions extracted from Pinot Noir wines. It was anticipated that these data would provide some insights into the nature of the polymeric material itself as well as information on their concentrations and the influence of vintage, age and style. Wine fractions were obtained using a variety of techniques, namely, a polymeric fraction (F-iso) remaining after isoamyl alcohol extraction of anthocyanins, three fractions (F1, F2, and F3) from solid phase extraction, and two polymeric fractions (Bu and Aq) using size exclusion chromatography.

5.2 Results

5.2.1 Polymeric material separated by isoamyl alcohol extraction of anthocyanins

5.2.1.1 Spectrophotometric analysis for polymeric material (F-iso)

As described above (Chapter 3), a previous protocol (Somers, 1971) was used to separate anthocyanins in wine from the polymeric phenolic material (F-iso). The contribution of that polymeric material to wine colour and its response to pH was investigated and compared with the findings of Somers (1971). Figure 5.1 shows the scattergram of the ratio of absorbance at 520 nm at pH 1.0 to that at pH 3.4 (correction factor) for polymeric material isolated by isoamyl alcohol from 2002, 2003, 2007, 2010, 2011, and 2014 to 2018 vintages. With increasing wine age, a generally decreasing response was seen in the ratio of absorbance at 520 nm at pH 1.0 to that at wine pH. The ratio varied from 2.6 to 1.1 for 2018 and 2002 wines, respectively.

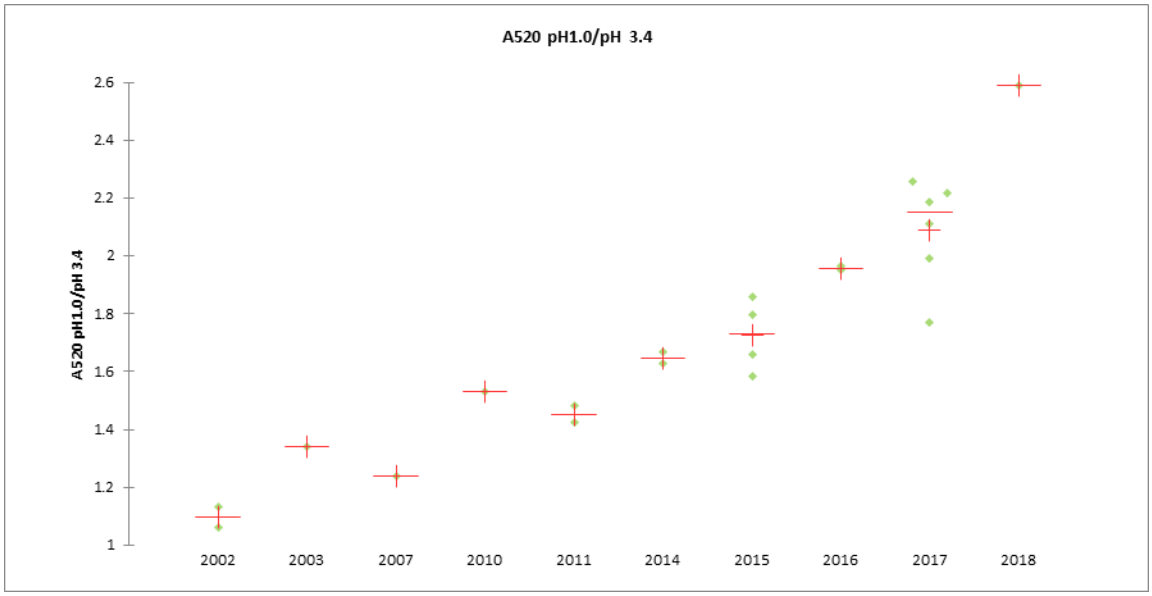


Figure 5.1 The scattergram of the ratio of absorbance 520 nm at pH 1.0 to that at pH 3.4 for polymeric material isolated as a result of isoamyl alcohol extraction of anthocyanins

Figure 5.2 shows scattergrams of two other calculated parameters, termed the degree of ionisation of anthocyanins (DegIofAn %) and total anthocyanins (TotAn mg/L) in the modified Somers assay described by Mercurio et al. (2007). Clearly, for polymeric material this designation is inappropriate. In reality TotAn mg/L is a measure of the difference between the total 520 nm absorbance measured at pH 1 and that assumed to be from SO_2-resistant pigments also at pH 1 (i.e. utilising a correction factor). In fact, if isoamyl alcohol extraction had been effective in removing anthocyanins, and all polymeric pigments (i.e. not extracted by isoamyl alcohol) were SO_2-resistant, this measure should have been zero for all wines. The actual correction factor for polymeric material varied; thus, Figure 5.2 shows results using the individually determined correction factors for each wine applied to the calculations for total anthocyanins and the degree of ionised anthocyanins rather than the assumed factor.

For the parameter designated TotAn mg/L, results varied from 9 to 28 mg/L, with some indication of lower values for older wines. The fact that these results were non-zero suggested that there was a component of polymeric pigment that was not SO_2-resistant, and that this component generally reduced with age. The parameter designated DegIofAn % is calculated as absorbance at 520 nm of

SO_2-bleachable pigments at wine pH as the numerator, and the total SO_2-bleachable absorbance measure at pH < 1.0 as the denominator. These values clearly increased with age from 39% for 2018 wines to 94% for 2002 wines. This indicates that material designated TotAn mg/L becomes increasingly ionised (i.e. coloured) at wine pH with age. Overall, these results demonstrated two features. First, there exists a component of polymeric pigment that is not SO_2-resistant (i.e. bleachable). Second, this material becomes increasingly ionised (i.e. coloured) with age. There is also some evidence that the concentration of this material tends to decline with age.

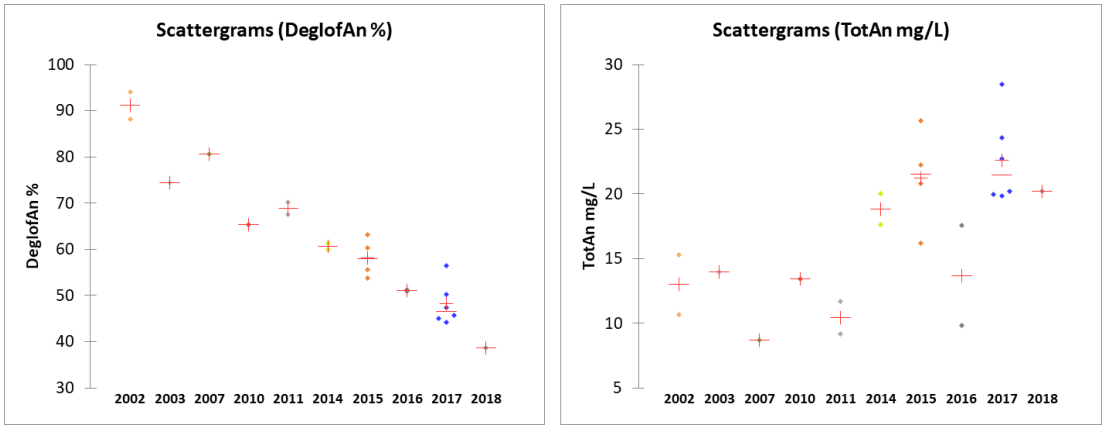


Figure 5.2 Scattergrams of the degree of ionised bleachable pigments (DeglofAn) and total bleachable pigments (TotAn) for polymeric material isolated as a result of isoamyl alcohol extraction of anthocyanins

The remaining colour and phenolic parameters, and MCP tannin concentration were plotted in Figure 5.3. With wine age increasing, general increasing patterns were seen with regard to chemical age (1 and 2), colour density, colour density corrected for SO_2, and hue. In addition, total phenolics and MCP tannin concentrations did not follow any age-related trends. Total phenolics for polymeric material ranged from 15 AU to 30 AU (absorbance unit), and they were lower than that in the whole wine, as we expected. The highest amounts of total phenolics were observed in wines SR10, SC03, and BL02, while the lowest amounts were in wines SC07, SL16, and SL11. MCP tannin concentrations ranged from around 550 mg/L to 1100 mg/L (epicatechin equivalent). The highest and lowest tannin concentrations were also found in the same wines as that of total phenolic content. When considering wine style, no clear patterns were observed.

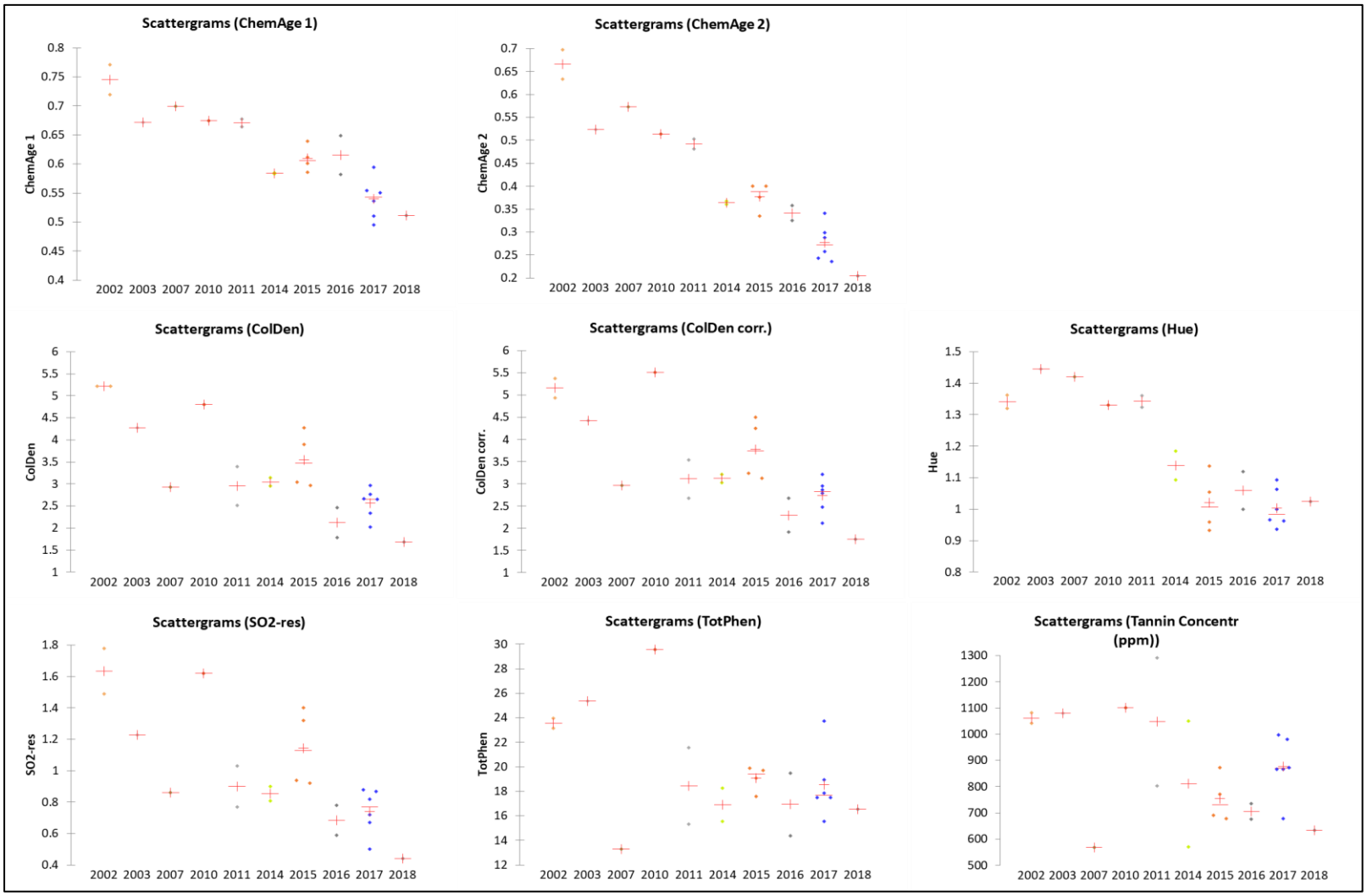


Figure 5.3 Scattergrams of related colour parameters, total phenolics and MCP tannin concentrations for polymeric material

5.2.1.2 Agglomerative Hierarchical Clustering analysis of F-iso

Figure 5.4 is the dendrogram of agglomerative clustering analysis for the F-iso polymeric fraction. Four clusters were identified. Five wines from vintages 2002 to 2011 appeared in a cluster (Group 1), with the exceptions of SC07 and SL11. Wines from 2014 to 2017 were grouped together (Group 2), while the remaining six wines from 2017 and 2018 vintages fell into a third group (Group 3). SC07 and SL11 were seen in a separate group (Group 4). Thus, similar to the results for whole wines (Chapter 4), an age-related pattern was revealed from Pinot Noir wines based on colour and tannin properties found in the polymeric material. Figure 5.5 shows the relationship between these chemical parameters. Three clusters were observed, as before for whole wines (Chapter 4). The central objects for these clusters were total anthocyanins, chemical age 2, and colour density corrected for SO_2. Chemical age was closely related to colour hue, while colour density and non-bleachable pigments were highly related.

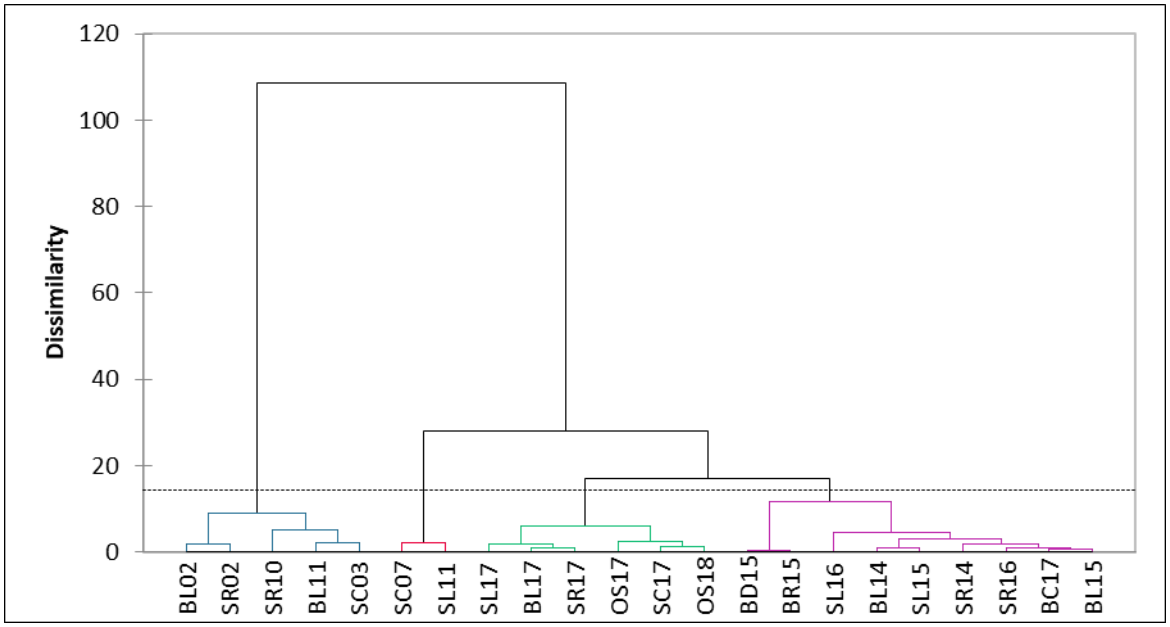


Figure 5.4 Dendrogram resulting from Agglomerative Hierarchical Clustering (AHC) of 22 studied wines for the polymeric fraction F-iso (Cophenetic correlation coefficient = 0.785)

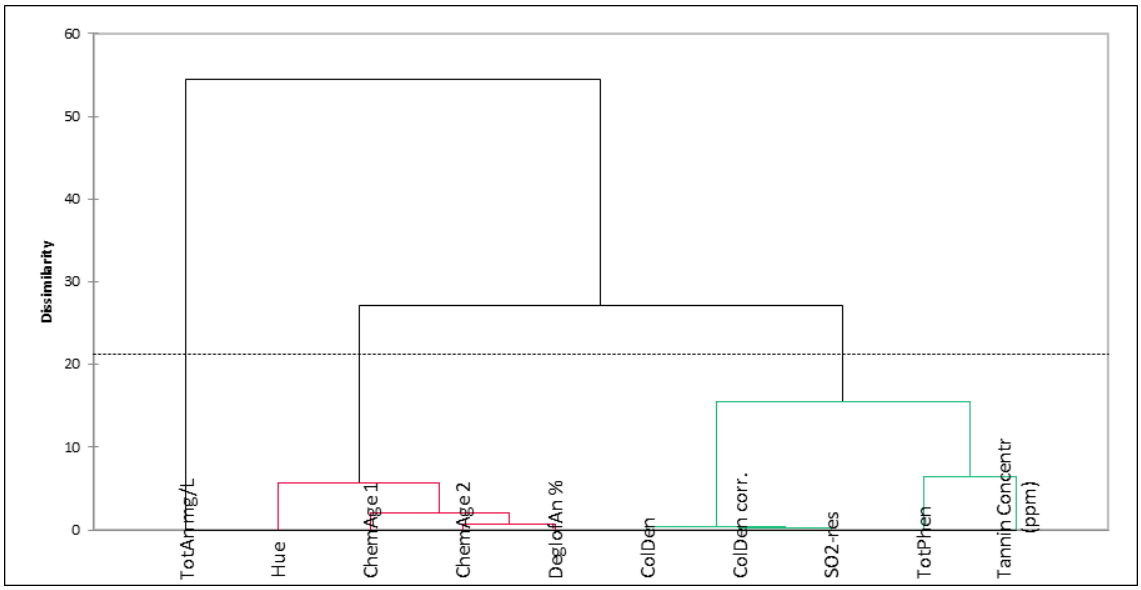


Figure 5.5 Dendrogram resulting from Agglomerative Hierarchical Clustering (AHC) of colour, phenolics, and tannin concentration (Cophenetic correlation coefficient = 0.916)

Table 5.1 shows the ANOVA analysis between the wine clusters for polymeric material F-iso in terms of the spectrophotometric parameters and tannin concentration. These clusters were significantly different ($P < 0.05$) for all 11 parameters. In relation to the age-related groups (3 to 1, increasing in age), TotAn (bleachable pigments) and A520 pH1/pH3.4 decreased, whilst chemical age, DegIofAn, colour density, hue, and SO_2-resistant pigments increased. These results mirror those for whole wines (Chapter 4). In contrast, MCP tannin and total phenolics did not follow age-related patterns, with the lowest values for the mid-aged group and the highest for the old-aged group. Considering SC07 and SL11, they were characterised by the lowest TotAn and tannin concentrations.

Table 5.1 ANOVA analysis for colour parameters, total phenolics, tannin concentration, and A520 pH 1.0/pH 3.4 of F-iso in accordance with the AHC clusters

Cluster	ChemAge 1	ChemAge 2	DeglofAn %	TotAn mg/L	ColDen	ColDen corr.	Hue	SO_2-res	TotPhen	MCP tannin (ppm)	A520 pH1/pH3.4
Group 1	0.70 a	0.57 a	78 a	6.9 c	4.58 a	4.76 a	1.36 a	1.43 a	24.7 a	1119 a	1.30 c
Group 4	0.68 a	0.53 a	74 a	3.8 c	2.72 b	2.83 b	1.37 a	0.82 b	14.3 b	686 b	1.36 c
Group 2	0.60 b	0.36 b	60 b	20.0 b	3.05 b	3.24 b	1.06 b	0.95 b	17.9 b	769 b	1.77 b
Group 3	0.53 c	0.26 c	45 c	28.6 a	2.36 b	2.49 b	1.00 b	0.67 b	18.4 b	839 b	2.23 a
Pr > F(Cluster)	<0.0001	<0.0001	<0.0001	<0.0001	0.000	0.000	<0.0001	0.001	0.000	0.001	<0.0001
Significant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

For each chemical parameters, only means followed by a different letter were significantly different using Tukey's test ($P < 0.05$).

5.2.2 Wine fractionation by SPE

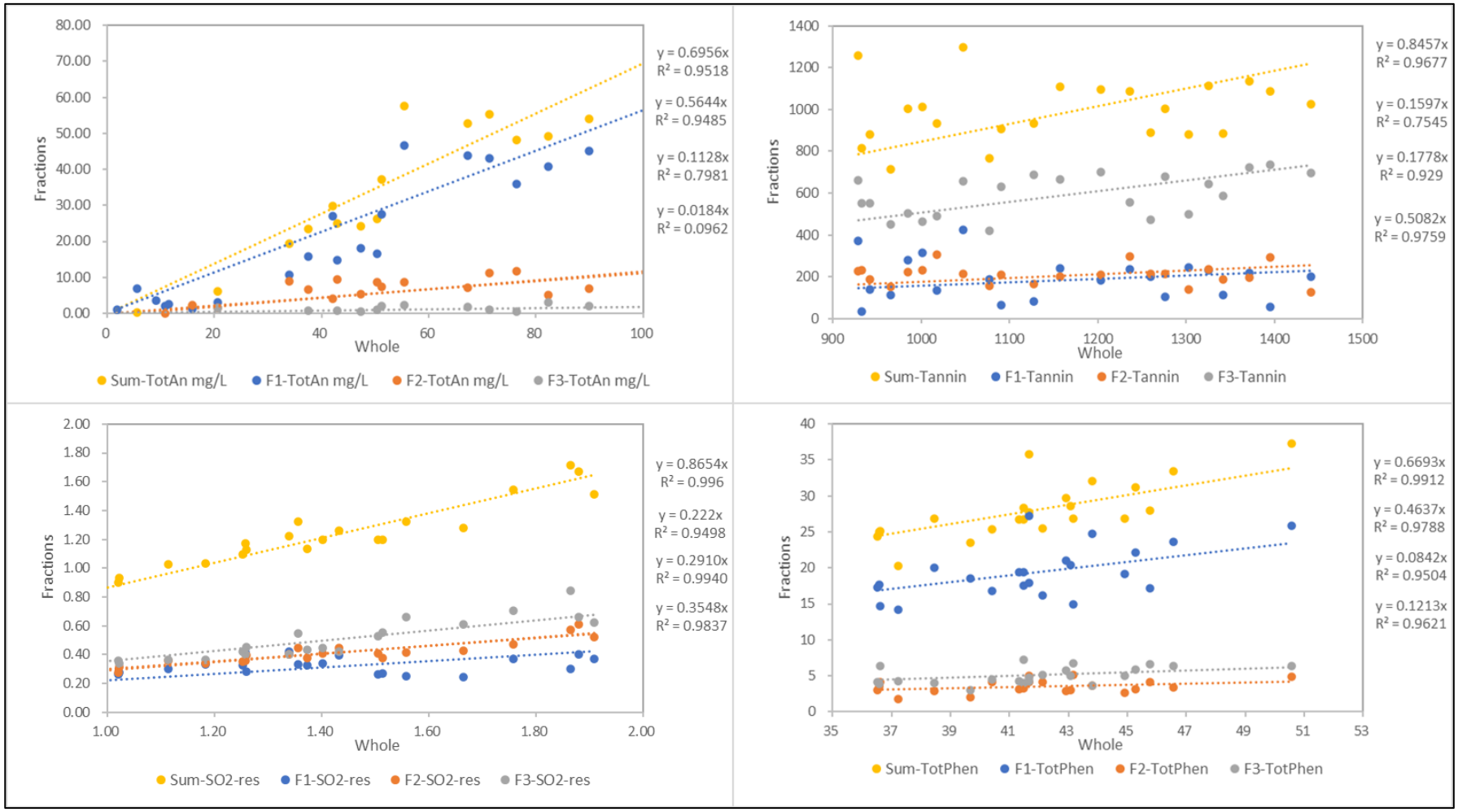
5.2.2.1 Spetrophotometric analysis for SPE fractions

As described above (Chapter 3), the method of McRae et al. (2013) was used to fractionate wines. Three fractions were obtained: F1, considered to be monomeric phenols; and F2 and F3, considered to be polymeric phenols distinguished by their degree of hydrophobicity. The colour properties, total phenolics, and tannin concentration were determined using standard methods as before.

The relationships of these parameters between the three SPE fractions and the whole (unfractionated) wine are given in Figure 5.6. Approximately 70% of total anthocyanins were measured in the three fractions in comparison with that in the unfractionated wine. The estimated anthocyanin concentration in F1 was the highest (56%), as expected. In addition, F2 had approximately 10% higher anthocyanin concentration than F3 (only 1%). F1 was strongly correlated with total anthocyanins measured in the unfractionated wine (0.95), whereas the correlations with F2 and F3 were less strong (0.80 and 0.09, respectively). Regarding MCP tannin, around 85% of tannins in the whole wine was extracted into the three SPE fractions, with F3 having the highest proportion, 51%, followed by F2 (18%) and F1 (16%). The linear regression results showed tannin concentration measured in fractions F2 and F3 had strong correlations with that measured in the whole wine (0.929 and 0.976, respectively), whereas there was a less strong correlation found with F1 (0.755). Similar patterns were also seen in SO_2-resistant pigments. Nearly 86% of the SO_2-resistant pigments were successfully eluted in total. F3 also had the highest proportion of SO_2-resistant pigments, around 35%, while around 29% of SO_2-resistant pigments were observed in F2. F1 had the lowest proportion of SO_2-resistant pigments, at nearly 22%. There were strong linear relationships between the three fractions and the whole wine ($R2 > 0.95$).

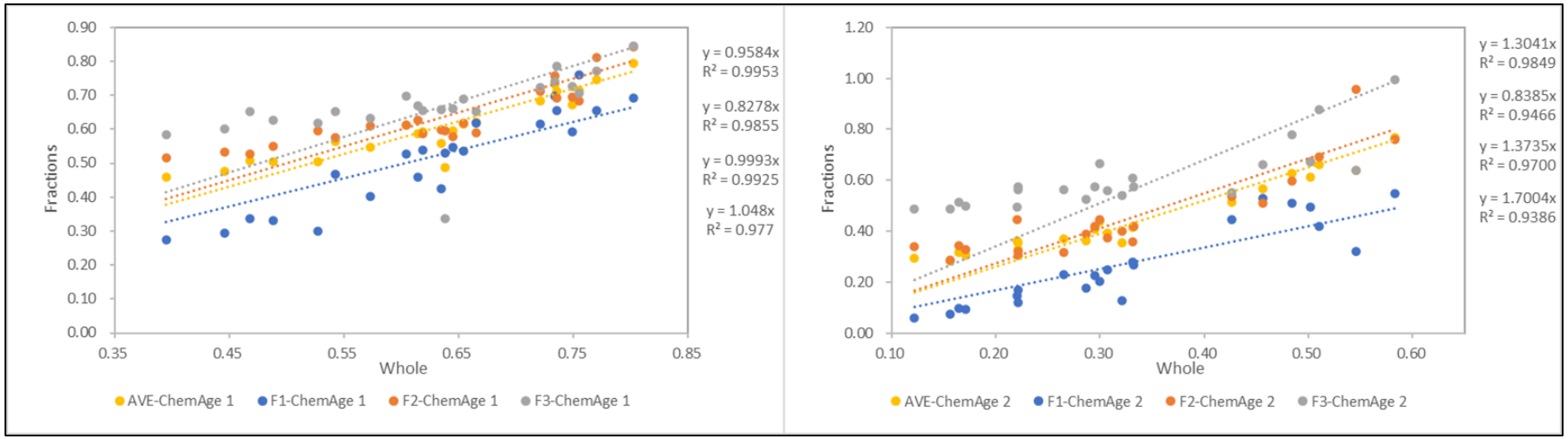
In contrast, only around 67% of total phenolics were observed in the total of three fractions. The highest proportion of total phenolics appeared in F1 (46%), while only 8% and 12% were found in F2 and F3, respectively, as we expected. F3 had the greatest chemical age (1 and 2), followed by F2 and F1. Generally, F1 had a lower chemical age than that whole wine, whilst F2 and F3 had higher chemical age than that of the whole wine. F3 and F1 had similar colour hue to the whole wine, whilst F2 had a

lower colour hue than the whole wine (Figure 5.7). Regarding colour parameters (Figure 5.8), colour density and corrected density for SO_2 of fractions accounted for 28% to 33% of the colour density determined in the whole wine. A slightly higher of colour density was present in F3. They displayed a strong correlation with that measured in the whole wine (0. 93-0.99). The hue of fractions was similar to the unfractionated wine, and strong relations were also observed between the three fractions and the unfractionated wine ($R2 > 0.98$). In summary, for the polymeric fractions, F2 was intermediate in most parameters F1 and F3. F3 had the greatest chemical age, and its colour related parameters such as SO_2-resistant pigments, colour density, and hue appeared to be the highest. F3 also had the highest concentrations of tannin.



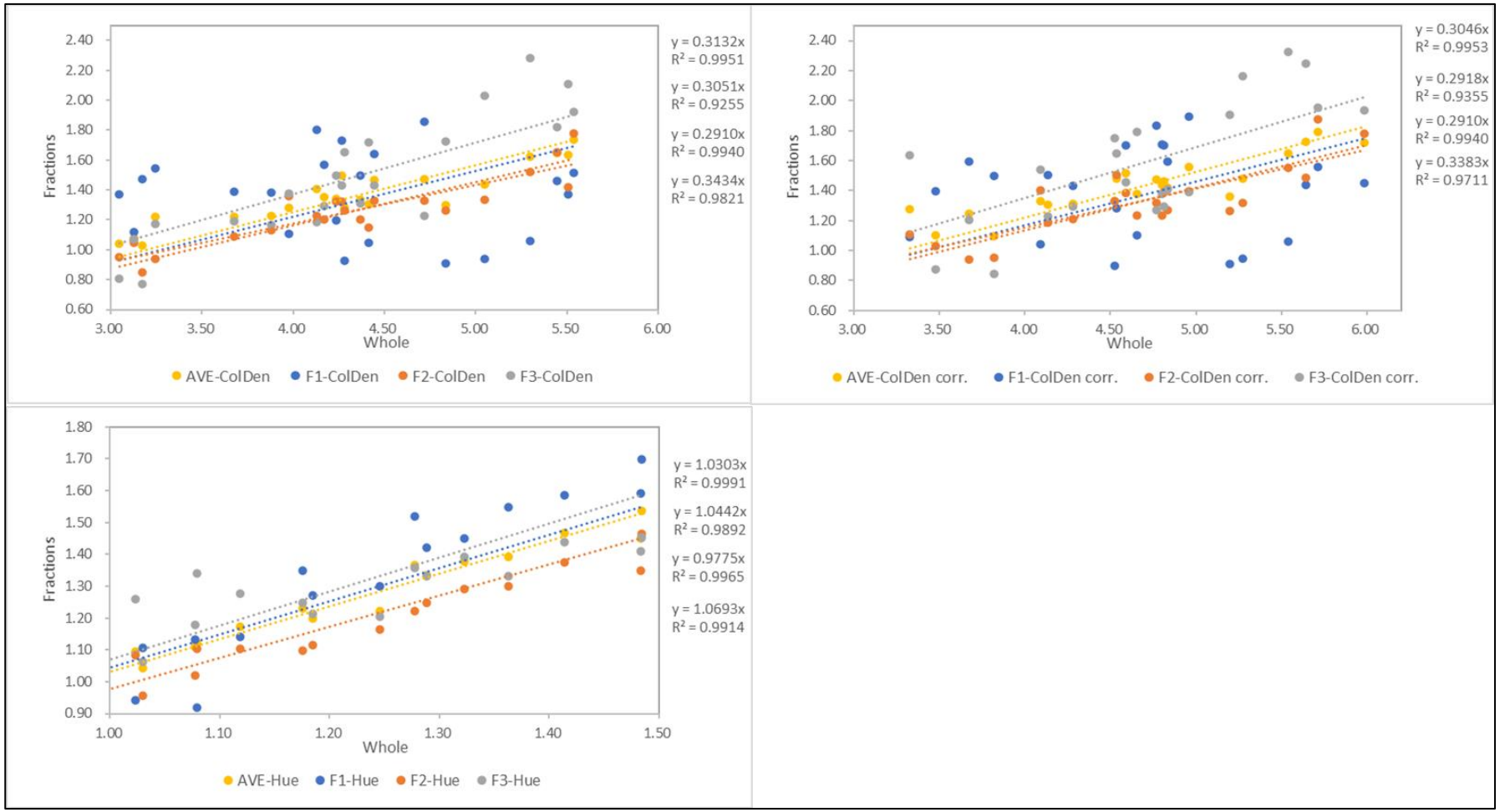
*The equations and R squared values displayed on the right side of the charts are, from the top to the bottom, for Sum, F1, F2, and F3

Figure 5.6 The relationships of total anthocyanin (TotAn), MCP tannin, SO_2-resistant pigments (SO_2-res), and total phenolics (TotPhen) between the whole wine and the three SPE fractions



*The equations and R squared values displayed on the right side of the charts are, from the top to the bottom, for Sum, F1, F2, and F3

Figure 5.7 The relationships of chemical age (1 and 2) and hue between the three SPE fractions and whole wine



*The equations and R squared values displayed on the right side of the charts are, from the top to the bottom, for Sum, F1, F2, and F3.

Figure 5.8 The relationships of colour density (ColDen), colour density corrected for SO_2 (ColDen corr.), and hue between the three SPE fractions and whole wine

5.2.2.2 Agglomerative Hierarchical Clustering for SPE fractions

Based on the spectrophotometric data for three SPE fractions, agglomerative clustering analysis of studied wines was carried out and the results are displayed in Figure 5.9. A distinct clustering pattern was discerned in relation to wine age as well as the previously defined (Chapter 4) wine style. The 22 wines were clustered into three groups, consisting of wines from 2002 to 2015 but excluding the two SP wines, wines from 2015 to 2018, and the SP wines. The SP wines, BD15 and BR15, separated from other wines but were more similar to the younger aged group.

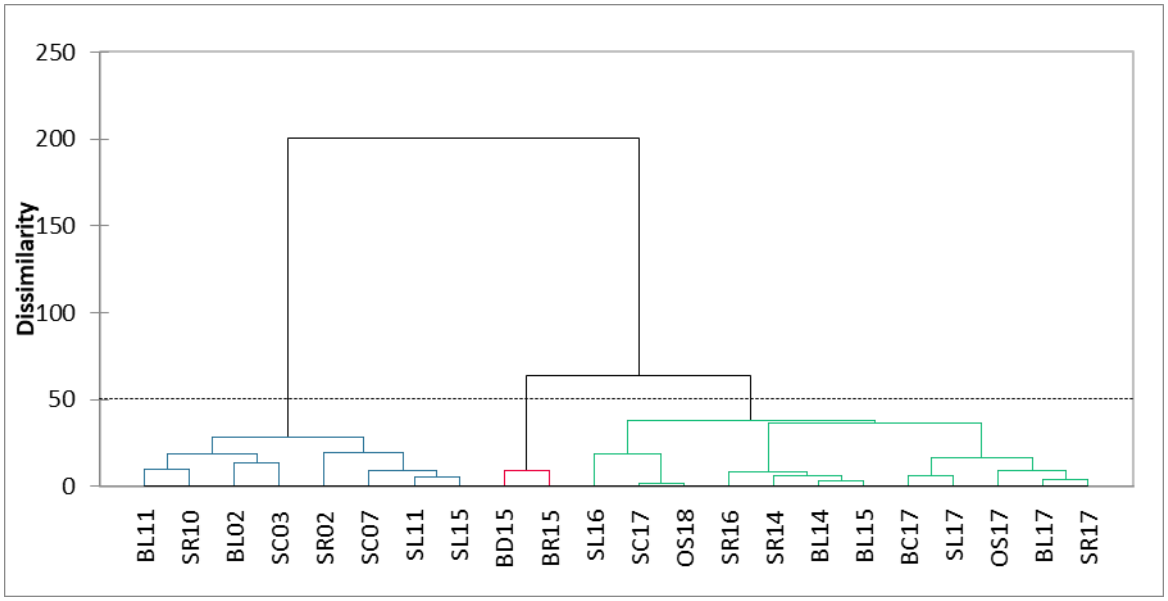


Figure 5.9 Dendrogram resulting from Agglomerative Hierarchical Clustering (AHC) analysis of study wines for three SPE fractions (Cophenetic correlation coefficient = 0.651)

Figure 5.10 shows the AHC analysis for the spectrophotometric parameters and tannin concentration. Three clusters were identified for the 25 chemical parameters determined for all the fractions. Total anthocyanin in F2 and F3 was excluded. Chemical age was associated with colour hue, while SO_2-resistant pigments were associated with colour density. In addition, total anthocyanins in F1 were also associated with colour density. The representative parameters for each individual cluster were colour density in F1, SO_2-resistant pigments in F2, and chemical age 1 in F2.

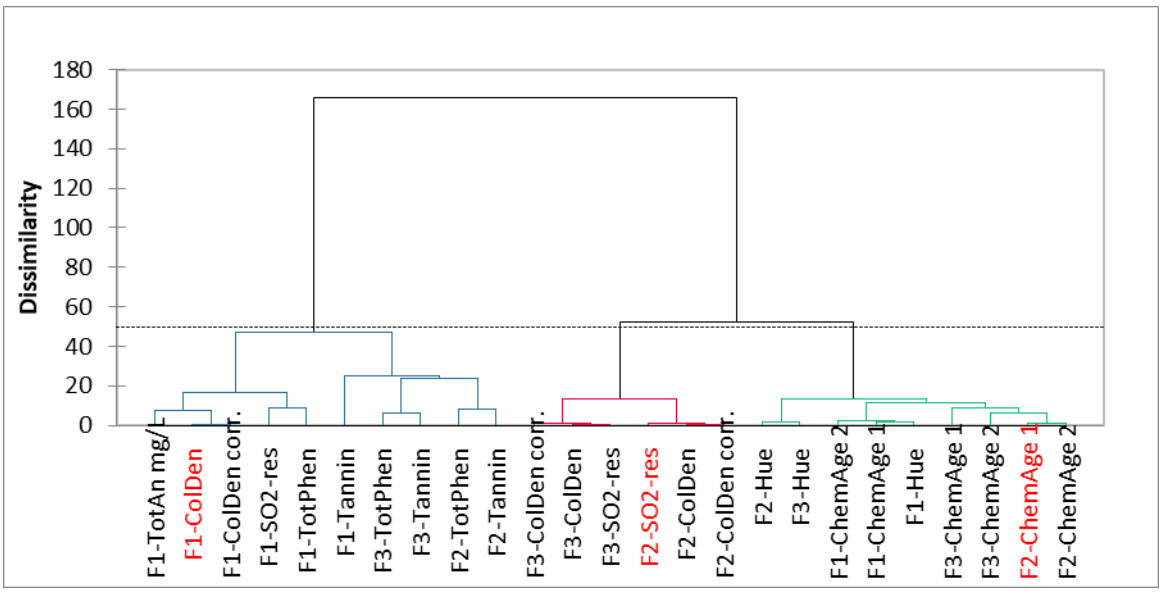


Figure 5.10 Dendrogram resulting from AHC analysis of chemical parameters for three SPE fractions (Cophenetic correlation coefficient = 0.760)

Table 5.2 shows the results of ANOVA analysis based on the clustering results of three SPE fractions. Statistically, a total of 19 parameters were significantly different ($P < 0.05$) between these groups; differences among tannin concentrations in three fractions, total phenolics in F2 and F3, and SO_2-resistant pigments in F1 were not significantly different ($P > 0.05$). For F2 and F3 there was an increase in chemical age (1 and 2), colour density, colour density corrected for SO_2, hue, and SO_2-resistant pigments from younger to older wines. This was in accordance with the results described in Chapter 4 for whole wines. However, parameters in F1 showed distinct patterns, such that colour density and colour density corrected for SO_2 decreased from younger to older wines.

SP wines appeared similar to the younger wine cluster with respect to parameters in F1 with significantly higher total anthocyanins, colour density, and total phenolics than older wines, as well as lower hue. Considering chemical parameters in F2, SP wines stood out from the other wines with the highest values for colour density, colour density corrected for SO_2, and SO_2-resistant pigments. Regarding parameters in F3, SP wines were similar to the older wines. In addition, despite not being significantly different, SP wines also had the highest concentrations of MCP tannin and total phenolics in F2, and the lowest tannin concentrations in F3. Therefore, the discriminant parameters for SP wines were determined mainly in F2.

Table 5.2 ANOVA analysis for three SPE fractions in terms of the colour parameters, total phenolics, and tannin concentration

Clusters	F1-ChemAge 1	F1-ChemAge 2	F1-TotAn mg/L	F1-ColDen	F1-ColDen corr.	F1-Hue	F1-SO_2-res	F1-TotPhen	F1-Tannin
1 (Old)	0.66 a	0.44 a	3.9 b	1.07 b	1.09 b	1.52 a	0.29	16.2 b	200
2 (SP)	0.54 ab	0.20 b	31.6 ab	1.49 a	1.50 a	1.10 b	0.39	22.0 a	169
3 (Mid-aged and Young)	0.41 b	0.15 b	37.7 a	1.53 a	1.58 a	0.97 b	0.32	21.1 a	187
Pr > F(Cluster)	<0.0001	<0.0001	0.000	0.000	0.000	<0.0001	0.088	0.002	0.921
Significant	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No

Clusters	F2-ChemAge 1	F2-ChemAge 2	F2-ColDen	F2-ColDen corr.	F2-Hue	F2-SO_2-res	F2-TotPhen	F2-Tannin
1 (Old)	0.72 a	0.64 a	1.33 b	1.39 b	1.29 a	0.44 b	3.7	192
2 (SP)	0.60 b	0.41 ab	1.72 a	1.83 a	0.93 b	0.57 a	4.1	262
3 (Mid-aged and Young)	0.58 b	0.36 b	1.14 c	1.20 c	1.05 b	0.34 c	3.3	214
Pr > F(Cluster)	<0.0001	0.000	<0.0001	<0.0001	<0.0001	0.000	0.425	0.172
Significant	Yes	Yes	Yes	Yes	Yes	Yes	No	No

Clusters	F3-ChemAge 1	F3-ChemAge 2	F3-ColDen	F3-ColDen corr.	F3-Hue	F3-SO_2-res	F3-TotPhen	F3-Tannin
1 (Old)	0.74 a	0.72 a	1.80 a	1.92 a	1.37 a	0.61 a	5.5	610
2 (SP)	0.68 ab	0.56 ab	1.87 a	1.94 a	1.05 b	0.64 a	4.4	481
3 (Mid-aged and Young)	0.62 b	0.54 b	1.17 b	1.27 b	1.19 b	0.37 b	4.9	599
Pr > F(Cluster)	0.007	0.003	<0.0001	<0.0001	<0.0001	<0.0001	0.330	0.258
Significant	Yes	Yes	Yes	Yes	Yes	Yes	No	No

For each chemical parameter, only means followed by a different letter were significantly different using Tukey's test ($P < 0.05$).

5.2.3 Wine fractionation by SEC

5.2.3.1 Spectrophotometric analysis for SEC fractions

As described in Chapter 3, wine tannin was isolated using SEC and fractionated using liquid-liquid separation from butanol to create two tannin subfractions, an aqueous fraction (Aq, a) and a butanol fraction (Bu, b) for each study wine (McRae et al., 2013). Their spectrophotometric properties and tannin concentrations were determined using standard methods as before (Mercurio et al., 2007).

Tannin and SO_2-resistant pigments measured for aqueous and butanol fractions are given in Table 5.3. Tannin concentrations and SO_2-resistant pigments of aqueous and butanol fractions fluctuated, and no trends were observed considering wine age and style. Tannin concentration of the aqueous fraction ranged from 271 mg/L epicatechin equivalent (SL15) to 782 mg/L (SL11), while that of the butanol fraction ranged from 125 mg/L (SC03) to 369 mg/L (BR15). Regardless of age and style, aqueous fractions contained higher tannin concentrations, between 18% and 76%, compared with butanol fraction. The ratio of total tannin of two fractions to that of the whole wine varied from 0.38 to 1.14. SO_2-resistant pigments in the aqueous fraction accounted for 15% to 96% of that in whole wine, while that in the butanol fraction accounted for 20% to 95%. Thirteen wines had greater SO_2-resistant pigments in butanol fractions compared with corresponding aqueous fractions, while the remaining had less SO_2-resistatant pigments in butanol fractions. For example, SL17 and BR15 had more than doubled SO_2-resistant pigments in butanol fractions compared with aqueous fractions, whilst SR10, OS18, SC07, and SR17 had respectively 73%, 43%, 33%, and 21% lower amounts of SO_2-resistant pigments in butanol fractions compared with their corresponding aqueous fractions.

Spectrophotometric measurements for aqueous and butanol fractions are given in Table 5.4. Trace amounts of total phenolics were determined in butanol fractions (< 5.1 AU), whilst the highest amounts were observed in BD15 and BR15 wines. Total phenolics measured in the aqueous fraction ranged from 1.2 to 14.4 AU, accounting for less than 35% of the total phenolics measured in the whole wine. Large variations between and within vintages were

seen in total phenolics in both fractions. A general increasing pattern of chemical age (1 and 2) with age was observed, as expected, but the largest value was found in BC17. SR10 also had greater chemical age than wines from 2002 and 2007. General increasing patterns with age were seen in colour density, colour density corrected for SO_2, and hue in the aqueous fractions, with some exceptions. SR10 had the highest colour density and colour density corrected for SO_2. BD15 and BC17 also showed higher colour density (3.23 and 3.66, respectively), compared with BL02 (1.34) and SC03 (1.79). In contrast, colour density, colour density corrected for SO_2, and hue in the butanol fluctuated across vintages. For example, SR10 had almost the lowest hue (1.1) compared with the highest hue in BC17 (1.5). BD15 and BR15 had the highest colour density whilst OS18 and Sl17 had the lowest colour density.

Table 5.3 Mean values of tannin and SO_2-resistant pigments amounts measured in aqueous and butanol fractions, and relative to whole wine (n=2)*

Wine code	Tannin-a mg/L	Tannin-b mg/L	Tannin (b/a)	Tannin (a+b)/w	SO_2-res-a AU	SO_2-res-b AU	SO_2-res b/a	SO_2-res (a+b)/w
BC17	629	314	0.50	0.71	1.38	1.16	0.84	1.77
BD15	802	360	0.45	1.14	0.91	0.99	1.10	1.01
BL02	435	239	0.55	0.60	0.46	0.73	1.61	0.64
BL11	301	201	0.67	0.48	0.23	0.59	2.60	0.54
BL14	490	228	0.46	0.55	0.59	0.59	1.01	0.85
BL15	405	176	0.43	0.59	0.54	0.44	0.82	0.70
Bl17	334	158	0.47	0.43	0.22	0.24	1.09	0.45
BR15	696	363	0.52	0.84	0.40	1.54	3.84	1.01
OS17	497	265	0.53	0.63	0.39	0.66	1.70	0.95
OS18	401	144	0.36	0.43	0.36	0.20	0.57	0.69
SC03	341	125	0.37	0.38	0.54	0.44	0.82	0.63
SC07	556	227	0.41	0.73	0.60	0.40	0.67	0.74
SC17	327	196	0.60	0.48	0.23	0.26	1.16	0.60
SL11	782	215	0.28	0.74	0.53	0.47	0.90	0.66
SL15	271	113	0.42	0.41	0.24	0.47	1.98	0.57
SL16	314	150	0.48	0.50	0.17	0.20	1.24	0.36
SL17	650	336	0.52	0.71	0.25	0.83	3.30	0.80
SR02	575	137	0.24	0.49	0.72	0.66	0.92	0.83
SR10	453	159	0.35	0.45	1.20	0.32	0.27	0.87
SR14	399	171	0.43	0.59	0.49	0.82	1.67	1.10
SR16	408	190	0.47	0.60	0.27	0.38	1.41	0.52
SR17	502	187	0.37	0.74	0.46	0.36	0.79	0.65

*a and b refer to the aqueous and butanol fractions, respectively; b/a, the ratio of b to a; (a+b)/w, the ratio of the total tannin of the two fractions to the whole wine.

Table 5.4 Mean values of colour and phenolic parameters determined in the aqueous and butanal fractions (n=2)

Wine code	ChemAge 1-a*	ChemAge 2-a	ColDen -a	ColDen corr.-a	Hue-a	TotPhen -a	ChemAge 1-b*	ChemAge 2-b	ColDen -b	ColDen corr.-b	Hue-b	TotPhen -b
BC17	0.90	0.06	3.66	3.72	1.40	10.16	0.80	0.03	2.66	3.51	1.50	1.43
BD15	0.57	0.04	3.23	3.24	1.09	8.46	0.64	0.05	3.30	3.51	1.16	5.04
BL02	0.79	0.07	1.34	1.38	1.41	1.88	0.80	0.07	2.09	2.28	1.36	3.15
BL11	0.64	0.05	0.86	0.82	1.34	1.18	0.60	0.05	2.14	2.11	1.09	3.68
BL14	0.53	0.02	2.06	2.19	1.00	8.49	0.61	0.03	2.00	2.14	1.30	1.38
BL15	0.53	0.02	1.95	2.01	0.98	7.31	0.59	0.02	1.65	1.64	1.45	1.63
BL17	0.58	0.01	0.85	0.83	1.19	2.84	0.54	0.01	0.83	0.98	1.38	0.55
BR15	0.66	0.05	1.30	1.26	1.16	1.51	0.74	0.07	4.82	4.81	1.21	5.09
OS17	0.48	0.02	1.45	1.58	0.97	7.19	0.52	0.02	2.22	2.62	1.32	1.46
OS18	0.77	0.01	0.92	1.05	1.27	5.73	0.43	0.01	0.76	0.98	1.50	0.80
SC03	0.72	0.05	1.79	1.83	1.46	8.77	0.65	0.05	1.70	1.70	1.26	1.60
SC07	0.69	0.04	2.03	2.07	1.35	7.07	0.69	0.04	1.48	1.43	1.22	0.36
SC17	0.51	0.01	0.89	0.91	1.13	5.08	0.51	0.02	0.96	1.13	1.36	1.26
SL11	0.50	0.06	1.77	2.23	1.14	12.29	0.68	0.04	1.59	1.70	1.23	1.66
SL15	0.52	0.02	0.92	0.97	1.18	4.40	0.68	0.03	1.68	1.68	1.14	1.14
SL16	0.52	0.01	0.69	0.74	1.38	3.33	0.60	0.01	0.77	0.81	1.28	1.61
SL17	0.43	0.01	0.82	1.22	1.12	10.99	0.69	0.04	2.85	2.79	1.16	5.05
SR02	0.65	0.04	2.51	2.58	1.41	10.16	0.82	0.07	2.51	2.17	1.09	1.27
SR10	0.73	0.14	4.42	3.89	1.41	14.37	0.78	0.03	1.19	1.09	1.10	-1.19
SR14	0.68	0.03	1.59	1.64	1.28	6.19	0.78	0.06	2.99	2.72	1.06	1.26
SR16	0.54	0.02	1.06	1.04	1.10	13.86	0.62	0.02	1.23	1.37	1.29	1.49
SR17	0.56	0.02	1.56	1.60	0.98	9.49	0.48	0.02	1.16	1.43	1.49	1.35

*a, aqueous fraction; b, butanol fractions; ChemAge, chemical age; ColDen, colour density; ColDen corr., colour density corrected for SO_2; TotPhen, total phenolics.

5.2.3.2 Agglomerative Hierarchical Clustering analysis for SEC fractions

Figure 5.11 is the dendrogram resulting from Agglomerative Hierarchical Clustering (AHC) analysis of SEC fractions of study wines. Four groups were identified related to age and style. Group 1 consisted of seven older wines from 2002 to 2014. The two SP wines clustered in Group 2, while BC17 and SR10 clustered in Group 3. The remaining elven wines from 2014 to 2018 fell into Group 4.

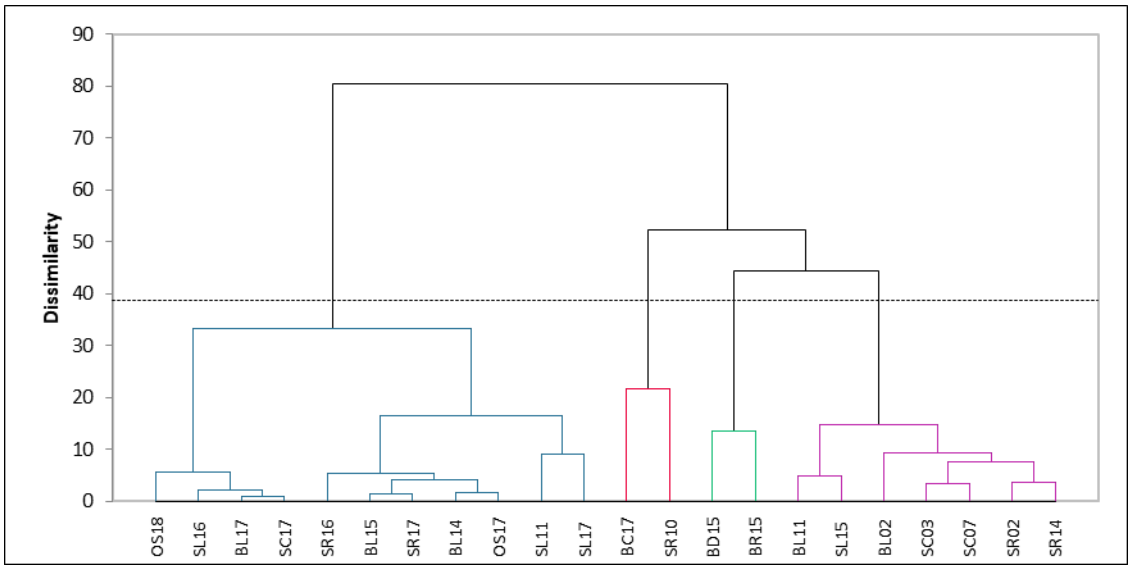


Figure 5.11 Dendrogram from AHC analysis of study wines for SEC fractions (Aq and Bu) (Cophenetic correlation coefficient = 0.513)

The results of ANOVA for the chemical measurements measured for aqueous and butanol fractions according to the AHC cluster results are given in Table 5.5. Statistically, significant differences were seen among 16 out of 18 parameters ($P < 0.05$), with only total phenolics in the aqueous fraction and hue of butanol fraction not significantly different between clusters. Regardless of fractionation, as wine age increased, the expected increasing patterns in most of colour parameters were seen between the two age-related groups 1 and 4. Chemical age (1 and 2), colour density, colour density corrected for SO_2, and SO_2-resistant pigments increased with age in both fractions, whilst hue decreased over ageing only in the aqueous fraction. The average tannin concentrations of the four clusters also showed significant differences in both fractions ($P < 0.05$). SP wines had the highest concentrations in both fractions, followed by the BC17/SR10 cluster, then young wines from 2014 to 2018. In contrast, old wines from 2002 to 2014 had the lowest amounts of MCP tannin. SP also had the highest mean amounts of total phenolics measured in butanol, whilst BC17/SR10 had the lowest amounts.

SP wines were characterised by the chemical parameters measured in the butanol fraction, particularly colour density, SO_2-resistant pigments, tannin concentration, and total phenolics, rather than aqueous fraction. Only tannin measured in aqueous fractions was seen significantly different between SP and

the other three groups. In contrast, the separation of BC17 and SR10 was associated with chemical parameters measured in the aqueous fraction since these wines had the highest chemical age, colour density, hue, SO_2-resistant pigments, and total phenolics in the aqueous fraction, whilst in butanol fraction, most of chemical parameters were not significantly different between BC17/SR10 and the other three groups.

Table 5.5 ANOVA analysis based on SEC clustering results on the basis on actual values

Clusters	ChemAge 1-a*	ChemAge 2-a	ColDen-a	ColDen corr.-a	Hue-a	SO_2-res-a	TotPhen-a	Tannin-a
3 (BC17/SR10)	0.82 a	0.10 a	4.04 a	3.80 a	1.41 a	1.29 a	12.27	541 ab
2 (SP)	0.61 ab	0.05 ab	2.26 b	2.25 ab	1.12 ab	0.65 b	4.99	749 a
1 (Old)	0.67 a	0.04 b	1.57 b	1.61 b	1.35 a	0.47 b	5.66	411 b
4 (Young)	0.54 b	0.02 b	1.27 b	1.40 b	1.12 b	0.36 b	7.87	465 b
Pr > F(Cluster)	0.002	0.000	0.000	0.001	0.001	<0.0001	0.128	0.034
Significant	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Clusters	ChemAge 1-b*	ChemAge 2-b	ColDen-b	ColDen corr.-b	Hue-b	SO_2-res-b	TotPhen-b	Tannin-b
3 (BC17/SR10)	0.79 a	0.03 ab	1.92 b	2.30 ab	1.30	0.74 ab	0.12 b	237 ab
2 (SP)	0.67 ab	0.06 a	4.06 a	4.16 a	1.18	1.27 a	5.06 a	361 a
1 (Old)	0.72 a	0.06 a	2.08 b	2.01 b	1.17	0.59 b	1.78 b	173 b
4 (Young)	0.57 b	0.02 b	1.46 b	1.60 b	1.34	0.42 b	1.66 b	204 b
Pr > F(Cluster)	0.002	<0.0001	0.001	0.002	0.056	0.002	0.004	0.006
Significant	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes

For each chemical parameter, only means followed by a different letter were significantly different using Tukey's test ($P < 0.05$). *a, aqueous phase; *b, butanol phase; ChemAge1, chemical age 1; ChemAge2, chemical age 2; DegIofAn, degree of ionisation of anthocyanins; TotAn, total anthocyanins; ColDen, colour density; COlDen corr., colour density corrected for SO_2; TotPhen, total phenolics; SO_2-res, SO_2-resistant pigments.

5.3 Discussion

In the present study, in response to wine acidification, absorbance at 520 nm of polymeric material increased confirming the presence of flavylium chromophores in polymeric pigments (Somers, 1971) regardless of age. Some chromophores in polymeric pigments were colourless at pH 3.4 and converted to the flavylium cation form at pH < 1.0, resulting in the increase of absorbance at 520 nm at pH < 1.0. Somers (1971) found that this factor for polymeric pigments varied from 1.5 to 2.0, and many studies have applied a factor of 5/3 to polymeric pigments in order to obtain an estimate of monomeric anthocyanins in wine (Mercurio et al., 2007; Somers & Evans, 1974; Somers & Evans, 1977). In the current study of Pinot Noir wines, a rather wider range of 1.1 to 2.6 was found. Moreover, this factor for polymeric pigments showed a general decrease from 2.6 to 1.1 as wine age increased. This age-related trend over ageing was first observed in the present study and it remains to be seen why polymeric pigments in old wine were more resistant to colour loss at higher pH. One possibility was that the structure and composition of polymeric pigments had been modified during ageing leading to

the formation of more stable structures (Bimpilas et al., 2016b; Heras-Roger et al., 2016). In addition, T-A-F (A type) are colourless at both wine pH and pH 1.0 and as a result polymeric pigments in old wine had less chromophores compared with young wines. In addition, the variations in this pH factor for polymeric pigments were not only affected by age because large variations were seen within vintages (Figure 5.1). This was likely caused by modulations during winemaking and maturation processes concerning different styles and quality achievements (Catania et al., 2021; Sener, 2018; Yang et al., 2021b).

Applying these individual pH factors to related colour parameters, the concentration of bleachable pigments (TotAn mg/L) decreased broadly over ageing, whilst the degree of ionisation of bleachable pigments (DegIofAn %) increased over ageing. This result confirmed the appearance of bleachable pigments in the polymeric material, which was supported by previous studies (Cheynier et al., 2006; Cucciniello et al., 2021; Laitila, 2021). Cucciniello et al. (2021) found that some types of non-bleachable pigments in acidic conditions may release monomeric anthocyanins. For example, the formation of acetaldehyde-bridged polymeric pigments such as M3G - acetaldehyde-(epi) catechin dimers was reversable and could release M3G. As a result, the colour attributed to bleachable pigments increased, whilst the colour attributed to polymeric pigments decreased. Some studies also showed that acetaldehyde linked pigments decreased during ageing and almost diminished in old wine (He et al., 2012). Another possibility was that around 2% of monomeric anthocyanins were likely to be retained in the aqueous phase, as studies showed that around 98% of anthocyanins could be removed by isoamyl alcohol (Somers, 1971).

A relatively high recovery (over 80%) concerning phenolic material was observed by SPE method (Jeffery et al., 2008). In contrast, large variations (38%-114%) of the total tannin extractability were observed from aqueous and butanol fractions, which was also confirmed by the previous studies (McRae et al., 2014; McRae et al., 2013). McRae et al. (2013) measured MCP tannin of 7-year-old wine isolated from SEC method and the total tannin of the two fractions was around 14% higher than the whole wine tannin. It was suggested that SPE method tended to be a robust extraction method concerning polymeric phenolics, whilst SEC and liquid-liquid separation methods may lack some consistency and reliability, especially for tannin measurements.

The discrimination of wine age was associated with colour and phenolic parameters, whilst the discrimination of style was not only associated with colour and phenolic parameters, but also associated with tannin concentration, which was confirmed by AHC and ANOVA results.

With wine age increasing, general increasing trends were seen concerning chemical age, hue, colour density, and SO_2-resistant pigments in F2, F3, and two SEC fractions. This was consistent with previous studies (Cheynier et al., 2006; Fulcrand et al., 2006; Laitila & Salminen, 2020; Mouls & Fulcrand, 2015)

and the results in Chapter 4. In the present study, SO_2-resistant pigments in F2 were lower than that in F3, which was also supported by previous studies (Jeffery et al., 2008). Some studies noted that F3 had a greater hydrophobicity than F2 (Jeffery et al., 2008), while butanol fractions had a greater hydrophobicity that the aqueous fractions (McRae et al., 2013). Previous studies found that polymeric pigments had a reduced infinity for water during ageing and this was attributed to the formation of more intermolecular or/and intramolecular linkages resulting from oxidation (Cheynier et al., 2006; Céline Poncet-Legrand et al., 2010). As a result, SO_2-resistant pigments in wine evolved during wine ageing. For example, flavanol-anthocyanins condensation products transformed into A-type polymeric pigments during ageing (Cheynier, 2012). This suggested that the composition and structure of polymeric pigments in F3 and the butanol fraction tended to be more modified compared with F2 and the aqueous fraction. Laitila (2021) also noted that colour density was related to different concentrations and sizes of those polymeric pigments.

In contrast, general decreasing patterns were seen in F1 concerning total anthocyanins, colour density and colour density corrected for SO_2, and total phenolics, whilst the increasing patterns were only seen in chemical age (1 and 2) and hue. This was attributed to the large ratio of monomeric pigments to polymeric material present in F1. Laitila (2021) noted that carboxypyranomalvidins, as monomeric pigments, were responsible for colour intensity. In addition to monomeric pigments, the presence of SO_2-resistant pigments and MCP tannin was also observed in F1, but they did not follow age-related trends. This was supported by previous studies (de Freitas & Mateus, 2011a; Jeffery et al., 2008). These polymeric phenolics were found to be low molecular pigments such as 4-vinylphenol derivatives of M3G (Jeffery et al., 2008), and de Freitas and Mateus (2011a) noted that 4-vinylphenol derivatives of M3G were found to be resistant to sulphite and remained constant during wine ageing. As a result, monomeric anthocyanins, 4-vinylphenol derivatives of M3G, and carboxypyranomalvidins potentially contributed to the colour intensity in F1.

Significant differences concerning tannin concentration were only found in SEC fractions. In the present study, SP had the greatest tannin concentration measured in SEC fractions, whilst there were no significant differences found between old and young wines (Carew et al., 2013; Gambacorta et al., 2011; Smith et al., 2015). As discussed in Chapter 4, MCP tannin concentrations could be affected by viticultural practices as well as winemaking interventions (Casassa et al., 2013; Casassa et al., 2015). Tannin concentration of aqueous fraction was much higher than butanol fraction, as expected (McRae et al., 2013).

Regarding total phenolics, significant differences were only found in F1 as well as SEC fractions. SP wines had the highest amounts, and young wines had higher amounts than old wines in both F1 and SEC fractions, which was agreed with previous results (Castellari et al., 2000; Gomez-Plaza et al., 2016).

Previous studies found that total phenolics had positively correlated with the levels of monomeric phenolics as well as wine quality (Gómez-Plaza et al., 2016; Kassara & Kennedy, 2011; Valentin et al., 2016).

SP (super premium) wines displayed distinct characteristics concerning colour and phenolic parameters and tannin concentration. With respect to some parameters, SP wines appeared similar to mid-aged and young wines with significantly higher levels of total anthocyanins, colour density, and total phenolics, and lower hue in F1. In contrast, with respect to other parameters SP wines appeared more similar to old wines with significantly higher levels of colour density and polymeric pigments observed in F2 and F3, although similar to mid-aged and young wines with the lowest hue in F3. In addition, SP wines were distinguished by the measurements for butanol fractions rather than aqueous fractions, with the highest colour density and colour density corrected for SO_2, SO_2-resistant pigments, total phenolics and tannin concentrations observed in butanol fractions. As described in Chapter 3, super premium wines spent the longest time in oak barrels, over 500 days. The micro oxygenation during oak ageing likely modified tannin structure and formed large polymeric phenolics in super premium wines (Sanchez-Iglesias et al., 2009; Yang et al., 2021a). The present study suggested that wine quality may be indicated by colour properties and tannin concentration, which was in accordance with previous studies observed in Syrah, Monastrell, and Pinot Noir wines (Gómez-Plaza et al., 2016; Kassara & Kennedy, 2011; Valentin et al., 2016). Gómez-Plaza et al. (2016) confirmed that wine quality was positively correlated with colour intensity, tannin concentrations, total phenolics, and greater polymeric pigments. Overall, colour intensity, SO_2-resistant pigments, total phenolics, tannin concentration tended to be significant contributors to the discrimination of wine quality.

Compared with the AHC result in Chapter 4 (three age-related clusters), the separation of super premium wines was related to measurements in SPE and SEC butanol fractions, whilst the separation of BC17 and SR10 wines were related to aqueous fractions. BC17 and SR10 wines appeared similar to the old wines because they possessed the highest chemical age, colour density, hue, and SO_2-resistant pigments in aqueous fractions indicating the increased colour incorporation in tannins in BC17 and SR10. This suggested that to some extent, wine phenolic fractionation might be a useful tool to differentiate wines in relation to quality.

5.4 Conclusion

In conclusion, red colour of polymeric phenolic material in old wines tended to be more resistant to changes in pH as indicated by a reduction in the factor A520 pH1/pH3.4 from 2.6 to 1.1 during ageing. These results for Pinot Noir wines are the first observations of an age-related trend for this factor in polymeric material. The degree of ionisation of these bleachable polymeric components increased during ageing, which was also observed for the first time. In addition, discrimination between wines in

terms of age and style was associated with colour and phenolic properties and tannin concentrations. Tannin concentration measured in SEC fraction and total phenolics measured in F1 and SEC fractions contributed to the discrimination of super premium wines. Super premium wines were characterised by significantly higher colour density, SO_2-resistant pigments, total phenolics, and tannin concentrations. Tannin fractionation to some extent contributed to the discrimination of quality. In addition, general increasing trends with increasing wine age were observed concerning colour properties in all polymeric fractions, whilst the monomeric fraction showed a decreasing trend in colour density. However, it remains to be seen which types of pigments caused the increased colour density in the monomeric and polymeric materials. Therefore, the compositional characterisation of different types of monomeric phenolics and polymeric pigments is the emphasis and focus of the following chapter.

Chapter 6

Chromatographic Analyses of the Composition of Monomeric and Polymeric Phenolic Material

6.1 Introduction

Phenolic composition, particularly anthocyanins and modified pigments, contribute to the colour, mouthfeel, and quality of red wine (Gutierrez-Escobar et al., 2021; Harrison, 2018; Huang & Xu, 2021; McRae et al., 2013). The contribution of monomeric anthocyanins and polymeric pigments to total pigments could be predictors of colour hue and density (Bimpilas et al., 2016b; Boulton, 2001; Heras-Roger et al., 2016). Various studies have investigated the evolutionary trends of polymeric pigments and tannin composition during winemaking and/or ageing as well as their impacts on colour and mouthfeel. However, few studies have focused on the compositional characterisation of key polymeric phenolics in Pinot Noir wines considering their impact on wine style/quality. Laitila (2021) studied the oligomeric pigments in wines from various varieties, vintages, and regions, found that for one-year-old Pinot Noir wines the average size of polymeric pigments was correlated with wine region, with Pfalz region showing higher average size than Beaune region, and for 1-6 years old wines dimeric adducts were the major compounds for T-A, T-A-(epi) catechin (A type), and T-methylmethine-A types of polymeric pigments. However, there is little information on the different types of polymeric phenolic material between young Pinot Noir wines and older wines. Currently, the methods for the quantification of individual anthocyanins, monomeric anthocyanin derivatives, and small dimers of anthocyanins-(epi) catechin are well established using liquid chromatography-mass spectrometry (LC-MS/MS) in numerous studies (Alcalde-Eon et al., 2006; Blanco-Vega et al., 2014; Delgado-Povedano et al., 2021; Willemse et al., 2015), whilst only a few methods available for the compositional quantification of polymeric pigments (Engström et al., 2014; Engström et al., 2015; Laitila, 2021; Laitila & Salminen, 2020; Laitila et al., 2019; Mouls & Fulcrand, 2012a; Stoj et al., 2020). Zeng et al. (2016) identified the dimeric and trimeric pigments as quantification markers released from polymeric pigments after depolymerisation reactions, which was also utilised in the present study.

As described in Chapter 4, correlations between age and colour properties have been established, while in Chapter 5 correlations between wine style/quality and colour and phenolic parameters, and tannin concentration were also established. The objective of this chapter was to identify any potential correlations between monomeric and polymeric phenolics and wine colour, and whether age and style could influence these in Pinot Noir wines. This chapter includes three main subsections: i) quantification of monomeric phenolics using RP-HPLC; ii) profile of tannin compositions using

phloroglucinolysis; iii) quantification of different types of polymeric phenolics using combined phloroglucinolysis and Orbitrap-UPLC-MS/MS.

6.2 Results

6.2.1 Monomeric phenols determined by HPLC

6.2.1.1 Quantification of monomeric phenols

The monomeric fraction (F1) of the twenty-two different Pinot Noir wines (see Chapter 3), was analysed by HPLC (section 3.2.2.7). In total, 17 monomeric phenolic compounds were quantified as well as a measure of total red pigments. The monomeric compounds included benzoic acids (gallic acid, syringic acid, protocatechuic acid, and hydroxybenzoic acid), hydroxycinnamic acids (caftaric acid, *cis*-coutaric acid, *trans*-coutaric acid, caffeic acid, *p*-coumaric acid, ferulic acid, and GRP), flavonols (quercetin and quercetin-3-glucoside), flavan-3-ols (catechin and epicatechin), resveratrol, and malvidin-3-glucoside (M3G).

The concentrations of the various monomeric phenolics determined (plus total red pigments) are given in Table 6.1. Flavan-3-ols were the most abundant, ranging from 68 to 331 mg/L, followed by hydroxycinnamic acids ranging from 35 to 63 mg/L, hydroxybenzoic acids from around 35 mg/L to 63 mg/L, and flavonols from 7 to 26 mg/L.

Regarding the hydroxybenzoic acids, gallic acid was found to be the most abundant, varying from 28.5 mg/L to 51.8 mg/L, while trace amounts of protocatechuic acid (2.64-7.28 mg/L), syringic acid (2.70-5.99 mg/L), and *p*-hydroxybenzoic acid (0.48-1.73) were also detected. The concentrations of *p*-hydroxybenzoic acid, protocatechuic acid, syringic acid, and gallic acid fluctuated across vintage and style. BD15 and SR10 had the lowest amounts of *p*-hydroxybenzoic acid, while OS17 and OS18 had the lowest of protocatechuic acid, syringic acid, and gallic acid. In contrast, SR17 had the highest amounts of *p*-hydroxybenzoic acid and protocatechuic acid, while BR15 and SR14 had the highest amounts of syringic acid, and SL17 had the highest of gallic acid.

Of the seven hydroxycinnamic acids quantified, caftaric acid was found in the highest concentration, ranging from 10.8 mg/L to 51.5 mg/L, followed by *trans*-coutaric (2.82-13.67 mg/L), caffeic acid (2.12-6.26 mg/L), *p*-coumaric acid (1.64-4.21 mg/L) and *cis*-coutaric acid (0.16-3.99 mg/L), GRP (0.07-2.72 mg/L), and hardly detectable concentrations were seen in ferulic acid (0.18-1.87 mg/L). OS17 had the highest amounts of GRP and the lowest amounts of caffeic acid, while OS18 had the lowest *p*-coumaric acid. Large variations in the concentrations of hydroxycinnamic acids were observed between vintages and styles.

The concentration of catechin varied between 52 and 237 mg/L, while that of epicatechin varied between 17 and 94 mg/L. Within vintages, large variations of catechin and epicatechin were seen in wines. For example, the concentration of catechin and epicatechin in 2017 wines varied between 125 and 225 mg/L, and 48 and 84 mg/L, respectively.

The concentrations of quercetin-3-glucoside and quercetin varied from 0 to 16.3 mg/L and 2.5 to 12.9 mg/L, respectively. Regarding resveratrol, their concentrations fluctuated over vintage and styles from 1.28 mg/L to 8.66 mg/L observed in BL02 and SC17, respectively. Substantial differences in resveratrol were shown within 2017, varying from 2.67 mg/L (OS17) to 8.66 mg/L (SC17). In addition, total red pigments ranged from 1.81 to 72.47 mg/L (M3G equivalent), while M3G ranged from 0.27 to 51.63 mg/L.

Fixed model REML (Residual Maximum Likelihood) analysis was carried out (see Chapter 3) to determine the effect of vintage and wine style on the concentrations of the monomeric phenols (Table 6.2). As previously described (Chapter 4), based on wine regions and anticipated quality, a total of six wine styles were categorised: Commercial (C), Super Premium (SP), Brancott Letter (BL), Stoneleigh Latitude (SL), Stoneleigh Rapaura (SR), and Central Otago (OS).

The statistical analysis indicated that 6 monomeric compounds plus total red pigments were significantly influenced by both vintage and style ($P < 0.05$). These monomeric phenolics were protocatechuic acid, hydroxycinnamates (caftaric acid, *trans*-coutaric acid, and GRP), quercetin-3-glucoside, and malvidin-3-glucoside. In addition, epicatechin and quercetin were also significantly ($P < 0.05$) influenced by vintage but not style. However, for both protocatechuic acid and quercetin, there was a significant interaction effect between vintage and style ($P < 0.05$). There was no statistically significant effect of vintage and style on the remaining 9 monomeric phenolics: hydroxybenzoic acids (gallic acid, syringic acid, and *p*-hydroxybenzoic acid), hydroxycinnamates (caffeic acid, ferulic acid, and GRP), resveratrol, quercetin-3-glucoside, and quercetin.

Table 6.1 Concentrations of phenolic compounds (mg/L) determined from SPE fraction F1

Wine code	*p*-Hydroxybenzoic acid	Protocatechuic acid	Syringic acid	Gallic acid	Caffeic acid	*p*-Coumaric acid	Ferulic acid	Caftaric acid	*cis*-coutaric acid	*trans*-coutaric acid	GRP
BC17	1.32 ± 0.10	3.91 ± 0.32	3.78 ± 0.30	46 ± 1.00	6.25 ± 0.53	3.67 ± 0.06	0.56 ± 0.07	51 ± 6.74	3.49 ± 0.16	12.12 ± 0.56	1.02 ± 0.00
BD15	0.47 ± 0.58	4.49 ± 0.14	4.61 ± 0.24	33 ± 0.35	3.52 ± 0.14	2.86 ± 0.38	0.28 ± 0.09	40 ± 2.83	2.39 ± 0.22	8.17 ± 0.06	0.82 ± 0.01
BL02	0.85 ± 0.02	3.56 ± 0.13	5.08 ± 0.38	38 ± 0.38	3.23 ± 0.09	2.96 ± 0.00	0.35 ± 0.00	10 ± 0.75	0.25 ± 0.01	2.96 ± 0.12	0.47 ± 0.13
BL11	0.87 ± 0.11	6.35 ± 0.49	4.87 ± 0.04	46 ± 0.29	5.21 ± 0.55	3.64 ± 0.09	0.44 ± 0.04	16 ± 2.64	1.40 ± 0.02	2.94 ± 0.10	0.13 ± 0.05
BL14	0.92 ± 0.06	4.30 ± 0.28	4.62 ± 0.00	38 ± 0.08	2.77 ± 0.16	2.76 ± 0.02	0.30 ± 0.07	37 ± 4.80	2.23 ± 0.02	8.65 ± 0.11	0.93 ± 0.14
BL15	1.12 ± 0.12	5.31 ± 0.23	4.38 ± 0.06	35 ± 0.51	2.61 ± 0.36	3.01 ± 0.03	0.3 ± 0.00	28 ± 6.47	2.20 ± 0.05	6.35 ± 0.01	0.60 ± 0.11
BL17	1.19 ± 0.13	7.04 ± 0.93	4.57 ± 0.06	47 ± 0.13	2.45 ± 0.22	2.43 ± 0.02	0.19 ± 0.01	43 ± 8.61	3.35 ± 0.16	10.7 ± 0.03	0.07 ± 0.08
BR15	0.89 ± 0.10	4.62 ± 0.11	5.56 ± 0.48	33 ± 2.10	3.41 ± 0.18	3.13 ± 0.08	0.35 ± 0.02	40 ± 1.78	0.22 ± 0.02	8.08 ± 0.42	0.6 ± 0.00
OS17	0.78 ± 0.05	2.64 ± 0.13	2.69 ± 0.16	28 ± 1.27	2.11 ± 0.11	2.34 ± 0.16	0.22 ± 0.00	41 ± 1.41	2.61 ± 0.17	8.83 ± 0.38	2.72 ± 0.29
OS18	1.06 ± 0.08	2.64 ± 0.3	3.46 ± 0.76	28 ± 2.42	2.39 ± 0.19	1.63 ± 0.10	0.27 ± 0.02	38 ± 2.59	2.88 ± 0.22	7.67 ± 0.68	1.98 ± 0.43
SC03	1.04 ± 0.19	6.06 ± 0.17	4.49 ± 0.93	48 ± 6.64	2.65 ± 0.39	2.94 ± 0.43	0.29 ± 0.02	10 ± 1.44	0.20 ± 0.07	3.12 ± 0.43	0.16 ± 0.00
SC07	1.28 ± 0.16	5.71 ± 0.03	4.49 ± 0.02	41 ± 0.90	4.34 ± 0.11	4.2 ± 0.21	0.50 ± 0.06	17 ± 0.36	1.61 ± 0.04	3.63 ± 0.06	0.13 ± 0.02
SC17	1.35 ± 0.11	4.01 ± 0.26	3.64 ± 0.14	40 ± 2.36	4.49 ± 0.27	2.92 ± 0.13	0.29 ± 0.00	49 ± 2.60	3.29 ± 0.33	12.93 ± 0.64	0.86 ± 0.18
SL11	0.89 ± 0.01	6.21 ± 0.77	4.96 ± 0.21	45 ± 0.85	5.99 ± 0.02	3.94 ± 0.20	0.41 ± 0.02	17 ± 0.67	2.05 ± 0.02	4.05 ± 0.19	0.71 ± 0.41
SL15	0.84 ± 0.10	3.49 ± 0.01	5.00 ± 0.16	31 ± 0.94	2.45 ± 0.18	2.79 ± 0.25	0.22 ± 0.05	28 ± 1.74	2.52 ± 0.04	6.55 ± 0.73	0.58 ± 0.13
SL16	0.87 ± 0.08	3.69 ± 0.16	5.45 ± 0.24	42 ± 0.42	3.24 ± 0.01	3.08 ± 0.13	0.24 ± 0.02	33 ± 1.38	3.36 ± 0.15	9.19 ± 0.64	1.01 ± 0.38
SL17	1.13 ± 0.03	3.69 ± 0.29	4.39 ± 0.30	51 ± 0.02	3.51 ± 0.04	3.20 ± 0.11	0.23 ± 0.00	46 ± 1.95	4.08 ± 0.21	13.28 ± 0.65	1.47 ± 0.57
SR02	0.83 ± 0.15	4.49 ± 0.93	4.40 ± 0.23	38 ± 1.37	3.58 ± 0.14	3.3 ± 0.11	1.86 ± 2.14	10 ± 0.40	0.19 ± 0.03	2.82 ± 0.12	0.97 ± 0.09
SR10	0.69 ± 0.19	3.58 ± 0.96	3.74 ± 1.17	29 ± 8.31	3.59 ± 1.04	3.13 ± 0.84	0.34 ± 0.03	25 ± 7.23	0.16 ± 0.11	5.09 ± 1.44	0.88 ± 0.21
SR14	1.13 ± 0.18	5.02 ± 0.19	5.98 ± 0.27	34 ± 0.03	3.50 ± 0.13	2.64 ± 0.03	0.43 ± 0.03	33 ± 0.68	0.25 ± 0.04	6.83 ± 0.23	1.05 ± 0.21
SR16	1.48 ± 1.03	3.32 ± 0.62	4.28 ± 2.64	46 ± 2.63	3.56 ± 0.31	3.21 ± 0.23	0.18 ± 0.25	24 ± 5.61	1.76 ± 2.19	6.89 ± 0.38	2.56 ± 1.90
SR17	1.73 ± 0.17	7.27 ± 0.36	4.89 ± 0.17	49 ± 0.19	2.38 ± 0.01	2.35 ± 0.00	0.28 ± 0.03	44 ± 0.05	3.12 ± 0.02	9.63 ± 0.09	0.70 ± 0.02

*Values reported as mean ± standard deviation (n= 2).

Table 6.1 (cont'd) Concentrations of phenolic compounds (mg/L) determined from SPE fraction F1

Wine code	Catechin	Epicatechin	Quercetin-3-glucoside	Quercetin	Resveratrol	Malvidin-3-glucoside	Total red pigments (M3G equiv.)
BC17	125 ± 4.1	57 ± 6.18	7.08 ± 0.15	5.93 ± 0.14	7.13 ± 0.62	28.64 ± 0.51	40 ± 0.43
BD15	86 ± 0.63	29 ± 2.65	1.91 ± 0.59	9.37 ± 0.05	4.35 ± 0.11	9.48 ± 0.17	16 ± 0.3
BL02	52 ± 3.55	17 ± 0.08	1.42 ± 0.16	6.56 ± 0.27	1.27 ± 0.00	0.23 ± 0.01	2 ± 0.42
BL11	95 ± 0.50	38 ± 0.24	1.22 ± 0.18	3.23 ± 0.04	2.73 ± 0.32	0.97 ± 0.05	3 ± 0.24
BL14	96 ± 0.21	45 ± 0.42	3.53 ± 0.00	12.86 ± 0.21	4.38 ± 0.28	8.66 ± 0.14	15 ± 0.28
BL15	113 ± 0.31	45 ± 0.05	4.20 ± 0.07	12.26 ± 0.16	4.42 ± 0.52	14.84 ± 0.29	23 ± 0.53
BL17	178 ± 0.33	70 ± 0.00	3.85 ± 0.03	2.45 ± 0.12	4.17 ± 0.38	37.08 ± 1.15	51 ± 1.73
BR15	84 ± 4.47	26 ± 1.19	1.04 ± 0.15	8.49 ± 0.30	4.71 ± 0.14	10.22 ± 0.46	16 ± 0.68
OS17	146 ± 9.76	48 ± 2.93	16.29 ± 0.74	6.94 ± 0.61	2.66 ± 0.20	51.62 ± 2.56	72 ± 3.9
OS18	223 ± 16.87	76 ± 6.10	9.36 ± 0.72	3.94 ± 0.31	1.56 ± 0.12	47.37 ± 4.27	63 ± 5.56
SC03	83 ± 9.25	34 ± 3.53	0.00 ± 0.00	3.37 ± 0.41	4.08 ± 0.49	0.26 ± 0.09	1 ± 0.24
SC07	71 ± 2.58	22 ± 1.26	0.00 ± 0.00	6.77 ± 0.20	6.86 ± 0.11	0.57 ± 0.04	2 ± 0.11
SC17	174 ± 0.57	64 ± 3.40	8.40 ± 0.49	6.44 ± 0.36	8.66 ± 0.37	30.52 ± 2.05	43 ± 2.82
SL11	87 ± 2.66	35 ± 2.89	3.35 ± 0.07	4.19 ± 0.33	4.83 ± 0.58	1.04 ± 0.01	3 ± 0.6
SL15	117 ± 2.16	46 ± 1.28	13.62 ± 0.47	6.82 ± 0.17	2.95 ± 0.52	6.58 ± 0.01	10 ± 0.03
SL16	164 ± 2.73	67 ± 1.84	12.47 ± 0.30	4.77 ± 0.06	3.60 ± 0.26	12.76 ± 0.08	19 ± 0.03
SL17	225 ± 7.25	84 ± 1.66	13.52 ± 0.12	12.36 ± 0.27	6.52 ± 0.31	26.64 ± 0.84	42 ± 1.35
SR02	51 ± 3.16	16 ± 0.40	1.00 ± 0.10	5.97 ± 0.32	2.07 ± 0.06	0.32 ± 0.06	2 ± 0.19
SR10	54 ± 15.21	17 ± 4.60	5.80 ± 1.63	6.26 ± 1.70	2.23 ± 0.65	0.65 ± 0.21	2 ± 0.56
SR14	89 ± 0.43	34 ± 1.56	2.40 ± 0.28	8.73 ± 0.74	5.32 ± 0.07	6.35 ± 0.32	11 ± 0.7
SR16	161 ± 1.43	70 ± 4.47	12.41 ± 2.54	5.48 ± 0.46	3.08 ± 0.92	14.55 ± 0.96	16 ± 8.19
SR17	178 ± 4.02	58 ± 0.81	1.06 ± 0.03	3.41 ± 0.03	4.56 ± 0.06	29.9 ± 1.06	46 ± 0.49

*Values reported as mean ± standard deviation (n=2).

Table 6.2 REML analysis of concentrations of monomeric phenolics in study wines

Monomeric phenolics	P (Vintage)	Av. SE	P (Style)	Av. SE	P (Vintage*Style)
Hydroxybenzoic acid	0.375	0.28	0.252	0.29	0.749
Protocatechuic acid	**0.004**	0.11	**0.002**	0.12	**0.006**
Syringic acid	0.286	0.64	0.282	0.67	0.406
Gallic acid	0.129	4.48	0.152	4.66	0.721
Caffeic acid	0.404	1.19	0.460	1.24	0.940
p-Coumaric acid	0.239	0.54	0.534	0.56	0.742
Ferulic acid	0.129	0.19	0.194	0.19	0.112
Caftaric acid	**0.003**	1.38	**0.021**	1.43	0.106
cis-Coutaric acid	0.322	1.47	0.66	1.52	0.932
trans-Coutaric acid	**0.007**	0.46	**0.036**	0.36	0.230
GRP	**0.033**	0.55	**0.023**	0.58	0.060
Catechin	0.096	33.2	0.404	34.5	0.747
Epicatechin	**0.016**	5.07	0.060	5.27	0.129
Quercetin	**0.022**	0.68	0.091	0.71	**0.017**
Quercetin-3-glucoside	**0.019**	1.07	**0.01**	1.10	0.057
Resveratrol	0.113	1.05	0.12	1.10	0.425
Malvidin-3-glucoside	**0.002**	1.36	**0.01**	1.42	0.141
Total red pigments	**0.003**	2.21	**0.02**	2.30	0.192

Statistically significant P values (< 0.05) are shown in bold. Standard errors (SE) are those for differences at the same level of the factor.

The scattergrams of nine compounds, including total red pigments, found to be significantly influenced by vintage and/or style are shown in Figures 6.1 and 6.2. The concentrations of caftaric acid, *trans*-coutaric acid, GRP, epicatechin, quercetin-3-glucoside, malvidin-3-glucoside, and total red pigments, showed general decreasing trends with increasing wine age. However, the concentrations of protocatechuic acid fluctuated across different vintages and wine styles.

Total red pigments were higher than M3G in each wine, indicating that besides M3G, other pigmented compounds were also retained in F1. The presence of other pigments was confirmed by 520 nm HPLC chromatograms. The concentration of these additional pigments also decreased with age. Young wines from 2017 and 2018 had the greatest amounts of these pigments, around 12 to 21 mg/L M3G equivalent, compared with 2 mg/L to 9 mg/L M3G equivalent observed in old wines. However, the proportion of the other pigments showed a general increase with age, from approximately 10.5% to 90.5%. Within vintages, some variations were observed. For example, higher amounts of other pigments were found in BL15, BR15 and BD15, compared with SL15.

Regarding wine styles, Central Otago wines OS17 and OS18 had the lowest concentrations of protocatechuic acid, and the highest mean amounts of GRP, epicatechin, M3G, Q3G plus total red pigments (Figures 6.1 and 6.2). SP wines had the greatest mean values of caftaric acid and the lowest values of M3G and Q3G. However, overall, wine age was more dominant on these six monomers plus total red pigments compared to wine style.

Regarding quercetin and epicatechin, quercetin concentrations showed large fluctuations among vintages, whilst epicatechin concentration decreased with age. In addition, variations of epicatechin within vintage were not related to wine style because epicatechin was affected by vintage, whilst the effect of style was not seen ($P = 0.060$).

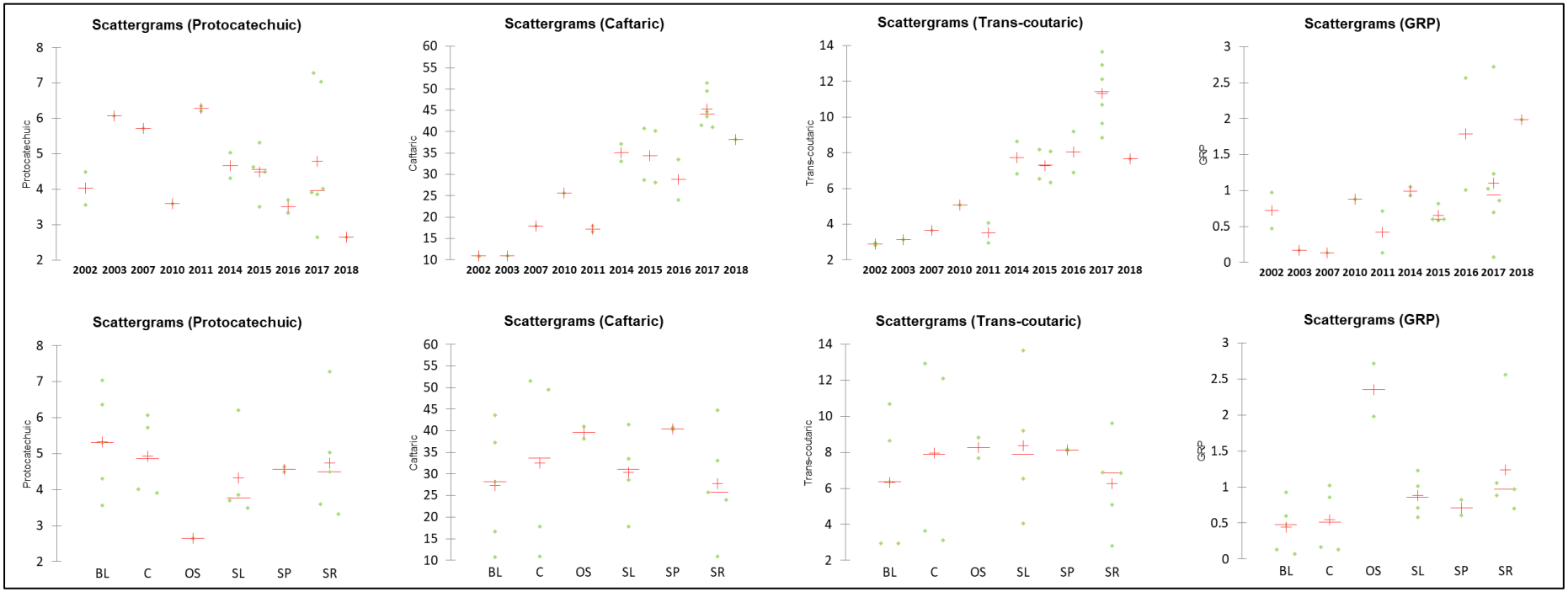


Figure 6.1 Scattergrams of protocatechuic acid, caftaric acid, *trans*-coutaric acid, and GRP according to vintages and wine styles

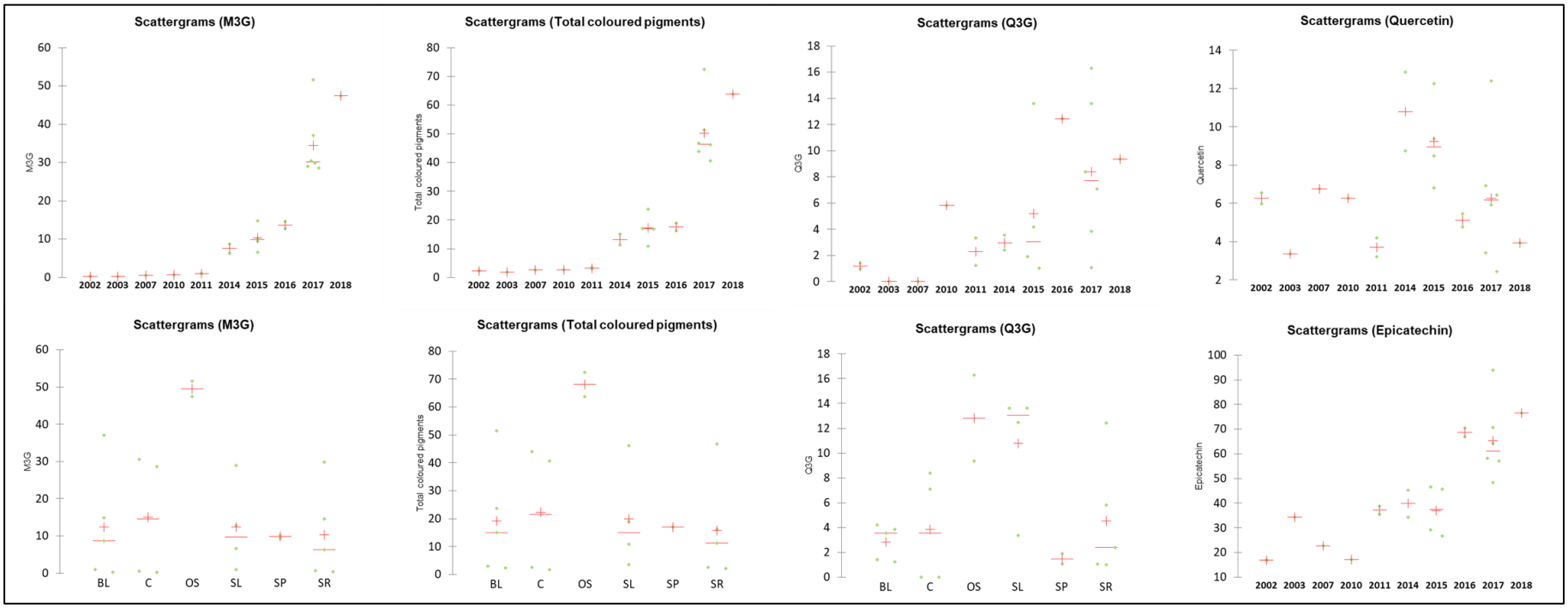


Figure 6.2 Scattergrams of malvidin-3-glucoside (M3G), total red pigments, quercetin-3-glucoside (Q3G), quercetin, and epicatechin according to vintages and wine styles

6.2.1.2 Agglomerative Hierarchical Clustering analysis

Figure 6.3 is the dendrogram of the Agglomerative Hierarchical Clustering analysis using the monomeric phenolics data. Discrimination was based primarily on wine age but also on wine style. Four clusters were identified: 7 older wines from 2002 to 2011 (cluster 1); 8 mid-aged wines from 2014 to 2016 (cluster 2); two young wines OS17 and OS18 from Central Otago (cluster 3); and the remaining 5 wines from vintage 2017 (cluster 4). Apart from cluster 3, different wine styles were seen in each age-related group.

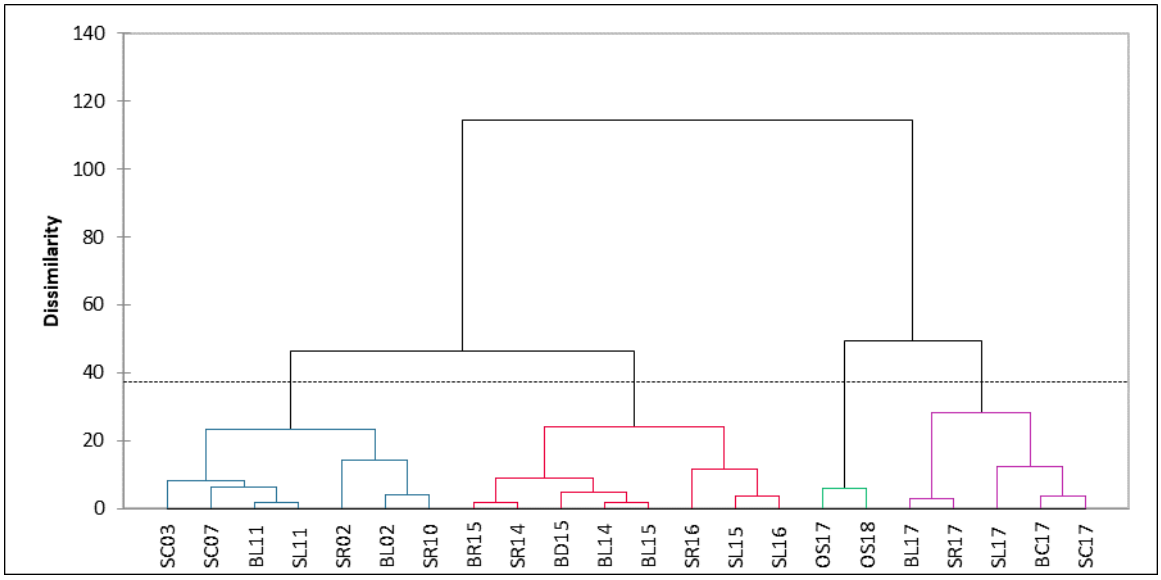


Figure 6.3 Dendrogram resulting from AHC analysis for wine observations according to HPLC result (Cophenetic correlation coefficient = 0.696)

ANOVA of the phenolic compounds based on these clusters is shown in Table 6.3. Statistically, fourteen out of eighteen phenolic compounds were significantly different between clusters ($P < 0.05$). The mean concentrations of protocatechuic acid, caffeic acid, ferulic acid, and quercetin were not significantly different ($P > 0.05$). Regarding the three age-related groups, 9 phenolic compounds and total red pigments declined with age, including *p*-hydroxybenzoic acid, caftaric acid, *trans*-coutaric, *cis*-coutaric, catechin, epicatechin, Q3G, resveratrol, and M3G, whilst only *p*-coumaric increased with age.

In summary, the main findings of REML analysis based on vintage and style, and ANOVA of groups identified by AHC (three age-related and Central Otago) were: i) protocatechuic acid, hydroxycinnamates (caftaric acid, *trans*-coutaric acid, and GRP), quercetin-3-glucoside, and malvidin-3-glucoside, and total red pigments were affected by vintage and style; ii) hydroxybenzoic acids (*p*-hydroxybenzoic acid, caftaric acid, *trans*-coutaric, and *cis*-coutaric), flavan-3-ols, Q3G, resveratrol, M3G, and total red pigments decreased during ageing, whilst only *p*-coumaric increased with age; iii)

The Central Otago wines displayed a distinct profile of monomeric phenolics, with the highest mean levels of GRP, M3G, and total red pigments, as well as the lowest level of syringic acid and gallic acid.

Table 6.3 ANOVA analysis for HPLC results based on four clusters

Cluster	Hydroxybenzoic acid	Protocatechuic acid	Syringic acid	Gallic acid	Caffeic acid	*p*-Coumaric acid	Ferulic acid	Caftaric acid	*cis*-Coutaric acid	*trans*-Coutaric acid	GRP
4 (OS)	0.93 b	2.64	3.08 c	28.7 c	2.26	1.99 c	0.25	39.6 ab	2.75 ab	8.25 b	2.35 a
3 (Young)	1.40 a	5.22	4.23 b	47.1 a	3.77	2.92 b	0.31	46.2 a	3.45 a	11.81 a	0.79 b
2 (Mid-aged)	0.97 b	4.29	4.99 a	37.1 bc	3.14	2.94 b	0.29	33.2 b	1.87 b	7.60 b	1.02 b
1 (Old)	0.93 b	5.14	4.58 ab	41.2 ab	4.09	3.45 a	0.60	15.8 c	0.84 c	3.52 c	0.49 b
Pr > F(Cluster)	0.035	0.061	0.002	0.002	0.143	0.003	0.28	<0.0001	0	<0.0001	0.003
Significant	Yes	No	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes

Cluster	Catechin	Epicatechin	Q3G	Quercetin	Resveratrol	M3G	Total red pigments
4 (OS)	185.2 a	62.4 ab	12.83 a	5.45	2.12 b	49.5 a	68.1 a
3 (Young)	178.8 a	68.8 a	6.80 ab	6.13	6.20 a	31.0 b	45.8 b
2 (Mid-aged)	114.1 b	45.7 b	6.45 ab	8.60	4.11 b	10.4 c	16.3 c
1 (Old)	70.8 c	26.0 c	1.83 b	5.20	3.44 b	0.6 d	2.6 d
Pr > F(Cluster)	<0.0001	0.001	0.029	0.131	0.014	<0.0001	<0.0001
Significant	Yes	Yes	Yes	No	Yes	Yes	Yes

For each chemical parameter, only means followed by a different letter were significantly different using Turkey's test ($P < 0.05$).

6.2.2 Tannin composition

6.2.2.1 Tannin composition characterisation

The composition of tannin extracted from 22 studied wines (see Chapter 3), was analysed using phloroglucinol (section 3.2.2.8). There were eleven tannin-related parameters determined (Table 6.4), including flavan 3-ol subunits, mean degree of polymerisation (mDP), yield of tannin (per cent of depolymerised tannin calculated based on wine MCP tannin concentration), and average molecular weight determined by phloroglucinolysis. In addition, the degree of gallolated tannins was determined by the percentage of skin-derived epigallocatechin extension subunit (EGC), while the degree of galloylated tannins was the percentage of combined seed-derived epicatechin gallate extension (P-ECG) and terminal subunits (ECG).

In terms of tannin composition, epicatechin extension and terminal subunits were dominant, accounting for 28.7% to 57.8% and 9.7% to 15.7%, respectively, of the total tannin subunits based on mole concentration. The proportion of catechin terminal subunits was larger than its extension subunits, 15.7%-27.0% and 6.8%-9.9%, respectively, which agreed with a previous study (Yang et al., 2021b). Catechin extension subunits and epicatechin terminal subunits also demonstrated large fluctuations within vintages 2015 and 2017. Skin-derived epigallocatechin extension units ranged from 6% to 20.6%, whilst only a small proportion of seed-derived galloylation subunits was observed, ranging from 0.12% to 3.72%. Approximately 3.5% of the difference was observed in gallolated tannins of wines from 2017, whilst only a minor difference (less than 0.2%) was seen in galloylated tannins in 2017.

The values of the mean degree of polymerisation were quite low, around 2.3 to 3.8 subunits, corresponding to an average molecular mass ranging from 862 to 1472 g/mol (relative to epicatechin). The yield of tannin varied substantially from 9.7% (SR02) to 56% (SR17), indicating that 56% of tannin in SR17 was characterised by phloroglucinol, whilst less than 10% of tannin in SR02 was cleavable. This suggested that SR17 had more acid-liable interflavanolic cleavages compared with SR02. Large variations of the yield of tannin were seen within vintage 2015 and 2017. SR17 had more than 10% of cleavable tannins compared with BL17 and SC17, while BL17 and SC17 had more than 6%-12% of cleavable tannins determined compared with SL17, OS17, and BC17.

Table 6.4 Compositional characterisation of the total tannin isolated from studied wines

Wine code	Extension unit (mole %)			Terminal unit (mole %)			% Gallo	% Galloyl	mDP	MW (g/mol)	% Yield
	P-ECG	P-CAT	P-EPI	ECG	CAT	EPI					
BC17	0.25 ± 0.08	8.54 ± 0.04	54.43 ± 0.25	0.13 ± 0.00	16.80 ± 0.21	11.95 ± 0.06	7.90 ± 0.01	0.38 ± 0.08	3.46 ± 0.02	1316 ± 7	35.45 ± 0.73
BD15	0.96 ± 0.00	7.74 ± 0.04	47.33 ± 0.11	0.09 ± 0.04	19.04 ± 0.01	10.66 ± 0.03	14.18 ± 0.10	1.05 ± 0.04	3.36 ± 0.00	1279 ± 1	22.97 ± 0.75
BL02	2.01 ± 0.05	6.80 ± 0.00	28.75 ± 0.15	0.15 ± 0.01	26.81 ± 0.09	14.87 ± 0.04	20.61 ± 0.07	2.16 ± 0.04	2.39 ± 0.00	881 ± 1	16.28 ± 0.00
BL11	1.14 ± 0.46	8.23 ± 0.37	44.14 ± 1.14	0.20 ± 0.07	23.43 ± 0.84	14.53 ± 0.24	8.33 ± 1.68	1.35 ± 0.53	2.62 ± 0.05	970 ± 21	25.20 ± 1.97
BL14	0.64 ± 0.00	8.32 ± 0.25	48.32 ± 0.09	0.15 ± 0.00	18.29 ± 0.08	11.87 ± 0.10	12.40 ± 0.16	0.79 ± 0.00	3.30 ± 0.02	1252 ± 8	23.44 ± 0.84
BL15	1.05 ± 0.55	8.20 ± 0.44	48.48 ± 0.21	0.15 ± 0.09	19.78 ± 0.81	12.14 ± 1.01	10.21 ± 1.57	1.20 ± 0.65	3.12 ± 0.16	1180 ± 71	28.69 ± 2.34
BL17	0.17 ± 0.0	9.18 ± 0.06	56.81 ± 0.60	0.10 ± 0.00	16.23 ± 0.10	9.71 ± 0.06	7.81 ± 0.82	0.26 ± 0.00	3.84 ± 0.02	1473 ± 11	45.76 ± 1.51
BR15	1.02 ± 0.09	7.81 ± 0.22	44.62 ± 1.90	0.09 ± 0.00	21.22 ± 0.49	11.71 ± 0.40	13.52 ± 0.70	1.11 ± 0.09	3.03 ± 0.08	1142 ± 33	16.77 ± 0.90
OS17	0.17 ± 0.03	9.37 ± 0.18	55.95 ± 1.20	0.05 ± 0.01	16.98 ± 0.02	10.00 ± 0.93	7.48 ± 0.50	0.22 ± 0.04	3.70 ± 0.12	1414 ± 51	37.31 ± 0.87
OS18	0.09 ± 0.0	9.95 ± 0.07	55.8 ± 0.21	0.03 ± 0.00	15.68 ± 0.21	10.57 ± 0.09	7.89 ± 0.02	0.12 ± 0.00	3.81 ± 0.02	1457 ± 7	54.47 ± 0.91
SC03	0.65 ± 0.02	8.15 ± 0.02	38.03 ± 0.26	0.11 ± 0.01	24.81 ± 0.25	15.73 ± 0.27	12.52 ± 0.79	0.76 ± 0.01	2.46 ± 0.03	902 ± 13	16.07 ± 1.51
SC07	1.49 ± 0.02	7.37 ± 0.04	30.58 ± 0.07	0.23 ± 0.08	26.99 ± 0.19	15.33 ± 0.08	18.01 ± 0.40	1.72 ± 0.10	2.35 ± 0.02	862 ± 8	13.69 ± 0.47
SC17	0.10 ± 0.00	9.08 ± 0.01	57.76 ± 0.02	0.05 ± 0.02	16.66 ± 0.02	10.28 ± 0.00	6.08 ± 0.02	0.15 ± 0.01	3.71 ± 0.00	1414 ± 1	47.76 ± 2.43
SL11	0.70 ± 0.02	8.47 ± 0.41	41.97 ± 0.50	0.07 ± 0.00	21.67 ± 0.23	14.36 ± 0.06	12.76 ± 0.60	0.78 ± 0.02	2.77 ± 0.02	1032 ± 9	20.85 ± 0.12
SL15	0.54 ± 0.01	8.55 ± 0.57	44.05 ± 0.00	0.04 ± 0.01	20.61 ± 0.33	13.97 ± 0.10	12.23 ± 0.79	0.58 ± 0.01	2.89 ± 0.02	1080 ± 9	31.16 ± 0.35
SL16	3.38 ± 0.09	8.28 ± 0.10	43.33 ± 0.26	0.34 ± 0.03	19.70 ± 0.13	12.98 ± 0.60	11.99 ± 0.01	3.72 ± 0.12	3.03 ± 0.04	1153 ± 17	35.58 ± 7.14
SL17	0.28 ± 0.04	8.74 ± 0.07	52.14 ± 0.31	0.05 ± 0.01	17.24 ± 0.15	12.21 ± 0.14	9.36 ± 0.07	0.32 ± 0.03	3.39 ± 0.03	1286 ± 13	39.28 ± 2.97
SR02	1.10 ± 0.05	7.75 ± 0.01	33.97 ± 0.14	0.19 ± 0.02	25.94 ± 0.36	15.00 ± 0.12	16.05 ± 0.41	1.29 ± 0.07	2.43 ± 0.03	894 ± 12	9.71 ± 0.22
SR10	1.04 ± 0.00	7.66 ± 0.26	38.51 ± 0.01	0.19 ± 0.05	22.83 ± 0.25	13.60 ± 0.11	16.17 ± 0.04	1.23 ± 0.05	2.73 ± 0.02	1019 ± 10	16.32 ± 0.19
SR14	0.50 ± 0.00	8.63 ± 0.08	45.51 ± 0.40	0.11 ± 0.02	21.49 ± 0.04	12.75 ± 0.09	11.01 ± 0.24	0.61 ± 0.02	2.91 ± 0.01	1089 ± 3	20.41 ± 2.04
SR16	2.82 ± 2.01	9.10 ± 0.42	45.76 ± 0.28	0.35 ± 0.20	19.16 ± 0.46	12.51 ± 0.37	10.31 ± 0.93	3.16 ± 2.21	3.12 ± 0.06	1190 ± 34	39.59 ± 0.66
SR17	0.29 ± 0.00	8.58 ± 0.24	54.66 ± 0.24	0.11 ± 0.01	17.32 ± 0.25	10.60 ± 0.13	8.44 ± 0.07	0.40 ± 0.01	3.57 ± 0.05	1360 ± 21	56.25 ± 2.78

Mean values ± SD; ECG, (–)-epicatechin gallate; CAT, (+)-catechin; EPI, epicatechin; EGC, (–)-epigallocatechin; % Gallo, percent epigallocatechin extension units; %Galloyl, percent galloylation (the total proportion of epicatechin gallate extension and terminal units); mDP, mean degree of polymerisation; MW, average molecular weight calculated using phloroglucinol; % Yield, percent mass conversion calculated based on wine MCP tannin concentration.

Table 6.5 shows the analysis of variance of tannin composition using the REML fixed model with vintage and style as factors. Statistically, five tannin subunits were significantly ($P < 0.05$) affected by vintage, whilst none were affected by wine style. The proportions of epicatechin gallate and epicatechin extension subunits, catechin terminal subunits, gallolated tannin, and galloylated tannin were significantly different between vintages. In addition, the interaction between vintage and style was not observed. The scattergrams of these five affected subunits are given in Figure 6.4. A general decreasing pattern with age was seen in the proportion of epicatechin extension units. In contrast, the remaining four subunits showed increased patterns. Substantial variations were also observed between and within vintage. For example, SL16 and SR16 had the greatest ratio of galloylated tannins, while SC03 had a lower ratio of gallolated tannin than SC07 and SR10. The proportion of skin-derived epigallocatechin subunits in SL11 was approximately 4% higher than that in BL11, while SR02 also had an around 4.6% higher gallolated tannin than BL02. This demonstrated the predominant effect of age on the modification of tannin composition and structure, regardless of wine style.

In contrast, the remaining tannin related parameters were not significantly different between vintages and styles. However, general age-related trends were still observed. A general increasing trend was obtained in the proportion of epicatechin terminal subunits, whilst catechin extension subunits, mDP, yield of tannin, and average molecular mass decreased broadly during ageing (Table 6.5). As only a minor proportion (< 0.5%) of epicatechin gallate terminal units was observed, their trends over ageing are not discussed. Considering their mole concentrations, all of the tannin compositions determined (in mole) were seen general decreases during ageing, as expected. In this case, the reduction degree of epicatechin and catechin extension units tended to be the largest. As a result, the proportions of epicatechin and catechin terminal subunits, skin-derived epigallocatechin, and seed-derived epicatechin gallate subunits were generally observed to increase with age. The reason why age showed no effects on these compositions could be associated with larger variations within vintage compared with variations between vintages. For example, SR17 and SC17 had 56% and 48% of tannin were cleavable, whilst BC17 and OS17 only had 35%-37% of tannin were cleavable. The average molecular weight was also seen nearly 200 g/mol (epicatechin equivalent) difference within vintages 2017 and 2015, with less than 0.4 units differences in terms of mDP.

When it comes to wine styles, there were no clear patterns observed between the six styles due to the large variations within style caused by the age effect. Central Otago wine, OS18 and OS17 had comparable proportions of depolymerised tannin (37% and 54%, respectively), gallolated tannins, and galloylated tannins compared with wines from 2017, implying that the effect of age was dominant rather than styles. SP wines BD15 and BR15, had around 10% lower yield of tannin and relatively a 1%

higher skin-derived gallolation than BL15 and SL15, although these variations were not significantly different.

Table 6.5 REML analysis of tannin composition in study wines

Tannin composition	P (Vintage)	SE	P (Style)	SE	P (Vintage*Style)
% P-ECG	**0.004**	0.11	0.080	0.12	0.078
% P-CAT	0.072	0.37	0.316	0.38	0.338
% P-EPI	**0.029**	2.89	0.535	3.00	0.588
% ECG	0.079	0.05	0.340	0.05	0.600
% CAT	**0.042**	1.47	0.950	1.53	0.507
% EPI	0.133	1.33	0.436	1.39	0.800
% Gallo	**0.032**	1.30	0.177	1.36	0.293
% Galloyl	**0.007**	0.16	0.131	0.17	0.137
mDP	0.080	0.27	0.715	0.29	0.687
MW (g/mol)	0.081	114	0.702	118	0.689
% Yield	0.117	9.29	0.564	9.66	0.732

Statistically significant P values (< 0.05) are shown in bold. Standard errors (SE) are those for differences at the same level of factor. Mean values ± SD; ECG, (–)-epicatechin gallate; CAT, (+)-catechin; EPI, epicatechin; EGC, (–)-epigallocatechin; % Gallo, percent epigallocatechin extension units; %Galloyl, percent galloylation (the total proportion of epicatechin gallate extension and terminal units); mDP, mean degree of polymerisation; MW, average molecular weight calculated using phloroglucinol; % Yield, percent mass conversion calculated based on wine MCP tannin concentration.

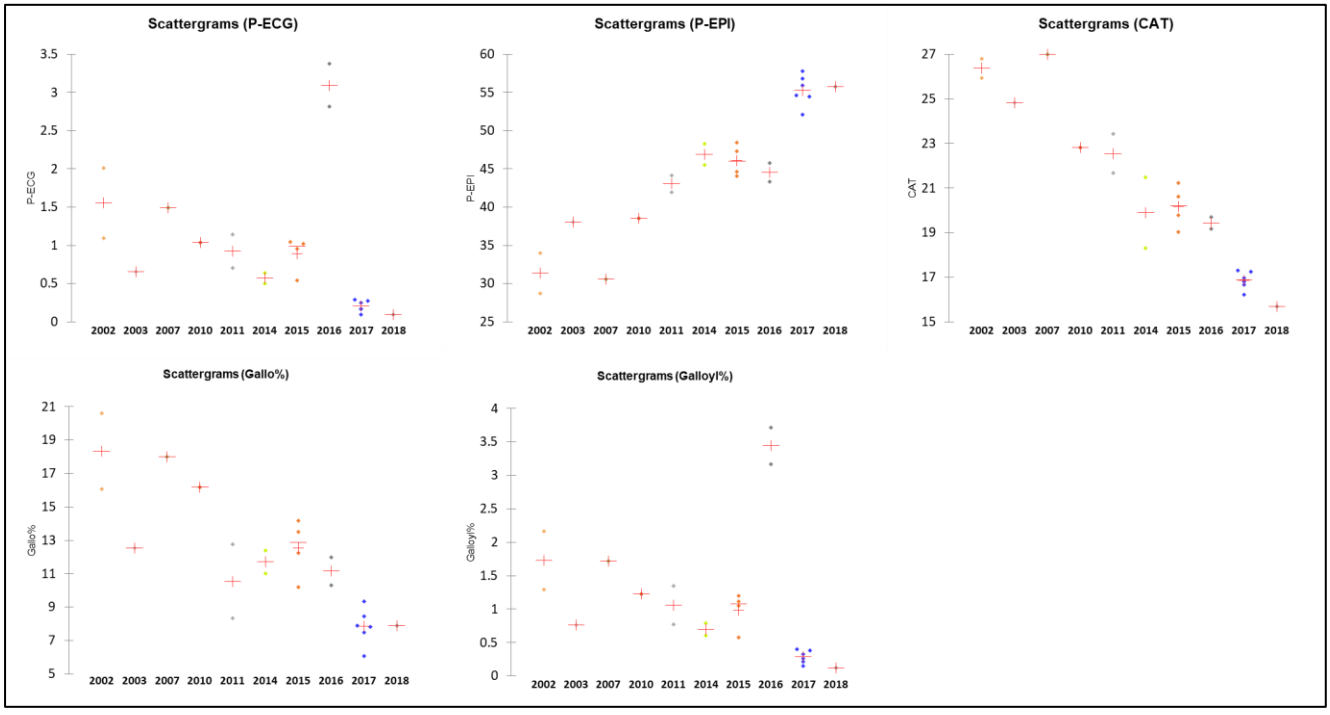


Figure 6.4 Scattergrams of percentages of epicatechin gallate extension unit (P-ECG), epicatechin extension unit (P-EPI), catechin terminal unit (CAT), skin-derived gallolated tannins (Gallo), and seed-derived galloylated tannin (Galloyl)

6.2.2.2 Agglomerative Hierarchical Clustering analysis

Figure 6.5 shows the dendrogram of agglomerative hierarchical analysis for the wines according to their tannin composition. Basically, four distinct clusters were distinguished by wine age. Old wines from 2002 to 2010, fell in cluster 1. Cluster 2, the most heterogeneous cluster, included nine wines spanning 3 years from 2011 to 2015, plus SC03. Two subgroups were also displayed in cluster 2, including one subgroup of Brancott wines, while the other of Stoneleigh wines excluding BL11. Brancott wines BL14, BL15, BD15, and BR15 had a lower proportion of epicatechin terminal units, a higher mean degree of polymerisation, and a higher average molecular mass compared with Stoneleigh wines SL11, SL15, SC03, and BL11. Cluster 3 included the seven youngest wines from 2017 and 2018. Cluster 4 consisted of SR16 and SL16.

The relationships between these tannin parameters are shown in Figure 6.6. These eleven tannin related parameters were classified into three separate groups. The yield of tannin, mDP, average molecular mass, and percentages of catechin and epicatechin extension subunits, were clustered together due to their strong negative correlations with age. The percentages of skin-derived epigallocatechin tannins, catechin and epicatechin terminal unit had strong positive correlations with age, while epicatechin gallate subunits and total seed-derived galloylated tannins were clustered together.

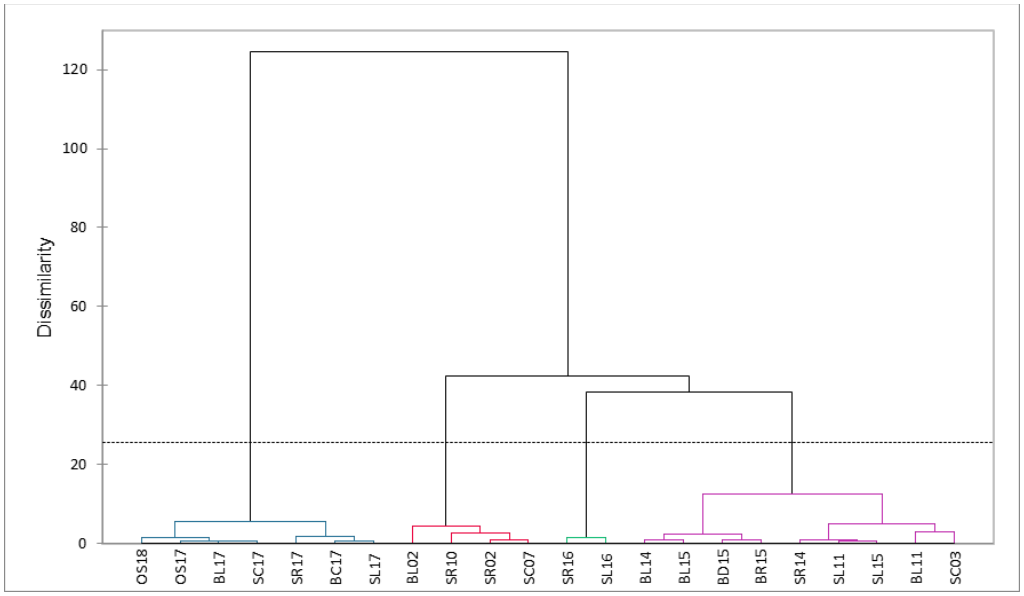


Figure 6.5 Dendrogram resulting from AHC analysis of studied wines according to their tannin composition (Cophenetic correlation coefficient = 0.625)

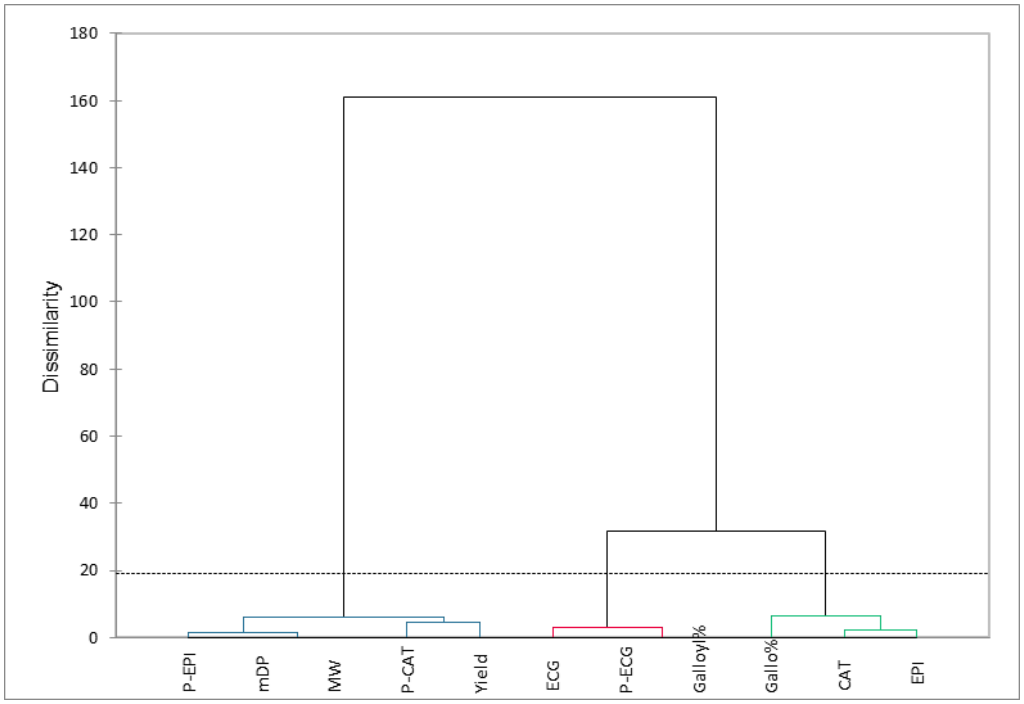


Figure 6.6 Dendrogram resulting from AHC analysis of eleven tannin parameters in relation to tannin composition (Cophenetic correlation coefficient = 0.946)

The analysis of variance of tannin compositions according to four clusters is displayed in Table 6.6. Statistically, significant differences were observed for all ten tannin related parameters between four age-related groups ($P < 0.05$). Both catechin and epicatechin extension units, mDP, average molecular weight, and yield of tannin decreased with wine age. In contrast, both catechin and epicatechin terminal unit, and gallolated tannins increased. In addition, the seed-derived tannin subunits had similar patterns: SL16 and SR16 had the highest ratio of galloylation, followed by older wines, mid-aged wines, and younger wines. The separation of SL16 and SR16 was attributed to the highest proportions of the seed-derived tannin subunits.

Overall, only 10%-56% of tannin composition was characterised based on the proportions of cleavable tannins. Old wine tannin had a significantly lower proportion of cleavable tannin, less than 14%, compared to 45% in young, indicating that around 86% of tannin composition was not characterised using phloroglucinolysis in old wines. This suggested tannin acid-liable inter-flavan linkages were substantially modified due to degradation, polymerization, rearrangement, and oxidation reactions (Cheynier et al., 2006; Fulcrand & Cheynier, 2004; Remy-Tanneau et al., 2003).

Table 6.6 ANOVA analysis according to clustering results of tannin composition

Cluster	P-ECG (%)	P-CAT (%)	P-EPI (%)	ECG (%)	CAT (%)	EPI (%)	Gallo (%)	Galloyl (%)	mDP	MW (g/mol)	Yield (%)
Young	0.19 d	9.06 a	55.4 a	0.07 c	16.7 c	10.8 b	7.9 c	0.26 d	3.6 a	1388 a	45.2 a
2016	3.10 a	8.69 ab	44.5 b	0.35 a	19.4 bc	12.7 ab	11.2 bc	3.4 a	3.1 b	1171 ab	37.6 a
Mid-aged	0.80 c	8.23 b	44.7 b	0.11 c	21.2 b	13.91 a	11.9 b	0.9 c	2.9 b	1103 b	22.8 b
Old	1.41 b	7.40 c	32.9 c	0.19 b	25.6 a	14.7 a	17.7 a	1.6 b	2.5 c	914 c	14.0 b
Pr > F(Cluster)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Significant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

For each chemical parameter, only means followed by a different letter were significantly different using Tukey's test ($P < 0.05$). ECG, (–)-epicatechin gallate; CAT, (+)-catechin; EPI, epicatechin; EGC, (–)-epigallocatechin; % Gallo, the proportion of epigallocatechin extension units; %Galloyl, the proportion of galloylation (the total proportion of epicatechin gallate extension and terminal units); mDP, the mean degree of polymerisation; MW, average molecular weight calculated using phloroglucinol; % Yield of tannin, percent mass conversion calculated based on wine MCP tannin concentration.

6.2.3 The polymeric pigments measured using Orbitrap-UPLC-MS/MS

6.2.3.1 Characterisation of polymeric pigments

As described in Chapter 3, the tannin content of 22 studied wines (section 3.2.2.1), was depolymerised using phloroglucinol (section 3.2.2.8), and the phloroglucinol adducts were characterised using UPLC-ESI- Q-Exactive quadrupole-orbitrap MS/MS analysis (Zeng et al., 2016).

Table 6.7 shows the 18 peaks and their corresponding compounds which were quantified by integrating peak areas based on the full scan (single ion monitoring mode, targeted MS) as well as product ion scan (parallel reaction monitoring mode, targeted MS^2). Their measured m/z, ppm range and m/z range used for integration were also listed. Peaks 3, 4, 9, and 10 were not visible on the MS scan, so these four peaks were only quantified based on the product ion MS/MS scan. The remaining 14 peaks were quantified on the MS scan.

Peaks were classified into seven different types of phenolic compounds based on measured m/z:

- C/EC-M3G consisted of two trimers measured at m/z 781.1956 and 781.1958 on full MS scan, separately, and was ascribed to catechin/epicatechin-malvidin-3-glucoside.

- M3G-C/EC were measured at m/z 781.1974 on MS/MS scan, consisting of malvidin-3-glucoside-catechin/epicatechin.

- M3G-C/EC (A type), measured at 783.2112 and 783.2116 on full MS scan, had an extra A type linkage and were ascribed to malvidin-3-glucoside-catechin/epicatechin (A type).

- C/EC-[M3G-C/EC (A type)], were measured at m/z 1071.2743 to 1071.2752 on MS scan and consisted of four trimers with A type linkages. They were ascribed to catechin/epicatechin-malvidin-3-glucoside-catechin/epicatechin (A type).

- [M3G-C/EC (A type)]-C/EC were measured at m/z around 1071.2748 on MS as well as on MS/MS, and included three trimers with A type linkages, and were ascribed to malvidin-3-glucoside-catechin/epicatechin (A type)-catechin/epicatechin.

- [M3G-C/EC (A type)]-phloroglucinol were measured at m/z 907.2287 and 907.2274, separately, and were ascribed to malvidin-3-glucoside-catechin/epicatechin (A type) extension subunit.

- M3G-methylmethine-C/EC-phloroglucinol were measured at m/z 933.2442 to 933.2432 and were ascribed to malvidin-3-glucoside-methylmethine-catechin/epicatechin extension subunits.

Note that the markers described by Zeng et al. (2016) are termed adducts of polymeric pigments although not all are coloured.

Table 6.7 UPLC-MS/MS data of the identified polymeric pigments markers

Identified Compound	No. of peaks	Integration range ppm	Integration range m/z	Calculated m/z	Measured m/z	Identified peaks	ppm difference	Retention time (min)
C/EC-M3G	2	5.12	781.1940-781.1980	781.1974	781.1956	Peak 1 FS	-2.3	5.5
					781.1958	Peak 2 FS	-2.05	6.2
M3G-C/EC	2		781.19 -> (329.0-329.1)	781.1974		Peak 3 PI		7.2-7.3
						Peak 4 PI		7.5-7.6
M3G-C/EC (A type)	2	5.11	783.2100-783.2140	783.2131	783.2112	Peak 5 FS	-2.43	7.3
					783.2116	Peak 6 FS	-1.92	8
C/EC-[M3G-C/EC (A type)]	4	5.6	1071.2730-1071.2790	1071.2765	1071.274	Peak 7 FS	-2.05	5.7
					1071.275	Peak 16 FS	-1.31	7.0-7.1
					1071.275	Peak 17 FS	-1.03	7.5
					1071.275	Peak 18 FS	-1.21	8.2
[M3G-C/EC (A type)]-C/EC	3	5.6	1071.2730-1071.2790	1071.2765	1071.275	Peak 8 FS	-1.59	6.4
			1071.27 -> (781.18-781.21)			Peak 9 PI		6.4-6.6
			1071.27 -> (781.18-781.21)			Peak 10 PI		7.1-7.2
[M3G-C/EC (A type)]-phloroglucinol	2	4.41	907.2265-907.2305	907.2291	907.2287	Peak 11 FS	-0.44	6.2
					907.2274	Peak 12 FS	-1.87	6.4
M3G-methylmethine-C/EC-phloroglucinol	3	6.43	933.2420-933.2480	933.2448	933.2441	Peak 13 FS	-0.75	8.1
					933.2437	Peak 14 FS	-1.18	8.4
					933.2432	Peak 15 FS	-1.71	8.5

FS, full scan; PI, product ion scan; C/EC-M3G, catechin/epicatechin-malvidin-3-glucoside; M3G-C/EC, malvidin-3-glucoside-catechin/epicatechin; C/EC-[M3G-C/EC (A type), catechin/epicatechin-malvidin-3-glucoside-catechin/epicatechin (A type); [M3G-C/CE (A type)]-C/EC, malvidin-3-glucoside catechin/epicatechin (A type)-catechin/epicatechin; [M3G-C/EC (A type)]-phloroglucinol, malvidin-3-glucoside-catechin/epicatechin (A type) extension subunit; M3G-methylmethine-C/EC-phloroglucinol, malvidin-3-glucoside-methylmethine-catechin/epicatechin extension subunits.

Compounds may have been liberated during acid-catalysed depolymerisation with phloroglucinol, in which case they could be considered to be markers for polymeric pigments markers. According to previous studies (Mouls & Fulcrand, 2012a; Poncet-Legrand et al., 2007), the phloroglucinol reactions for polymeric pigments are given in Figure 6.7. Another possibility was that oligomeric pigments were naturally present in wine.

C/EC-M3G dimers could act as terminal units and were regarded as the quantification markers of coloured T-A^+ type polymeric pigments (Reaction 1). M3G-C/EC and M3G-C/EC (A type) could be related to the depolymerisation of colourless T-A-F polymeric pigments with respective B type and A type linkages involved between M3G and catechin or epicatechin (Reaction 2 and 3). This led to the quantification differences in their mass-to-charge ratios (781 and 783, respectively). As a result, M3G-C/EC and M3G-C/EC (A type) were possibly the quantification markers of T-A-F or T-A-F (A type) polymeric pigments, respectively.

C/EC-[M3G-C/EC (A type) were ascribed to colourless F-A-F (A type) trimers, and they could be the markers for T-F-A-F (A type) polymeric pigments (Reaction 4). In addition, T-F-A-F (A type) and T-A-F (A type) were considered to be different types of polymeric pigments because the F-A linkage appeared not cleavable in the case of T-F-A-F (A type), whilst the F-A linkage was cleavable in the case of T-A-F (A type) polymeric pigments.

[M3G-C/EC (A type)]-C/EC were ascribed to colourless A-F (A type)-F trimer and could be quantification markers for T-A-F (A type)-F polymeric pigments (Reaction 5). [M3G-C/EC (A type)]-phlor was the quantification marker for colourless A-F (A type)-T type pigments, namely A-T (A type) polymeric pigments (Reaction 6), whilst M3G-methylmethine-C/EC-phloroglucinol were the markers for coloured A-methylmethine-T type polymeric pigments (Reaction 7). As a result, five different oligomeric pigments including C/EC-M3G, M3G-C/EC, M3G-C/EC (A type), C/EC-[M3G-C/EC (A type), and [M3G-C/EC (A type)]-C/EC existed in wines and were potentially quantified. On the other hand, seven types of polymeric pigments could be quantified by seven individual markers, including T-A, T-A-F, T-A-F (A type), T-F-A-F (A type), T-A-F (A type), A-F (A type)-T, and A-methylmethine-T types.).

In addition, the coloured A-T type polymeric pigments (Reaction 8) were not observed due to the absence of the markers, A-F extension subunits. The absence of A-T coloured polymeric pigments was potentially attributed to the formation of A-F (A type)-T polymeric pigments (quantified by A-F (A type)-T markers). Another possibility was that flavylium forms of A-T underwent cyclization to produce xanthylium formed pigments showing yellow or brown colour during wine ageing and oxidation.

1. $T-A+Phloro \rightarrow F-A+nF-Phloro$
2. $T-A-F+Phloro \rightarrow A-F+nF-Phloro$
3. $T-A-F\,(A\,type)+Phloro \rightarrow A-F(A\,type)+nF-Phloro$
4. $T-F-A-F\,(A\,type)+Phloro \rightarrow F-A-F(A\,type)+nF-Phloro$
5. $T-A-F\,(A\,type)-F+Phloro \rightarrow A-F(A\,type)-F+nF-Phloro$
6. $A-F\,(A\,type)-T+Phloro \rightarrow A-F(A\,type)-Phloro+nF$
7. $A-methylmethine-T+Phloro \rightarrow A-methylmethine-F-Phloro+(n-1)F-phloro+F$
8. $A-T+Phloro \rightarrow A-F-Phloro+nF$

T, tannin; A, anthocyanins; F, monomeric flavan-3-ol; Phloro, phloroglucinol; A type, the linkages between A and F including C4-C6 as well as C2-O-C7.

Figure 6.7 Phloroglucinol reactions for different types of polymeric pigments

Data for these 7 types of quantification markers are given in Table 6.8. Based on their relative molar contribution to the total, the most dominant adducts were C/EC-M3G and the corresponding T-A polymeric pigments, accounting for 17.5%-73.6%, followed by M3G-C/EC (A type) and T-A-F (A type) (12.0%-72.3%), A-methylmethine-T (0.1%-18.3%), A-F (A type)-T (4.1%-10.8%), C/EC-[M3G-C/EC (A type) and T-F-A-F (A type) (1.1%-4.3%), [M3G-C/EC (A type)]-C/EC and T-A-F (A Type)-F (0.07%-0.56%), and M3G-C/EC and T-A-F (0.0%-0.07%).

The Fixed model REML analysis was carried out (see Chapter 3) to determine the effect of vintage and wine style on the relative molar proportions of oligomeric and polymeric pigments (Table 6.9). The statistical analysis indicated that 5 types of oligomeric and polymeric phenolic adducts were significantly affected by age ($P < 0.05$). These adducts included C/EC-M3G and T-A, M3G-C/EC (A type) and T-A-F (A type), [M3G-C/EC (A type)]-C/EC and T-A-F (A Type)-F, A-F (A type)-T, and A-methylmethine-T. In contrast, M3G-C/EC and T-A-F, and C/EC-[M3G-C/EC (A type) and T-F-A-F (A type),

were not affected by vintage ($P > 0.05$). In addition, only [M3G-C/EC (A type)]-C/EC and the corresponding T-A-F (A type)-F were significantly affected by wine style ($P < 0.05$), whilst the remaining six types of compounds were not affected by style ($P > 0.05$). There were no interaction effects between vintage and style observed.

Table 6.8 Quantification* of 7 types of phloroglucinol adducts

Code	C/EC-M3G T-A	M3G-C/EC T-A-F	M3G-C/EC (A type) T-A-F (A type)	C/EC-[M3G-C/EC (A type) T-F-A-F (A type)	[M3G-C/EC (A type)]-C/EC T-A-F (A type)-F	[M3G-C/EC (A type)]-phlor A-F (A type)-T	M3G-methylmethine-C/EC-phloroglucinol A-methylmethine-T
BC17	66.45±2.93	0.04±0.04	16.33±1.08	2.29±0.37	0.2±0.07	6.30±1.05	8.39±0.32
BD15	60.22±3.00	0.06±0.06	23.73±1.47	3.13±0.28	0.22±0.09	5.00±0.73	7.63±0.37
BL02	17.30±2.93	0.00±0.00	70.52±1.99	3.75±0.20	0.43±0.20	7.88±0.56	0.11±0.02
BL11	47.66±2.15	0.00±0.00	36.88±0.67	4.48±0.11	0.61±0.16	9.79±1.27	0.58±0.26
BL14	63.37±1.96	0.02±0.02	22.78±0.57	2.34±0.16	0.26±0.03	7.10±1.21	4.13±0.02
BL15	61.28±4.28	0.02±0.02	23.28±2.31	2.51±0.31	0.30±0.03	7.81±1.70	4.81±0.03
BL17	66.33±3.79	0.02±0.02	14.98±1.47	2.02±0.31	0.35±0.12	5.77±1.28	10.54±0.83
BR15	59.50±3.23	0.06±0.05	24.62±1.54	2.36±0.19	0.18±0.07	5.96±1.08	7.31±0.30
OS17	69.96±1.93	0.02±0.02	13.28±0.81	1.51±0.18	0.16±0.06	5.79±0.76	9.27±0.09
OS18	65.72±2.09	0.01±0.01	11.62±0.81	1.52±0.14	0.09±0.02	5.27±0.82	15.76±0.29
SC03	26.44±0.38	0.00±0.00	55.52±0.25	5.63±0.41	0.76±0.22	11.51±0.13	0.15±0.08
SC07	26.99±1.16	0.00±0.00	58.52±0.46	3.55±0.25	0.22±0.04	10.08±0.61	0.64±0.13
SC17	71.94±0.49	0.00±0.00	14.83±0.06	1.94±0.25	0.17±0.04	5.83±0.15	5.29±0.10
SL11	47.14±1.65	0.00±0.00	37.52±0.72	3.57±0.29	0.22±0.01	10.33±0.60	1.22±0.02
SL15	56.38±1.13	0.00±0.01	27.12±0.51	2.71±0.39	0.21±0.06	7.46±0.29	6.10±0.13
SL16	62.99±1.45	0.00±0.00	21.61±0.90	2.53±0.18	0.15±0.03	5.04±0.37	7.69±0.03
SL17	63.54±1.47	0.02±0.02	13.04±0.50	1.91±0.23	0.11±0.06	5.83±0.55	15.57±0.10
SR02	24.77±1.29	0.00±0.00	59.60±0.79	5.37±0.06	0.76±0.22	8.98±0.72	0.52±0.06
SR10	37.03±2.60	0.00±0.00	45.49±1.08	3.80±0.04	0.48±0.17	12.00±1.72	1.19±0.01
SR14	57.50±2.04	0.01±0.01	26.72±0.77	2.50±0.05	0.29±0.06	8.38±1.28	4.61±0.01
SR16	63.51±2.56	0.01±0.01	18.25±1.22	2.65±0.28	0.31±0.10	6.75±1.07	8.53±0.08
SR17	60.89±1.21	0.04±0.05	11.50±1.09	1.80±0.10	0.27±0.15	4.56±0.72	20.93±0.61

*Data were calculated as integrated peak area divided by m/z as a proportion of the total for the 7 adducts

Table 6.9 REML analysis of 7 types of phloroglucinol adducts based on vintage and wine style

Compound	P (Vintage)	Ave of SE	P (Style)	Ave of SE	P (vintage *style)
C/EC-M3G, T-A	**0.011**	3.561	0.420	3.705	0.388
M3G-C/EC, T-A-F	0.577	0.03	0.478	0.031	0.932
M3G-C/EC (A type), T-A-F (A type)	**0.001**	1.28	0.138	1.332	0.066
C/EC-[M3G-C/EC (A type), T-F-A-F (A type)	0.063	0.437	0.694	0.454	0.433
[M3G-C/EC (A type)]-C/EC, T-A-F (A type)-F	**0.011**	0.026	**0.031**	0.027	0.052
[M3G-C/EC (A type)]-phlor, A-F (A type)-T	**0.029**	0.662	0.222	0.689	0.338
M3G-methylmethine-C/EC-phlor, A-methylmethine-T	**0.043**	1.836	0.088	1.91	0.276

Statistically significant P values (< 0.05) are shown in bold. Standard errors (SE) are those for differences at the same level of factor.

The scattergrams for the quantification markers according to vintage and style are shown in Figure 6.8. Seven types of polymeric pigments were plotted against vintage, whilst only [M3G-C/EC (A type)]-C/EC marker was plotted against wine style. General declining trends were seen in the two types of polymeric pigments, including C/EC-M3G and T-A, and A-methylmethine-T. In contrast, general increasing trends were seen in the remaining four types of polymeric pigments, including M3G-C/EC (A type) and T-A-F (A type), [M3G-C/EC (A type)]-C/EC and T-A-F (A Type)-F, C/EC-[M3G-C/EC (A type) and T-F-A-F (A type), and A-F (A type)-T. In addition, there were no age-related trends in M3G-C/EC and T-A-F.

T-A or C/EC-M3G adducts decreased from 74% to 17% for studied wines aged from 3 to 19 years. Young wines OS17 and SC17 (aged 4 years old) had around 72% and 74% of T-A type polymeric pigments and C/EC-M3G, separately. As wine age increased, T-A type polymeric pigments and C/EC-M3G decreased gradually from approximately 68% to 58% in wines aged 3-7 years. After 7 years, T-A pigments decreased rapidly to about 30% observed in wine aged 14 years (SC07). After this, a slow proportional decrease was observed from 28% to 17%.

The general proportional decreasing pattern was also observed in M3G-methylmethine-T polymeric pigments, although large variations were observed within vintage 2017. Considering M3G-methylmethine-T, over the 3 to 10 years of ageing (2011-2018), their proportions decreased gradually from 18.37% (SR17) to around 1.1 % (SL11). Wines aged over 10 years had less than 0.6% of M3G-

methylmethine-T and further diminished over 19 years of ageing, with only 0.45% and 0.09% observed in SR02 and BL02, respectively. In addition, considerable variation of M3G-methylmethine-T was observed in wines from 2017, varying from 4.5% (SC17) to 18.4% (SR17). In contrast, M3G-C/EC (A type) and the corresponding T-A-F (A type) polymeric pigments showed a steady increase with age, from about 12.03% to 72.27%. Again, however, considerable variation was also seen, this time in the oldest wines BL02 (72.3%) and SR02 (61.4%).

C/EC-[M3G-C/EC (A type) and the corresponding T-F-A-F A (type) polymeric pigments increased slowly from 1.1% to 4.3% during ageing. However, variations were observed within multiple vintages, although the variations were not large (< 1.5%). This may explain why the effect of age was not significant in REML analysis. OS17 and OS18 had the lowest proportions (1.1%), whilst the highest proportion was seen in SC03.

Regarding [M3G-C/EC (A type)]-C/EC trimers and the corresponding polymeric pigments T-A-F (A type)-F, their proportions in wine were lower than 0.6%, although a general increasing pattern was also observed. Variations were also seen between styles. BL and SR had higher proportions of [M3G-C/EC (A type)]-C/EC and T-A-F (A type)-F, ranging from around 0.2% to 0.6%, whilst OS wines had the lowest proportion, less than 0.1%.

A general proportional increasing pattern was seen in A-F (A type)-T polymeric pigments, although some variations were observed. A-F (A type)-T increased gradually from 4.1% to 10.3% in wines aged from 3 to 11 years old. After 11 years of ageing, A-F (A type)-T polymeric pigments fluctuated. SR10 and SC03 had the highest proportion of A-F (A type)-T, accounting for 10.3% to 10.8%, compared with SR02 and BL02 (8.0% and 7.0%, respectively). Within vintages, A-F (A type)-T varied from 4.5% to 7.0% in wine aged 6 years old (2015). BD15 and BR15 had a lower proportion than SL15 and BL15.

Regarding M3G-C/EC and the corresponding T-A-F polymeric pigments, they did not follow any aged-related trend, with only a minor proportion (< 0.1%) seen in all studied wines. BD15 and BR15 had the highest proportion, 0.07%.

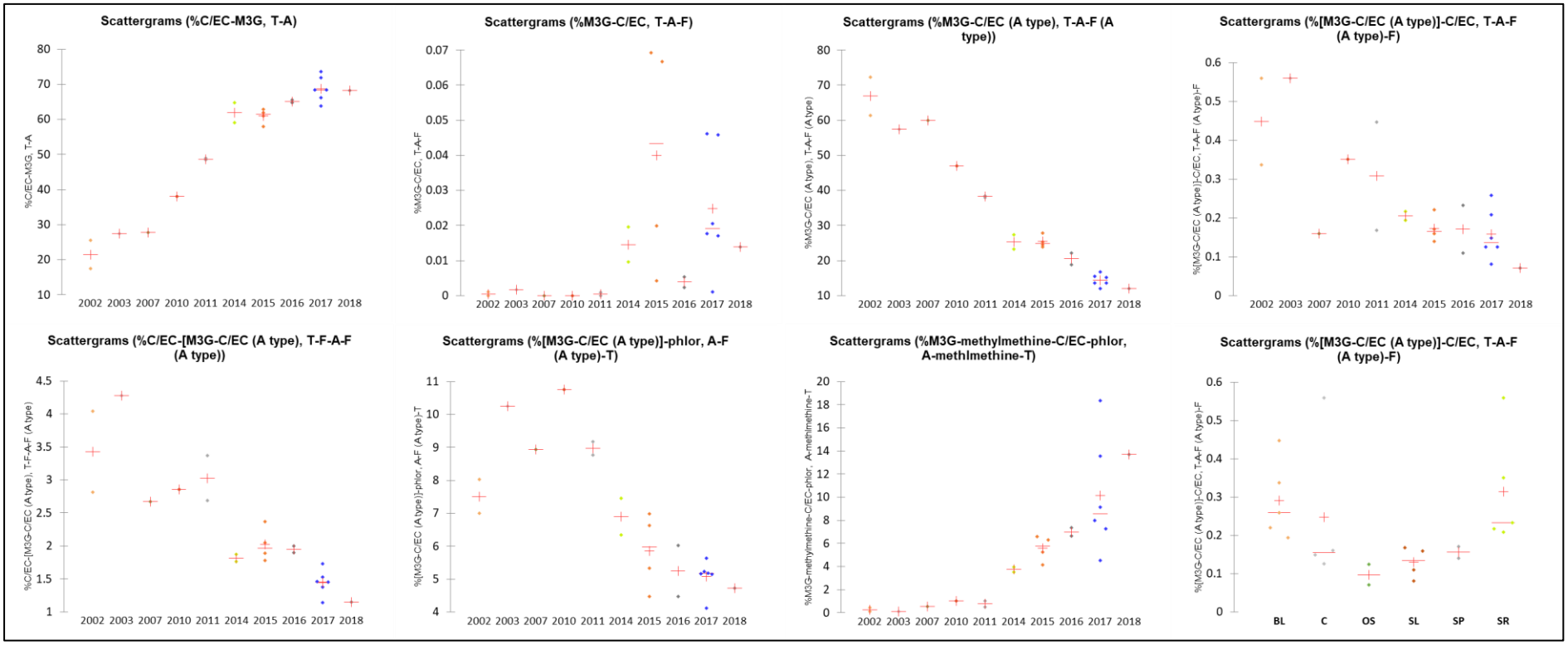


Figure 6.8 Scattergrams of proportions of seven quantification markers according to 10 vintages plus the scattergram of M3G-C/CE (A type)-C/EC marker according to 6 wine style

6.2.3.2 Agglomerative Hierarchical Clustering (AHC) analysis

Figure 6.9 shows the dendrogram of the Agglomerative Hierarchical Clustering analysis using the markers for polymeric pigments. The discrimination of 22 studied wines was based primarily on wine age but also on wine style. Four clusters were identified: 7 older wines from 2002 to 2011 (cluster 1); 10 mid-aged and young wines from 2014 to 2017 (cluster 2); 3 young wines, SR17, SL17, and OS18 (cluster 3); and the remaining 2 wines from designated super premium style, BR15 and BD15 (cluster 4). Apart from cluster 4, different wine styles were seen in each age-related group.

Figure 6.10 shows the relationships between seven markers of polymeric pigments. Four types of A-type oligomeric and polymeric pigments were clustered together, which corresponded with the scattergram results that A-type polymeric pigments showed general increasing patterns against wine age increasing. In contrast, M3G-methylmethine-T and E/EC-M3G and T-A were clustered, which was also consistent with the scattergram results, with general decreasing patterns observed over ageing.

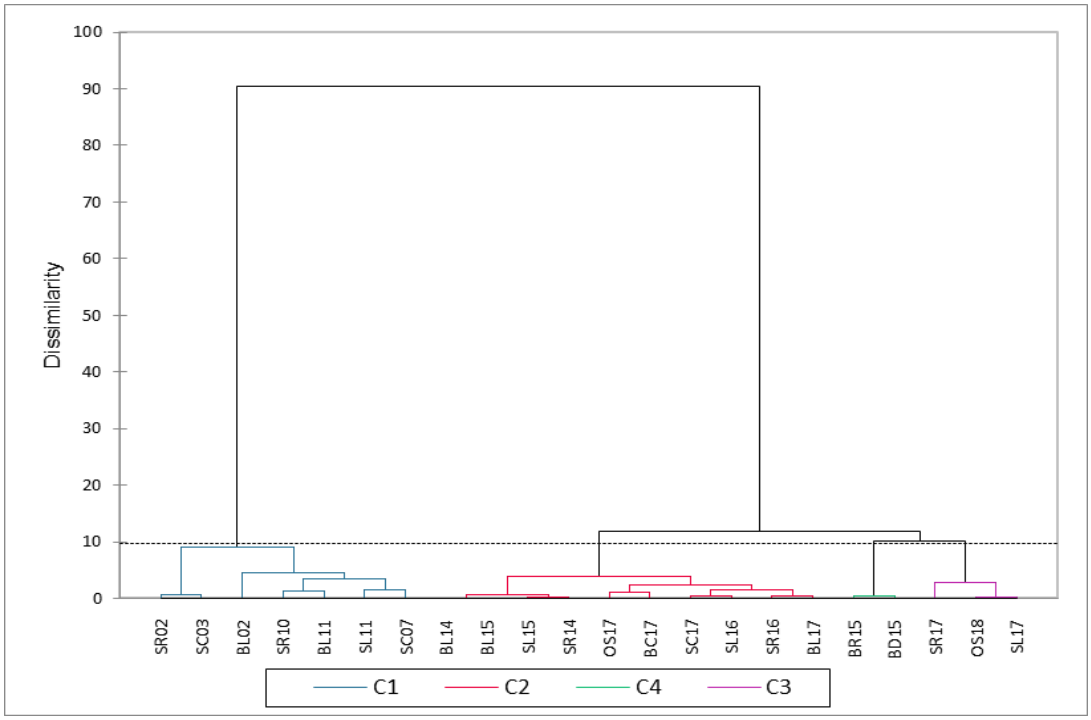


Figure 6.9 Dendrogram resulting from AHC analysis for wine observations according to UPLC-MS/MS (Cophenetic correlation coefficient = 0.800)

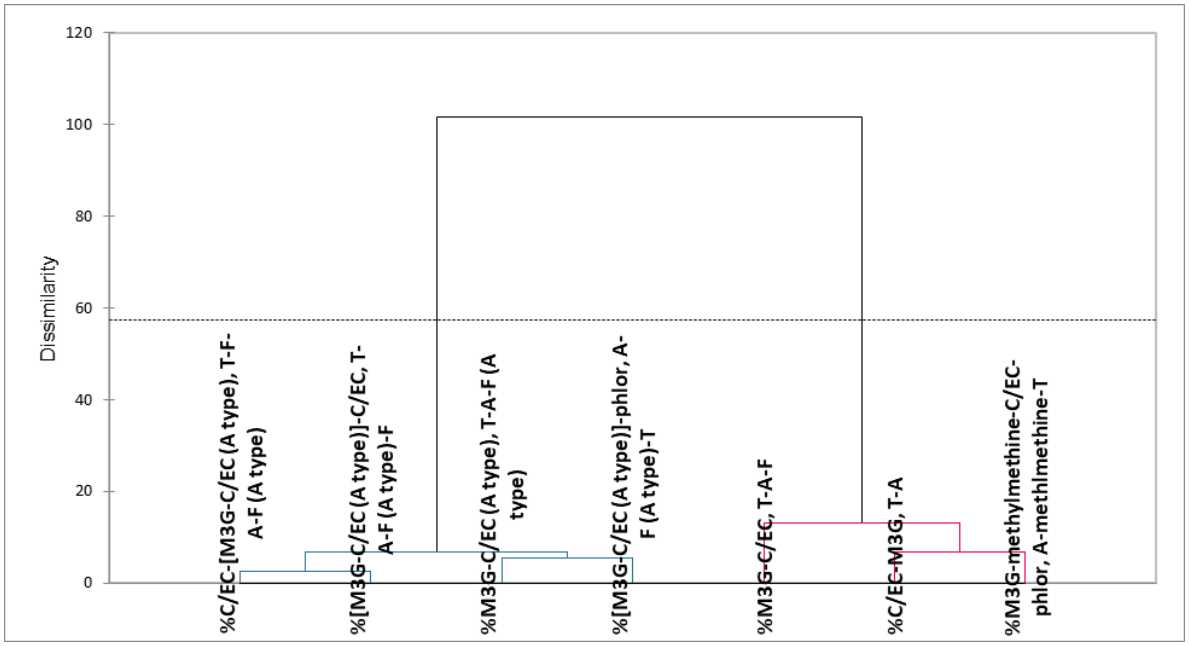


Figure 6.10 Dendrogram resulting from AHC analysis for seven types of polymeric pigments identified by UPLC-MS/MS (Cophenetic correlation coefficient = 0.972)

ANOVA of the seven types of polymeric pigment based on these clusters is shown in Table 6.10. Statistically, all seven markers were significantly different between clusters ($P < 0.05$). Two types of polymeric pigment decreased with age, C/EC-M3G and T-A, and A-methylmethine-T, whilst the remaining four A-type polymeric pigments increased. In addition, M3G-C/EC and T-A-F also showed a slight decrease. However, the separation of SP was mainly attributed to the high proportions of M3G-C/EC and T-A-F (0.068%), although the variations of these pigments tended to be minor between the four clusters. Although not statistically significant, SP also had a higher proportion of A-methylmethine-T, and a lower proportion of A-F (A type)-T compared with the other mid-aged wines.

Table 6.10 ANOVA analysis for Orbitrap-UPLC-MS/MS results based on four clusters

Cluster	% C/EC-M3G	% M3G-C/EC	% M3G-C/EC (A type)	% C/EC-[M3G-C/EC (A type)	% [M3G-C/EC (A type)]-C/EC	% [M3G-C/EC (A type)]-phlor	% M3G-methylmethine-C/EC-phlor
	T-A	T-A-F	T-A-F (A type)	T-F-A-F (A type)	T-A-F (A type)-F	A-F (A type)-T	A-methylmethine-T
2002-2011	33.35 b	0.001 c	53.50 a	3.25 a	0.37 a	8.99 a	0.55 c
2014-2017	65.72 a	0.015 bc	20.47 b	1.73 b	0.18 ab	5.90 b	5.98 b
2017-2018	66.08 a	0.026 b	12.54 b	1.33 b	0.12 b	4.69 b	15.22 a
SP	61.44 a	0.068 a	24.91 b	2.07 b	0.16 ab	4.90 b	6.45 b
Pr > F(Cluster)	<0.0001	<0.0001	<0.0001	<0.0001	0.004	<0.0001	<0.0001
Significant	Yes	Yes	Yes	Yes	Yes	Yes	Yes

For each chemical parameter, only means followed by a different letter were significantly different using Turkey's test ($P < 0.05$).

6.3 Discussion

All monomeric compounds as well as total red pigments determined were within the ranges that had been previously reported for Pinot Noir wine (Kolota et al., 2020; Wimalasiri, 2020; Yang et al., 2021). Considering tannin composition, the most abundant tannin subunits in studied wines were epicatechin and catechin, followed by skin-derived epigallocatechin and seed derived epicatechin-o-gallate, which was also in accord with the previous observations (Aron & Kennedy, 2007; Cheynier et al., 2006; Smith et al., 2015; Yang et al., 2021b). Considering polymeric pigments, five predominate markers were quantified (> 1.0%), including coloured T-A (18%-74%), three types of colourless A type polymeric pigments (T-A-F (A type) (10%-70%), T-F-A-F (A type), and A-F (A type)-T), as well as coloured A-methylmethine-T adducts (0.1%-18.3%), which was also supported by the previous studies (Durner et al., 2015; Fulcrand et al., 2006; Mouls & Fulcrand, 2012b).

The present study highlighted the importance of vintage and style to different phenolic compounds. REML analysis found that a number of monomeric phenolics and polymeric pigments were significantly influenced by vintage and styles ($P < 0.05$).

Considering wine styles, six monomeric phenolics and total red pigments were significantly affected by wine style: i.e. protocatechuic acid, caftaric acid, *trans*-coutaric acid, GRP, and quercetin-3-glucoside, and malvidin-3-glucoside. In addition, the M3G-C/EC (A type)-F trimers and T-A-F (A type)-F adducts were the only type of polymeric pigments affected by wine style, although only a minor proportion was observed (< 0.6%) in the current study. Brancott Letter (BL) and Stoneleigh Rapaura (SR) had higher proportions of T-A-F (A type)-F adducts, whilst the Central Otago wines (OS) had the lowest proportion, indicating oxidative reactions differed in terms of A-F (A type)-F trimers as well as the corresponding T-A-F (A type)-F adducts. This is the first report on the effect of wine style on monomeric phenolics and total red pigments as well as T-A-F (A type)-F adducts in Pinot Noir wine. However, future investigation is required to properly understand how wine style influenced these compounds because producing distinctive styles of wines depends on a range of natural and human factors. Previous studies highlighted the effect of terroir on the profiles of monomeric anthocyanins, flavan-3-ols, stilbenes, phenolic acids, and polymeric pigments in wines (Karaoğlan et al., 2015; Kelebek et al., 2011; Knight et al., 2015; Roullier-Gall et al., 2014). Some studies also suggested that canopy management influenced the content of hydroxycinnamic acids, anthocyanins, flavonols, and polymeric pigments (Cortell et al., 2005; Downey et al., 2006; Lemut et al., 2011). For example, sun exposure was found to affect caftaric acid negatively in Pinot Noir wine (Downey et al., 2006; Price et al., 1995). Flavonols had been more influenced by light rather than other factors, in agreement with the suggestion that canopy management could significantly modulate the production of flavonols (Cortell et al., 2005; Downey et al., 2006). Winemaking interventions also play a role in modulating wine style,

thus affecting monomeric and polymeric phenolics (Harrison, 2018; Yang et al., 2022). For example, Yang et al. (2021) evaluated that post-MLF micro-oxygenation resulted in the disappearance of coutaric acid and the conversion of caftaric acid to caffeic acid as well as polymeric pigments.

Considering wine vintages, age-related trends were observed in a range of monomeric and polymeric phenolics and tannin composition in the present study.

With respect to monomeric phenolics, the general decreasing trends seen in caftaric acid, *trans*-coutaric acid, GRP, epicatechin, quercetin-3-glucoside, malvidin-3-glucoside, and total pigments with increasing age agreed with previous studies (Garciafalcon et al., 2007; Gomez-Plaza et al., 2001, 2002). In addition, the concentration of total phenolic acids, hydroxycinnamic acids, and flavan-3-ols also decreased mostly over ageing, which was in accord with previous studies (Garciafalcon et al., 2007; Gomez-Plaza et al., 2002). During ageing, the expected decreases were seen in caftaric acid, coutaric acid and GRP (Perestrelo et al., 2020). The hydrolysis of caftaric acid and coutaric acid produces caffeic acid and coumaric acid (Casassa et al., 2016). Consequently, the general increasing pattern of coumaric acid over ageing was expected (Table 6.1), which also agreed with previous studies (Garciafalcon et al., 2007). In contrast, caffeic acid did not increase with age. This may be ascribed to the fact that caffeic acid could participate in oxidation reactions and react with M3G to form pinotin (de Freitas & Mateus, 2011). The decrease of GRP may relate to the content of caftaric acid amounts because the chemical reaction between caftaric acid quinone and glutathione (GSH) leads to the formation of grape reaction product (Cheynier, 2012). Based on the REML result, the concentration of gallic acid was affected by other factors during ageing rather than vintage and styles. The formation of gallic acid was attributed to the hydrolysis of hydrolysable tannin during oak ageing, and the concentration of gallic acid may be related to the time spent in oak ageing (Cadahia et al., 2001), toasting intensity of oak, and fermentation modulation (Sanz et al., 2011; Uzkuc et al., 2021).

The reduction of phenolic compounds may be associated with the formation of copigmentation complexes with anthocyanins (Asenstorfer et al., 2006; Morata et al., 2007). Previous studies found that flavonols had the highest binding capability with anthocyanins, followed by hydroxycinnamic acid, flavan-3-ols, and hydroxybenzoic acid (Ferreira da Silva et al., 2005; Galland et al., 2007; Leydet et al., 2012; Nave et al., 2012; Tsutomu, 1992). Moreover, the reduction of epicatechin, catechin, and malvidin-3-glucoside was also associated with the formation of polymeric pigments such as T-A, A-methylmethine-T, and T-A-F (A type), which was supported by the current and previous studies (Cheynier et al., 2006; Fulcrand & Cheynier, 2004; Remy-Tanneau et al., 2003). However, REML analysis showed that catechin was not significantly different between vintages ($P > 0.05$). This was attributed to large variations within vintage, which likely caused discrimination bias for the analysis of variance. Variations within vintage 2017 (130 mg/L) were dominant compared with the variations between

vintage (< 60 mg/L), and thus age effect was not seen on catechin. These variations were affected by many factors, such as the selection of Pinot Noir clones (Casassa, Vega-Osorno, et al., 2021) and maceration (Gordillo et al., 2021). Casassa et al. (2021) found that Pinot Noir wines of clones 777 and 2A had higher amounts of phenolic contents than those of clone 115. As shown in Chapter 3 (Table 3.4), wines BC17 with the lowest catechin and epicatechin had the lowest proportions of clones 115, whilst SR17 with the highest of catechin and epicatechin was seen a larger proportion of clones 777 and UCD5.

The presence of monomeric pigments and their general decreasing trends were seen in F1. Previous studies confirmed that those pigments tended to be mainly 4-vinylphenol pyranoanthocyanins in F1 of Cabernet Sauvignon and Shiraz wines using LC-MS (Jeffery et al., 2008). Boido et al., (2006) found that the concentration of 4-vinylphenol pyranoanthocyanins remained constant but their contribution to total pigments increased from 1% to 21% during storage in Tannat wines. Another possibility was that other pyranoanthocyanins, such as vitisin A and vitisin B, decreased with age since they contribute to red tonality in old wine, whilst 4-vinylphenol of M3G pyranoanthocyanins were responsible for the orange-red colour in older wines (Jeffery et al., 2008; Rivas et al., 2006).

The yield of tannin (tannin mass conversion) decreased gradually over ageing, which was in accord with previous studies (McRae et al., 2012; Yang et al., 2021b). Some studies suggested that the decrease of tannin mass conversion was a result of the gradual decrease in the ratio of acid-catalysed cleavage of inter-flavan bonds, due to an increase of intramolecular (A type) and intermolecular linkages (biaryl-type) among flavan-3-ols, consistent with the oxidation and rearrangement reactions (Mouls & Fulcrand, 2012b; C. Poncet-Legrand et al., 2010; Vernhet et al., 2011). This was also supported by the present findings in that older (10-19 years) wines had increased proportions of A type polymeric pigments such as T-A-F (A type) and A-F (A type)-T, and decreased proportions of T-A and A-methylmethine-T adducts compared with younger (3-4 years) wines. As a result, these reactions would have resulted in the decrease of cleavable tannin, as well as red colour loss (increased hue over ageing) and colour stability (increased SO_2-resistant pigments) during wine ageing found in Chapter 4. Although in the present study, no age effect was observed in the yield of tannin by REML analysis, likely this was because of the large variations within vintage 2017. For example, SR17 had an approximately 20% higher depolymerised tannin compared with BC17, implying that more tannins modification occurred in BC17 compared with SR17.

Regarding the mean degree of polymerisation, mDP values for studied wines were quite low (2.0-4.0). Aron and Kennedy (2007) reported that the mDP of young Pinot Noir wines was 5.0 to 8.6, whilst Yang et al. (2021b) obtained a lower value (4.2) for Pinot Noir wine after alcoholic fermentation, which fell to 3.1 after 60-day microoxygenation treatment. A reduction in mDP over ageing has also been

reported by other authors (McRae et al., 2012; Yang et al., 2021b). However, C. Poncet-Legrand et al. (2010) found the mean degree of polymerisation increased caused by oxidation according to small angle X-ray scattering, suggesting that intermolecular reactions occurred to form large macromolecules of tannin. In addition, this decrease in mDP may not represent a decrease in tannin size during wine ageing, because a substantial proportion of inter-flavan linkages were not cleavable by phloroglucinol due to the formation of A type and biaryl-type linkages during ageing. In the present study, older wines had less than 13% of cleavable tannin subunits that could be characterised by phloroglucinolysis compared with over 45% in younger wines. During ageing, the reduction of determined average molecular mass and its strong positive correlation with mDP were also observed in the current study, which was in accord with previous studies (Jorgensen et al., 2004). However, this determined average molecular mass was not representative especially in aged wine due to the low yield of tannin. Jorgensen et al. (2004) found that the size distribution of tannins changed little during oxidative degradation determined by gel permeation chromatography in Pinot Noir wine. In contrast, McRae et al. (2014) noted that old wine had a larger average molecular size determined by both GPC and small angle X-ray scattering methods in Cabernet Sauvignon wines. Therefore, future work is required to understand the impact of wine age on the size distribution of tannin in Pinot Noir wine.

In terms of skin-derived epigallocatechin tannin, epigallocatechin extension units were in larger proportion compared to seed-derived galloylated tannin, which was in accord with a previous study of Pinot Noir wine (Carew et al., 2020). In the present study, a proportional increase in skin-derived gallolated tannin was found over ageing, but the concentration of epigallocatechin subunits saw a general decrease. This agreed with a previous study (Yang et al., 2022) with Pinot Noir wines. However, in a study of Cabernet Sauvignon wines, McRae et al. (2012) reported that the proportion of epigallocatechin was 10% greater in young wines (4-19 years old) compared to older wines (22-49 years old). One probable reason for the proportional increases in skin-derived epigallocatechin could link to the greater reduction of epicatechin and catechin extension subunits, implying that more intermolecular and intramolecular linkages occurred among epicatechin and catechin than that in epigallocatechin subunits. Consequently, the observed declining rate of the epicatechin and catechin subunits was larger than the rate of epigallocatechin and increased the contribution of epigallocatechin subunits. This was also supported by UPLC-MS/MS data that a larger proportion of A-type polymeric pigments, such as T-A-F (A type), were formed between (epi)catechin. In contrast, a minor percentage of seed-derived epicatechin gallate tannin was observed for studied wines (0.12% to 3.72%), in comparison with 7.6% of galloylated tannin observed in finished Pinot Noir wine (Yang et al., 2021). McRae et al. (2012) also found a quite low proportion (2-3%) of galloylated tannin in Cabernet Sauvignon and Syrah wines aged 4-49 years old, and old wine had a slightly higher ratio of seed-derived galloylated tannin, which was in line with the present study. Significant variations in

proportions of epicatechin gallate extension unit and combined galloylated tannins were found between vintages, although their proportional variations were not large (less than 5%). Previous studies suggested that minor differences in epicatechin-O-gallate could result from seed-derived tannin extractability during winemaking rather than the effect of age (Carew et al., 2020; Carew et al., 2013). This was also supported by the present study. For example, 2016 wines (6 years old) had a significantly higher proportion of epicatechin-O-gallate subunits (Table 6.4) than young wines, indicating that a higher proportion of epicatechin-O-gallate could extract from grape seeds in wines SR16 and SL16, compared with wines from 2017 and 2018. This also contributed to the separation of 2016 wines from 2017 and 2018 wines.

With respect to polymeric pigments, general decreasing patterns with age were seen in T-A and A-methylmethine-T adducts, whilst increasing patterns were seen in all A-type adducts including T-A-F (A type), A-F (A Type)-T, T-F-A-F (A type), and F-A-F (A type)-F. In addition, no age-related patterns were seen in T-A-F adducts.

Coloured T-A and A-methylmethine-T polymeric pigments decreased dramatically with age, which was in accord with the previous studies (Blanco-Vega et al., 2014; Drinkine et al., 2005; Drinkine et al., 2007b). In the case of T-A adducts, anthocyanins in their neutral pseudobase form react at the electrophilic C4 position of the flavan-3-ol acting as an extension unit. In contrast, A-T adducts are formed by the reaction of anthocyanin in the flavylium at the electrophilic C4 position. As a result, T-A type polymeric pigments were more abundant than the A-T type pigments (Cheynier et al., 2006; Dueñas et al., 2006). Some studies noted that the methylmethine linkage was attributed to the production of acetaldehyde from yeast metabolism during fermentation as well as the oxidation of ethanol during ageing (Drinkine et al., 2007a, 2007b). In the present study, A-methylmethine-T almost disappeared after 10 years of ageing (< 1.0%) because of the instability of the ethyl linkage, which was supported by the previous studies (Blanco-Vega et al., 2014). The declining rate of A-methylmethine-T was faster for 4-7 years of ageing than T-A, whilst T-A decreased more rapidly after 7 years of ageing, indicating that A-methylmethine-T and T-A adducts were relatively unstable and evolved over ageing, which was in line with the previous observations (Laitila & Salminen, 2020).

Laitila and Salminen (2020) also noted that T-A and A-methylmethine-T polymeric pigments were the primary contributors to the colour intensity for wines aged 1 to 6 years old. In the present study around 60% of T-A and 4%-6% of A-methylmethine-T were still observed after 6 years (2015 wines). This suggested that T-A and A-methylmethine-T polymeric pigments as well as malvidin-3-glucoside and the other pigments measured by HPLC contributed to colour change regarding hue and intensity during ageing. Decreased proportions of coloured T-A and A-methylmethine-T as well as decreased malvidin-3-glucoside, and increased other red pigments measured in F1 suggested the reduction of the degree

of redness in older wines. As a result, an increased colour hue was observed in aged wines in Chapter 4 and 5. Proportional decreases in coloured T-A and A-methylmethine-T as well as decreased malvidin-3-glucoside also suggested increased proportions of other pigments which were responsible for yellow/orange colour, such as xanthylium pigments and pyranoanthocyanins (He et al., 2012; Vallverdu-Queralt et al., 2017). As a result, aged wines had increased colour density compared with young wines from 2017 and 2018 found in Chapters 4 and 5. Laitila (2021) found the average sizes of the T-A adducts increased in wines over 1-6 years of ageing, although the main compounds in T-A adducts were the dimers, (epi)catechin-malvidin-3-glucoside. This suggested that during ageing T-A polymeric pigments were depolymerised regardless of their size and the smallest adducts were more prone to depolymerisation than the larger ones, leading to the increased average size of the adducts. In addition, previous studies also found that A-methylmethine-T dimer was not stable at wine pH due to the acid-catalysed cleavage of the methylmethine linkages (Drinkine et al., 2007a; Escribano-Bailón et al., 2001). As a result, A-methylmethine-T polymeric pigments could be depolymerised under excessive phloroglucinol, but it remains to be seen how well these acetaldehyde intermediate pigments were resistant to depolymerisation.

In contrast, the expected increasing patterns were seen in the proportions of A-type polymeric pigments, as a consequence of decreased direct inter-flavan linkages in T-A adducts and increased intramolecular linkages during ageing (Cheynier et al., 2006; Fulcrand et al., 2006; He et al., 2012). T-A-F (A type) (10%-70%) were higher than A-F (A type)-T (4%-11%) and T-F-A-F (A type) (1%-4%). The main compounds in T-A-F (A type) adducts were malvidin-3-glucoside-(epi) catechin (A type) dimers in wine aged 1-6 years, although the average size of T-A-F (A type) adducts increased over ageing (Laitila, 2021), which was to some extent supported by the present study because the proportions of the dimers, malvidin-3-glucoside-(epi) catechin (A type) were 9%-66% higher, compared with the trimers, (epi)catechin- malvidin-3-glucoside-(epi) catechin (A type). It remains to be seen how the size of A-type polymeric pigments transformed over a longer term of ageing (4-19 years) because a range of chemical reactions potentially occurred during ageing, such as degradation, polymerisation, rearrangements, and oxidation reactions (Cheynier, 2012; He et al., 2012). The evolutionary trends of A-F (A type)-T and T-F-A-F (A type) have been first observed in Pinot Noir wines in the current study. Laitila (2021) suggested that the concentration of T-A-F (A type) adducts almost remained unchanged over 1-6 years of ageing, which was different to the present study where the relative concentration of T-A-F (A type) adducts fluctuated, although the proportion decreased generally. In addition, large variations of A-type polymeric pigments were seen within vintages in the present study, which were possibly caused by oxidative reactions during oak and bottling ageing (Aleixandre-Tudo & du Toit, 2020; Durner et al., 2015). Durner et al. (2015) noted that the ratio of flavanol to anthocyanin was positively correlated with the formation of large polymeric pigments.

AHC results were also consistent with the REML results and highlighted the importance of age and styles to monomeric and polymeric phenolics and tannin composition. The discrimination of age-related groups was attributed to the variations observed between monomeric phenolics, tannin composition, and the proportions of polymeric pigments. In addition, the discrimination of Central Otago wines (OS) was related to monomeric phenolics, whilst the discrimination of super premium wines (SP) was attributed to polymeric pigments.

Central Otago (OS) wines were distinguished by the lower levels of hydroxybenzoic acids (p-hydroxybenzoic acid, protocatechuic acid, syringic acid, and gallic acid), p-coumaric acid, *trans*-coutaric acid, resveratrol, and relatively higher levels of GRP, M3G, and total red pigments, compared with young wines from 2017. This result was in accord with the result in Chapter 5 that Central Otago wines showed higher total anthocyanins and corrected colour density for SO_2. Malvidin-3-glucoside and total red pigments were responsible for colour density and hue observed in F1 from OS wines. In addition to that, the proportions of T-A and A-methylmethine-T adducts also contributed to colour density and hue in OS wines. OS wines spent less than 100 days in barrels than other young wines from 2017, resulting in a lower level of gallic acid (Waterhouse, 2016).

Considering super premium wines, SP wines were distinguished by the highest concentration and proportion of malvidin-3-glucoside-(epi) catechin dimers and the corresponding T-A-F adducts, although their proportions were minor (around 0.07%). The large proportion of malvidin-3-glucoside-(epi) catechin (A type) adducts and xanthylium formed adducts were responsible for the minor proportions of malvidin-3-glucoside-(epi) catechin dimers (Dueñas et al., 2006; He et al., 2012). The formation of malvidin-3-glucoside-(epi)catechin dimers required oxygen to regenerate the flavylium cation from the colourless A-T flavene form. In contrast, a colourless A-T flavene could react to form the colourless A-type dimer under anaerobic conditions or could undergo cyclization to form a coloured xanthylium pigments (yellow/brown) with excessive oxygen (Cheynier et al., 2006; Dueñas et al., 2006; Remy-Tanneau et al., 2003). As a result, SP wines had more coloured malvidin-3-glucoside-(epi)catechin dimers and T-A-F adducts. This suggested that the oak ageing was modified for the SP wines to improve the perceived quality of the wine, highlighting the importance of oak ageing and colour to wine quality. In addition, although wine styles did not exert any influence on tannin compositions, some distinct variations were also revealed between styles. For example, SP wines BD15 and BR15 showed a lower proportion of cleavable tannin, catechin extension subunits, and epicatechin terminal subunits, accompanied by a higher percent of skin-derived gallolated tannins compared with premium wines BL15 and SL15, implying that tannin structure and composition in SP had been modified significantly by a long-term barrel ageing (over 540 days) with a higher proportion of 1-year-old wood involved, as described in chapter 3.

Considering the AHC result based on tannin composition, the separation of 2016 wines was linked to the greater seed-derived epicatechin gallate subunits. In addition, the subgroup of Brancott wines from 2014 and 2015 was separated from Stoneleigh wines from 2014 and 2015. This separation was potentially attributed to the relatively higher mean ratios of epicatechin terminal units as well as lower mDP and average molecular mass observed in Stoneleigh wines compared with Brancott wines (Table 6.4), implying that different tannin modifications took place for Stoneleigh and Brancott wines from 2014 and 2015.

6.4 Conclusion

In the present study, six monomeric phenolics, total monomeric red pigments and one type of polymeric pigment were associated with vintage, age, and style: i.e. protocatechuic acid, caftaric acid, *trans*-coutaric acid, GRP, and quercetin-3-glucoside, and malvidin-3-glucoside, total red pigments, as well as the M3G-C/EC (A type)-F trimers and T-A-F (A type)-F adducts. In contrast, tannin composition was only associated with vintage and age. During ageing, the general decreasing patterns were seen in p-hydroxybenzoic acid, caftaric acid, coutaric acid, flavan-3-ols, resveratrol, malvidin-3-ols, total red pigments, as well as yield of tannin, mDP, average molecular weight, and catechin and epicatechin extension units, and coloured T-A and A-methylmethine-T adducts, whilst the general increasing patterns were seen in p-coumaric acid, proportions of catechin and epicatechin terminal units, seed-derived epicatechin gallate subunits, and skin-derived epigallocatechin subunits, as well as proportions of A-type polymeric pigments.

When it comes to colour, the decreased proportion of malvidin-3-glucoside, T-A and A-methylmethine-T adducts, as well as the increased proportion of other red pigments measured in HPLC contributed to the changes in colour density and hue during bottle ageing (3-19 years). In contrast, increased proportions of T-A-F (A type), A-F (A type)-T, T-F-A-F (A type) and T-A-F (A type)-F adducts resulted in colour reduction and compositional stability in old wines. The discrimination of OS wines was driven by monomeric phenolics, whilst the discrimination of SP wines was driven by the highest concentration and proportion of malvidin-3-glucoside-(epi) catechin dimers and the corresponding T-A-F adducts. It remains to be seen how the size of T-A, A-methylmethine-T, as well as A-type polymeric pigments transformed over long-term ageing and whether the dimeric pigments were the main compounds in each type of polymeric pigments.

Chapter 7

Overall Discussion and Conclusion

Pinot Noir wines possess a range of phenolic compounds that evolve during ageing, affecting colour, taste, mouthfeel, and overall wine quality. Numerous studies have considered the impact of wine age on colour and phenolic composition as discussed in Chapter 2 (Boido et al., 2006; de Freitas et al., 2004; Gutierrez-Escobar et al., 2021; Laitila & Salminen, 2020). Whilst revealing information about the phenolic composition of Pinot Noir wine, there still exists a significant research gap in differentiating Pinot Noir wine style/quality based on the chemical composition of wine. This research is the first of its kind to carry out an in-depth investigation concerning the effects of age and style in terms of phenolic composition.

In this study, many of the results relating to age were not unexpected. This included trends observed by previous studies (Blanco-Vega et al., 2014; Drinkine et al., 2007b; He et al., 2012; Laitila & Salminen, 2020; McRae et al., 2012), as well as others reported in this study for the first time such as the measurements of markers of polymeric pigments in Pinot Noir wines differing in age and style. Wine age exerts the dominant impact on colour and phenolic composition, regardless of the effects of vintage and style. Many age-related trends were observed in respect of colour, tannin composition, and polymeric pigments. General decreasing patterns with increased wine age were seen in the concentrations of a number of monomeric phenolics (*p*-hydroxybenzoic acid, caftaric acid, coutaric acid, flavan-3-ols, resveratrol, anthocyanins), total red pigments, as well as parameters related to tannin composition (tannin mass conversion, mean degree of polymerisation, average molecular weight, catechin and epicatechin extension units, and coloured T-A and A-methylmethine-T adducts). Furthermore, general increasing patterns with increased wine age were seen in other phenolic attributes, i.e. *p*-coumaric acid, the proportions of catechin and epicatechin terminal units, seed-derived epicatechin gallate subunits, and skin-derived epigallocatechin subunits, as well as proportions of A-type polymeric pigments. The age-related trends in A-F (A type)-T and T-F-A-F (A type) species in Pinot Noir wines have been reported in the current study for the first time. These results highlighted the importance of wine age in modifying the structure and composition of monomeric and polymeric phenolics.

The current study also established an association between the concentrations of monomeric and polymeric pigments and wine colour during ageing. The decreased proportion of malvidin-3-glucoside, T-A and A-methylmethine-T adducts, as well as the increased proportion of other red pigments measured in HPLC, contributed to the change of colour density and hue during wine ageing; whereas increased proportions of T-A-F (A type), A-F (A type)-T, T-F-A-F (A type) and T-A-F (A type)-F adducts

resulted in colour loss and compositional stability in old wines. The compounds listed above were considered important compounds for overall colour properties and helped to explain colour evolution in red wine from purple/red to orange hue during ageing. Future investigation should focus on these compounds and include additional Pinot Noir wines that were not available in this study so that colour evolution can be comprehensively depicted. The impact of these compounds on mouthfeel attributes could also be further investigated. In addition, other pigments responsible for yellow/brown colour, such as pyranoanthocyanins in red wine, require further investigation.

The impact of styles was also investigated considering colour and phenolic parameters and phenolic composition. Significant differences were observed in six of the monomeric phenolics and total red pigments: i.e. protocatechuic acid, caftaric acid, *trans*-coutaric acid, GRP, quercetin-3-glucoside, and malvidin-3-glucoside. In addition, significant differences in M3G-C/EC (A type)-F trimers and T-A-F (A type)-F adducts were also observed, although only a minor proportion was found in the current study. Central Otago (OS) wines were mainly distinguished by monomeric phenolics, while super premium (SP) wines were distinguished by higher colour density, SO_2-resistant pigments, total phenolics, and tannin concentrations, malvidin-3-glucoside-(epi) catechin dimers and the corresponding T-A-F adducts. Wines from the remaining four styles Brancott Letter, Commercial, Stoneleigh Latitude and Stoneleigh Rapaura could not be differentiated in the current study. Nevertheless, overall, it was concluded that style can affect the phenolic composition. This result provides some guidelines to direct viticultural and winemaking practices with the aim of improving the colour and phenolic profile and thus improving the perceived quality of Pinot Noir wine. In particular, concerning some parameters, SP wines appeared similar to mid-aged and young wines with significantly higher levels of total anthocyanins, colour density, total phenolics, as well as lower hue in F1. In contrast, concerning other parameters, SP wines appeared more similar to old wines with significantly higher levels of colour density and polymeric pigments observed in F2 and F3, although similar to mid-aged and young wines with the lowest hue in F3. Future studies evaluating how style could influence these phenolic compounds of Pinot Noir by eliminating the effect of age would enhance the knowledge gained from this study.

Consideration should be given to vintage variations. Due to the predominant effect of wine age, the variations caused by vintage were generally not significant. The differences in tannin concentration were thought to be caused by vintage, viticultural management, and winemaking practices (Carew et al., 2013; Casassa et al., 2013; Casassa et al., 2015; Gambacorta et al., 2011; Smith et al., 2015).

This study has also investigated polymeric phenolic fractions. The red colour of polymeric phenolic material in old wines tended to be more resistant to changes in pH as indicated by a reduction in the factor A520 pH1/pH3.4 from 2.6 to 1.1 during ageing, with the degree of ionisation of these bleachable

polymeric components increasing during ageing. This result for Pinot Noir wines is the first report of this age-related trend in the polymeric material. It was likely to be associated with the disruption of the conjugation system in colourless polymeric pigments (Cheynier et al., 2006; Cucciniello et al., 2021; Laitila, 2021).

Considering tannin fractionation, the solid phase extraction method appeared to be more effective and efficient, with a high recovery of phenolic material observed, compared with size exclusion chromatography combined with liquid-liquid fractionation. Tannin fractionation was beneficial to the discrimination of style. In addition, the method used for the quantification of seven markers of polymeric pigments was very useful. Phloroglucinolysis combined with HPLC, or Orbitrap-UPLC-MS/MS revealed a more detailed analysis of the average tannin subunits composition and small oligomeric phenolics. However, the phloroglucinolysis method is limited by the degree to which de-polymerisation occurs. In the current study, aged Pinot Noir wine had around 90% of polymeric phenolics which could not be characterised by phloroglucinolysis. Recently, a group-specific UPLC–MS/MS method was developed, which utilised in-source collision-induced dissociation to achieve the depolymerisation step and larger polymeric pigments could be semi-quantified (Laitila, 2021; Laitila & Salminen, 2020; Laitila et al., 2019). Future investigations could be conducted using this novel method without depolymerisation, to obtain detailed chromatographic fingerprints of anthocyanins and the composition evolution of polymeric pigments in red wine.

This study has investigated the phenolic composition in Pinot Noir commercial wines and how they were influenced by age, vintage, and style. These observations are starting points and pave the way for modulating phenolic composition and wine colour through viticultural and winemaking interventions, thus improving the perceived quality and the ageing potential of Pinot Noir wines. However, several aspects of this research should be further investigated as this would enhance the knowledge gained in this study. Potential future investigations include:

- To determine tannin composition in polymeric fractions and to examine how they change regarding age and style.

- To measure the size distribution of tannin subfractions by different methods such as gel permeation chromatography and small angle X-ray scattering and compare results to those obtained by phloroglucinolysis in order to explore the potential effects of age and style on average molecular weight and size distribution of tannin subfractions.

- To identify and quantify other red pigments eluted after the peak of malvidin-3-glucoside in monomeric fraction F1 and to understand their roles in colour intensity and stability.

- To measure the average size of each type of polymeric pigments and examine the main individual compounds in each type of polymeric pigments during ageing.

- To investigate the evolutionary trends of pyranoanthocyanins and to examine the potential effect of style on pyranoanthocyanins and their roles in colour intensity.

- To characterise the sensory attributes of polymeric fractions and try to establish the correlations between perceived astringency and phenolic composition obtained in this study.

Appendix

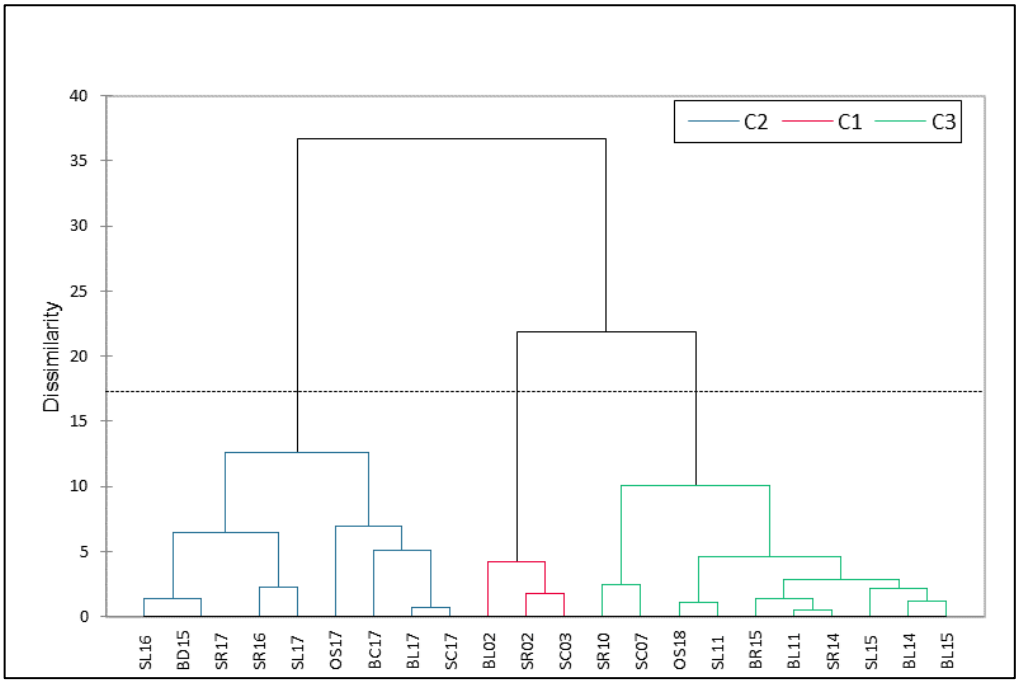


Figure A.1 Dendrogram resulting from agglomerative hierarchical (AHC) analysis based on the general chemical wine parameters (Cophenetic correlation coefficient = 0.591)

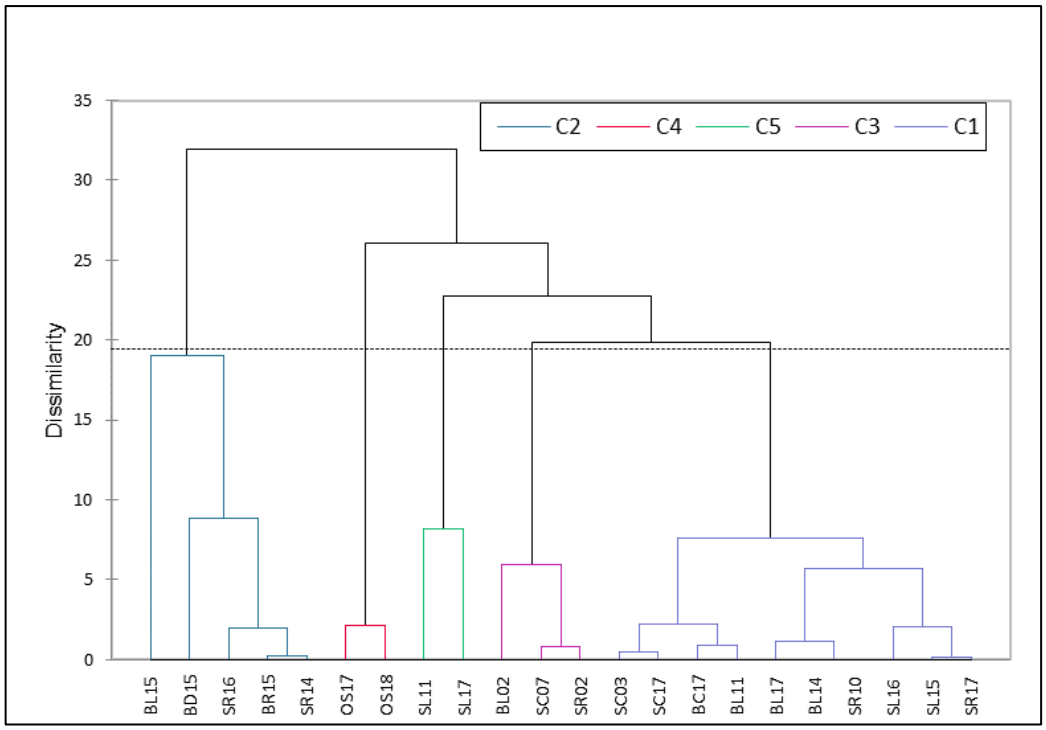


Figure A.2 Dendrogram resulting from AHC analysis based on the percentages of clone selections for individual wines (Cophenetic correlation coefficient = 0.652)

Table A.1 ANOVA type III SS analysis for the general chemical parameters based on the above clustering result

Cluster	Alcohol (ABV)	Titratable acidity (g/L)	pH	Volatile acid (g/L)	Free SO_2	Molecular SO_2
Young (C2)	13.03 b	5.40 b	3.57 b	0.83 a	37 a	0.43 b
Mid-aged (C3)	13.93 a	6.01 a	3.53 b	0.68 b	30 b	0.54 a
Old (C1)	14.06 a	5.77 ab	3.65 a	0.66 b	25 b	0.28 c
Pr > F(Cluster)	0.001	0.001	0.005	0.013	0.006	0.000
Significant	Yes	Yes	Yes	Yes	Yes	Yes

For each chemical parameter, means followed by a different letter were significantly different using Tukey's test ($P < 0.05$)

Table A.2 Average proportions of the clones contributing to 8 wine series

Series	828, 923	clone 667	clone 777	10/5	clone 115	UCD 5	UCD 6	Not recorded
B* Classic	0	5	18	9	31	34	3	0
Dror	2	0	40	0	58	0	0	0
Letter	7	0	39	0	8	32	13	0
Rows	2	0	73	0	25	0	0	0
Last Shepherd	0	1	6	5	1	6	12	69
S* Classic	1	0	17	13	13	39	13	3
Latitude	0	30	28	14	9	19	0	0
Rapaura	0	3	60	5	6	20	5	0

*B, Brancott; S, Stoneleigh

Table A.3 ANOVA type III SS analysis for the different clone selections in wines according to the five clusters

Clusters	828, 923	clone 667	clone 777	10/5	clone 115	UCD 5	UCD 6	Not recorded
C1	0.2	6.9 b	38.6 b	2.8 c	11.9	34.7 a	3.6 bc	1.4 b
C5	0.0	35.9 a	19.2 bc	27.3 a	10.6	7.1 b	0.0 c	0.0 b
C3	0.0	0.0 b	10.2 c	15.7 ab	3.3	38.9 a	31.9 a	0.0 b
C2	8.0	0.2 b	67.5 a	0.0 c	22.1	2.2 b	0.0 c	0.0 b
C4	0.0	1.0 b	5.7 c	5.3 bc	1.2	5.8 b	12.2 b	68.8 a
Pr > F(Cluster)	0.415	0.002	0.002	0.006	0.343	<0.0001	<0.0001	<0.0001
Significant	No	Yes	Yes	Yes	No	Yes	Yes	Yes

For each chemical parameter, means followed by a different letter were significantly different using Tukey's test ($P < 0.05$).

References

Addinsoft. (2022). *XLSTAT statistical and data analysis solution* https://www.xlstat.com/en

Alcalde-Eon, C., Escribano-Bailón, M. T., Santos-Buelga, C., & Rivas-Gonzalo, J. C. (2006). Changes in the detailed pigment composition of red wine during maturity and ageing. *Analytica Chimica Acta, 563*(1-2), 238-254. https://doi.org/10.1016/j.aca.2005.11.028

Alegria, M., Jara, M., Cataldo, A., Aguirre, P., Gonzalez, A., & Bordeu, E. (2014). Influence of freezing skin grapes to extract phenolic compounds during red wine maceration. *37th World Congress of Vine and Wine and 12th General Assembly of the Oiv (Pt 1), 3*. https://doi.org/10.1051/bioconf/20140302011

Aleixandre-Tudo, J. L., Buica, A., Nieuwoudt, H., Aleixandre, J. L., & Du Toit, W. (2017). Spectrophotometric Analysis of Phenolic Compounds in Grapes and Wines. *Journal of Agricultural and Food Chemistry, 65*(20), 4009. https://doi.org/10.1021/acs.jafc.7b01724

Aleixandre-Tudo, J. L., & du Toit, W. (2020, Aug 15). A chemometric approach to the evaluation of the ageing ability of red wines. *Chemometrics and Intelligent Laboratory Systems, 203*. https://doi.org/10.1016/j.chemolab.2020.104067

Alvarez, I., Aleixandre, J. L., Garcia, M. J., & Lizama, V. (2006, Mar 23). Impact of prefermentative maceration on the phenolic and volatile compounds in Monastrell red wines. *Analytica Chimica Acta, 563*(1-2), 109-115. https://doi.org/10.1016/j.aca.2005.10.068

Araujo, L. D., Parr, W. V., Grose, C., Hedderley, D., Masters, O., Kilmartin, P. A., & Valentin, D. (2021, Nov). In-mouth attributes driving perceived quality of Pinot noir wines: Sensory and chemical characterisation. *Food Research International, 149*, 110665. https://doi.org/10.1016/j.foodres.2021.110665

Aron, P. M., & Kennedy, J. A. (2007, Jul 11). Compositional investigation of phenolic polymers isolated from Vitis vinifera L. Cv. Pinot noir during fermentation. *Journal of Agricultural and Food Chemistry, 55*(14), 5670-5680. https://doi.org/10.1021/jf0704817

Arts, M. J., Haenen, G. R., Wilms, L. C., Beetstra, S. A., Heijnen, C. G., Voss, H. P., & Bast, A. (2002, Feb 27). Interactions between flavonoids and proteins: effect on the total antioxidant capacity. *J Agric Food Chem, 50*(5), 1184-1187. https://doi.org/10.1021/jf010855a

Asenstorfer, R. E., Lee, D. F., & Jones, G. P. (2006). Influence of structure on the ionisation constants of anthocyanin and anthocyanin-like wine pigments. *Analytica Chimica Acta, 563*(1-2), 10-14. https://doi.org/10.1016/j.aca.2005.09.040

Baiano, A., De Gianni, A., Mentana, A., Quinto, M., Centonze, D., & Del Nobile, M. A. (2016, Oct). Colour-related phenolics, volatile composition, and sensory profile of Nero di Troia wines treated with oak chips or by micro-oxygenation. *European Food Research and Technology, 242*(10), 1631-1646. https://doi.org/10.1007/s00217-016-2662-8

Bavaresco, L. (2003). Role of viticultural factors on stilbene concentrations of grapes and wine. *Drugs Exp Clin Res, 29*(5-6), 181-187.

Berradre, M. N., Sulbaran, B. D., Ojeda, G. J., Fernandez, V. C., Soto, L., & Alcala, J. O. (2020, Jul-Sep). Total phenols content during alcoholics fermentations of pure and mixed cultures of wine

yeasts. *Revista De La Facultad De Agronomia De La Universidad Del Zulia, 37*, 195-200. <Go to ISI>://WOS:000564812900030

Berrueta, L. A., Rasines-Perea, Z., Prieto-Perea, N., Asensio-Regalado, C., Alonso-Salces, R. M., Sanchez-Ilarduya, M. B., & Gallo, B. (2020). Formation and evolution profiles of anthocyanin derivatives and tannins during fermentations and ageing of red wines. *European Food Research & Technology, 246*(1), 149-165. https://doi.org/10.1007/s00217-019-03405-x

Bimpilas, A., Panagopoulou, M., Tsimogiannis, D., & Oreopoulou, V. (2016a, 2016/04/15/). Anthocyanin copigmentation and color of wine: The effect of naturally obtained hydroxycinnamic acids as cofactors. *Food Chemistry, 197*, 39-46. https://doi.org/https://doi.org/10.1016/j.foodchem.2015.10.095

Bimpilas, A., Panagopoulou, M., Tsimogiannis, D., & Oreopoulou, V. (2016b). Anthocyanin copigmentation and color of wine: The effect of naturally obtained hydroxycinnamic acids as cofactors. *Food Chemistry, 197*(Pt A), 39-46. https://doi.org/10.1016/j.foodchem.2015.10.095

Bimpilas, A., Tsimogiannis, D., Balta-Brouma, K., Lymperopoulou, T., & Oreopoulou, V. (2015, 2015/07/01/). Evolution of phenolic compounds and metal content of wine during alcoholic fermentation and storage. *Food Chemistry, 178*, 164-171. https://doi.org/https://doi.org/10.1016/j.foodchem.2015.01.090

Bindon, K. A., McCarthy, M. G., & Smith, P. A. (2014, Dec). Development of wine colour and non-bleachable pigments during the fermentation and ageing of (Vitis vinifera L. cv.) Cabernet Sauvignon wines differing in anthocyanin and tannin concentration. *Lwt-Food Science and Technology, 59*(2), 923-932. https://doi.org/10.1016/j.lwt.2014.05.051

Blanco-Vega, D., Gómez-Alonso, S., & Hermosín-Gutiérrez, I. (2014, 2014/09/01/). Identification, content and distribution of anthocyanins and low molecular weight anthocyanin-derived pigments in Spanish commercial red wines. *Food Chemistry, 158*, 449-458. https://doi.org/https://doi.org/10.1016/j.foodchem.2014.02.154

Blanco-Vega, D., López-Bellido, F. J., Alía-Robledo, J. M., & Hermosín-Gutiérrez, I. (2011, 2011/09/14). HPLC–DAD–ESI-MS/MS Characterization of Pyranoanthocyanins Pigments Formed in Model Wine. *Journal of Agricultural and Food Chemistry, 59*(17), 9523-9531. https://doi.org/10.1021/jf201546j

Bloomfield, D., Heatherbell, D., & Pour Nikfardjam, M. (2003, 01/01). Effect of p-coumaric acid on the color in red wine. *Mitteilungen Klosterneuburg, 53*, 195-198.

Bloomfield, D. G. (2006). Some aspects of colour and phenolic evolution in Vitis vinifera L. cv. Pinot noir wine.

Boido, E., Alcalde-Eon, C., Carrau, F., Dellacassa, E., & Rivas-Gonzalo, J. C. (2006, 2006/09/01). Ageing Effect on the Pigment Composition and Color of Vitis vinifera L. Cv. Tannat Wines. Contribution of the Main Pigment Families to Wine Color. *Journal of Agricultural and Food Chemistry, 54*(18), 6692-6704. https://doi.org/10.1021/jf061240m

Boulton, R. (2001). The Copigmentation of Anthocyanins and Its Role in the Color of Red Wine: A Critical Review. *American Journal of Enology and Viticulture, 52*(2), 67-87.

Božič, J. T., Butinar, L., Albreht, A., Vovk, I., Korte, D., & Vodopivec, B. M. (2020). The impact of Saccharomyces and non-Saccharomyces yeasts on wine colour: A laboratory study of

vinylphenolic pyranoanthocyanin formation and anthocyanin cell wall adsorption. *Food science & technology, 123*, 109072. https://doi.org/10.1016/j.lwt.2020.109072

Burns, T. R., & Osborne, J. P. (2015). Loss of Pinot noir Wine Color and Polymeric Pigment after Malolactic Fermentation and Potential Causes. *American Journal of Enology and Viticulture, 66*(2), 130. https://doi.org/10.5344/ajev.2014.14061

Cadahia, E., Varea, S., Munoz, L., de Simon, B. F., & Garcia-Vallejo, M. C. (2001, Aug). Evolution of ellagitannins in Spanish, French, and American oak woods during natural seasoning and toasting. *Journal of Agricultural and Food Chemistry, 49*(8), 3677-3684. https://doi.org/DOI 10.1021/jf010288r

Campos, F. M., Couto, J. A., & Hogg, T. A. (2003). Influence of phenolic acids on growth and inactivation of Oenococcus oeni and Lactobacillus hilgardii. *J Appl Microbiol, 94*(2), 167-174. https://doi.org/10.1046/j.1365-2672.2003.01801.x

Canas, S., Anjos, O., Caldeira, I., Fernandes, T. A., Santos, N., Lourenco, S., Granja-Soares, J., Fargeton, L., Boissier, B., & Catarino, S. (2022, Jan 15). Micro-oxygenation level as a key to explain the variation in the colour and chemical composition of wine spirits aged with chestnut wood staves. *Lwt-Food Science and Technology, 154*. https://doi.org/10.1016/j.lwt.2021.112658

Carew, A. L., Kerslake, F. L., Bindon, K. A., Smith, P. A., Close, D. C., & Dambergs, R. G. (2020). Viticultural and Controlled Phenolic Release Treatments Affect Phenolic Concentration and Tannin Composition in Pinot noir Wine. *American Journal of Enology and Viticulture, 71*(4), 256. https://doi.org/10.5344/ajev.2020.19003

Carew, A. L., Smith, P., Close, D. C., Curtin, C., & Dambergs, R. G. (2013). Yeast effects on Pinot noir wine phenolics, color, and tannin composition. *Journal of Agricultural and Food Chemistry, 61*(41), 9892. https://doi.org/10.1021/jf4018806

Caridi, A., De Bruno, A., De Salvo, E., Piscopo, A., Poiana, M., & Sidari, R. (2017, Mar). Selected yeasts to enhance phenolic content and quality in red wine from low pigmented grapes. *European Food Research and Technology, 243*(3), 367-378. https://doi.org/10.1007/s00217-016-2750-9

Carrasco-Sanchez, V., Marican, A., Vergara-Jaque, A., Folch-Cano, C., Comer, J., & Laurie, V. F. (2018, Nov 1). Polymeric substances for the removal of ochratoxin A from red wine followed by computational modeling of the complexes formed. *Food Chemistry, 265*, 159-164. https://doi.org/10.1016/j.foodchem.2018.05.089

Casassa, L. F., Beaver, C. W., Mireles, M., Larsen, R. C., Hopfer, H., Heymann, H., & Harbertson, J. F. (2013, Dec). Influence of Fruit Maturity, Maceration Length, and Ethanol Amount on Chemical and Sensory Properties of Merlot Wines. *American Journal of Enology and Viticulture, 64*(4), 437-449. https://doi.org/10.5344/ajev.2013.13059

Casassa, L. F., Bolcato, E. A., Sari, S. E., & Barda, N. (2021, Dec). Effects of maceration length after prefermentative cold soak: Detailed chromatic, phenolic and sensory composition of cabernet sauvignon, malbec and merlot wines. *Journal of Food Composition and Analysis, 104*. https://doi.org/10.1016/j.jfca.2021.104168

Casassa, L. F., & Harbertson, J. F. (2014). Extraction, Evolution, and Sensory Impact of Phenolic Compounds During Red Wine Maceration. *Annual Review of Food Science and Technology, Vol 5, 5*, 83-109. https://doi.org/10.1146/annurev-food-030713-092438

Casassa, L. F., Huff, R., & Steele, N. B. (2019, Dec 1). Chemical consequences of extended maceration and post-fermentation additions of grape pomace in Pinot noir and Zinfandel wines from the Central Coast of California (USA). *Food Chemistry, 300*. https://doi.org/10.1016/j.foodchem.2019.125147

Casassa, L. F., Keller, M., & Harbertson, J. F. (2015, May). Regulated Deficit Irrigation Alters Anthocyanins, Tannins and Sensory Properties of Cabernet Sauvignon Grapes and Wines. *Molecules, 20*(5), 7820-7844. https://doi.org/10.3390/molecules20057820

Casassa, L. F., Larsen, R. C., & Harbertson, J. F. (2016, Jul). Effects of Vineyard and Winemaking Practices Impacting Berry Size on Evolution of Phenolics during Winemaking. *American Journal of Enology and Viticulture, 67*(3), 257-268. https://doi.org/10.5344/ajev.2016.15105

Casassa, L. F., Vega-Osorno, A. A., & Hernandez, J. P. (2021, Oct). Chemical and chromatic effects of saignee combined with extended maceration and microwaved stem addition on three Pinot Noir clones from the Central Coast of California. *Australian Journal of Grape and Wine Research, 27*(4), 540-552. https://doi.org/10.1111/ajgw.12511

Castellari, M., Matricardi, L., Arfelli, G., Galassi, S., & Amati, A. (2000, Apr). Level of single bioactive phenolics in red wine as a function of the oxygen supplied during storage. *Food Chemistry, 69*(1), 61-67. https://doi.org/Doi 10.1016/S0308-8146(99)00240-X

Catania, A., Lerno, L., Sari, S., Fanzone, M., Casassa, F., & Oberholster, A. (2021, Sep). Impact of micro-oxygenation timing and rate of addition on color stabilization and chromatic characteristics of cabernet sauvignon wines. *Lwt-Food Science and Technology, 149*. https://doi.org/10.1016/j.lwt.2021.111776

Cheynier, V. (2000). Tannins in grape and grape products. *Tannins in Livestock and Human Nutrition, Proceedings*(92), 90-94. <Go to ISI>://WOS:000180710800017

Cheynier, V. (2012, Jun). Phenolic compounds: from plants to foods. *Phytochemistry Reviews, 11*(2-3), 153-177. https://doi.org/10.1007/s11101-012-9242-8

Cheynier, V., Duenas-Paton, M., Salas, E., Maury, C., Souquet, J. M., Sarni-Manchado, P., & Fulcrand, H. (2006). Structure and properties of wine pigments and tannins. *American Journal of Enology and Viticulture, 57*(3), 298-305. <Go to ISI>://WOS:000240934100007

Chira, K., Jourdes, M., & Teissedre, P. L. (2011). Cabernet sauvignon red wine astringency quality control by tannin characterization and polymerization during storage. *European Food Research & Technology, 234*(2), 253-261. https://doi.org/10.1007/s00217-011-1627-1

Chira, K., Pacella, N., Jourdes, M., & Teissedre, P. L. (2011). Chemical and sensory evaluation of Bordeaux wines (Cabernet-Sauvignon and Merlot) and correlation with wine age. *Food Chem, 126*(4), 1971-1977. https://doi.org/10.1016/j.foodchem.2010.12.056

Choi, K. O., Lee, D. H., Park, S. J., Im, D., & Hur, Y. Y. (2020, Nov). Correlations Between Phenolic Composition and Perceived Astringency of Wines. *Applied Sciences-Basel, 10*(22). https://doi.org/10.3390/app10228020

Cliff, M. A., King, M. C., & Schlosser, J. (2007). Anthocyanin, phenolic composition, colour measurement and sensory analysis of BC commercial red wines. *Food Research International, 40*(1), 92-100. https://doi.org/10.1016/j.foodres.2006.08.002

Cojocaru, G. A., & Antoce, A. O. (2017). Influence of Fermentor Type on Polyphenol Extraction in Red Wines Produced from Cabernet Sauvignon. *Scientific Papers-Series B-Horticulture, 61*, 249-256. <Go to ISI>://WOS:000414551900036

Cooper, M. (2022). *Michael cooper wines*. Retrieved July 5, 2022 from https://michaelcooperwines.com/

Cortell, J. M., Halbleib, M., Gallagher, A. V., Righetti, T. L., & Kennedy, J. A. (2005, Jul 13). Influence of vine vigor on grape (Vitis vinifera L. Cv. Pinot Noir) and wine proanthocyanidins. *J Agric Food Chem, 53*(14), 5798-5808. https://doi.org/10.1021/jf0504770

Cruz, L., Brás, N. F., Teixeira, N., Mateus, N., Ramos, M. J., Dangles, O., & De Freitas, V. (2010, 2010/03/10). Vinylcatechin Dimers Are Much Better Copigments for Anthocyanins than Catechin Dimer Procyanidin B3. *Journal of Agricultural and Food Chemistry, 58*(5), 3159-3166. https://doi.org/10.1021/jf9037419

Cucciniello, R., Forino, M., Picariello, L., Coppola, F., Moio, L., & Gambuti, A. (2021, Dec). How acetaldehyde reacts with low molecular weight phenolics in white and red wines. *European Food Research and Technology, 247*(12), 2935-2944. https://doi.org/10.1007/s00217-021-03841-8

de Beer, P., Strever, A., & du Toit, W. J. (2017). Do Differences in the Colour and Phenolic Composition of Young Shiraz Wines Reflect During Ageing? *South African Journal of Enology and Viticulture, 38*(1), 29-34. <Go to ISI>://WOS:000407770400004

de Freitas, V., & Mateus, N. (2011a, 2011/09/01). Formation of pyranoanthocyanins in red wines: a new and diverse class of anthocyanin derivatives. *Analytical and Bioanalytical Chemistry, 401*(5), 1463-1473. https://doi.org/10.1007/s00216-010-4479-9

de Freitas, V., & Mateus, N. (2011b). Formation of pyranoanthocyanins in red wines: a new and diverse class of anthocyanin derivatives : Wine Analysis. *Analytical and Bioanalytical Chemistry, 401*(5), 1463-1473.

de Freitas, V., Sousa, C., Silva, A. M. S., Santos-Buelga, C., & Mateus, N. (2004, Dec 13). Synthesis of a new catechin-pyrylium derived pigment. *Tetrahedron Letters, 45*(51), 9349-9352. https://doi.org/10.1016/j.tetlet.2004.10.132

del Alamo, M., Casado, L., Hernandez, V., & Jimenez, J. J. (2004, Sep 17). Determination of free molecular phenolics and catechins in wine by solid phase extraction on polymeric cartridges and liquid chromatography with diode array detection. *Journal of Chromatography A, 1049*(1-2), 97-105. https://doi.org/10.1016/j.chroma.2004.08.017

Del Fresno, J. M., Morata, A., Loira, I., Escott, C., & Lepe, J. A. S. (2020, Apr 7). Evolution of the Phenolic Fraction and Aromatic Profile of Red Wines Aged in Oak Barrels. *Acs Omega, 5*(13), 7235-7243. https://doi.org/10.1021/acsomega.9b03854

del Rio, J. L. P., & Kennedy, J. A. (2006). Development of Proanthocyanidins in Vitis vinifera L. cv. Pinot noir Grapes and Extraction into Wine. *American Journal of Enology and Viticulture, 57*(2), 125-132.

Delgado-Povedano, M. D., de Villiers, A., Hann, S., & Causon, T. (2021, Feb). Identity confirmation of anthocyanins in berries by LC-DAD-IM-QTOFMS. *Electrophoresis, 42*(4), 473-481. https://doi.org/10.1002/elps.202000274

Downey, M. O., Dokoozlian, N. K., & Krstic, M. P. (2006). Cultural Practice and Environmental Impacts on the Flavonoid Composition of Grapes and Wine: A Review of Recent Research. *American Journal of Enology and Viticulture, 57*(3), 257-268. https://www.ajevonline.org/content/ajev/57/3/257.full.pdf

Downey, M. O., Harvey, J. S., & Robinson, S. P. (2004). The effect of bunch shading on berry development and flavonoid accumulation in Shiraz grapes. *Australian Journal of Grape and Wine Research, 10*(1), 55-73. https://doi.org/10.1111/j.1755-0238.2004.tb00008.x

Drinkine, J., Glories, Y., & Saucier, C. (2005, 2005/09//). (+)-Catechin-aldehyde condensations: competition between acetaldehyde and glyoxylic acid. *Journal of Agricultural and Food Chemistry, 53*(19), 7552-7558. https://doi.org/10.1021/jf0504723

Drinkine, J., Lopes, P., Kennedy, J. A., Teissedre, P. L., & Saucier, C. (2007a, Feb 21). Analysis of ethylidene-bridged flavan-3-ols in wine. *J Agric Food Chem, 55*(4), 1109-1116. https://doi.org/10.1021/jf0626258

Drinkine, J., Lopes, P., Kennedy, J. A., Teissedre, P. L., & Saucier, C. (2007b, Jul 25). Ethylidene-bridged Flavan-3-ols in red wine and correlation with wine age. *J Agric Food Chem, 55*(15), 6292-6299. https://doi.org/10.1021/jf070038w

Dueñas, M., Fulcrand, H., & Cheynier, V. (2006, 2006/03/23/). Formation of anthocyanin–flavanol adducts in model solutions. *Analytica Chimica Acta, 563*(1), 15-25. https://doi.org/https://doi.org/10.1016/j.aca.2005.10.062

Duley, G., Dujourdy, L., Klein, S., Werwein, A., Spartz, C., Gougeon, R. D., & Taylor, D. K. (2021, Mar 15). Regionality in Australian Pinot noir wines: A study on the use of NMR and ICP-MS on commercial wines. *Food Chemistry, 340*. https://doi.org/10.1016/j.foodchem.2020.127906

Durner, D., Nickolaus, P., Weber, F., Trieu, H. L., & Fischer, U. (2015). Evolution of Anthocyanin-Derived Compounds during Micro-Oxygenation of Red Wines with Different Anthocyanin-Flavanol Ratios. *Advances in Wine Research, 1203*, 253-274. <Go to ISI>://WOS:000371199300016

Engström, M. T., Pälijärvi, M., Fryganas, C., Grabber, J. H., Mueller-Harvey, I., & Salminen, J.-P. (2014, 2014/04/16). Rapid Qualitative and Quantitative Analyses of Proanthocyanidin Oligomers and Polymers by UPLC-MS/MS. *Journal of Agricultural and Food Chemistry, 62*(15), 3390-3399. https://doi.org/10.1021/jf500745y

Engström, M. T., Pälijärvi, M., & Salminen, J.-P. (2015, 2015/04/29). Rapid Fingerprint Analysis of Plant Extracts for Ellagitannins, Gallic Acid, and Quinic Acid Derivatives and Quercetin-, Kaempferol- and Myricetin-Based Flavonol Glycosides by UPLC-QqQ-MS/MS. *Journal of Agricultural and Food Chemistry, 63*(16), 4068-4079. https://doi.org/10.1021/acs.jafc.5b00595

Es-Safi, N.-E., Fulcrand, H., Cheynier, V., & Moutounet, M. (1999). Studies on the acetaldehyde-induced condensation of (-)-epicatechin and malvidin 3-O-glucoside in a model solution system. *Journal of Agricultural and Food Chemistry, 47*(5), 2096-2102. https://doi.org/10.1021/jf9806309

Escott, C., Del Fresno, J. M., Loira, I., Morata, A., Tesfaye, W., González, M. D. C., & Suárez-Lepe, J. A. (2018). Formation of polymeric pigments in red wines through sequential fermentation of flavanol-enriched musts with non-Saccharomyces yeasts. *Food Chemistry, 239*, 975-983. https://doi.org/10.1016/j.foodchem.2017.07.037

Escott, C., Morata, A., Loira, I., Tesfaye, W., & Suarez‐Lepe, J. A. (2016). Characterization of polymeric pigments and pyranoanthocyanins formed in microfermentations of non‐Saccharomyces

yeasts. *Journal of Applied Microbiology, 121*(5), 1346-1356. https://doi.org/10.1111/jam.13255

Escribano-Bailón, T., Álvarez-García, M., Rivas-Gonzalo, J. C., Heredia, F. J., & Santos-Buelga, C. (2001). Color and stability of pigments derived from the acetaldehyde-mediated condensation between malvidin 3-O-glucoside and (+)-catechin. *Journal of Agricultural and Food Chemistry, 49 3*, 1213-1217.

Escribano-Viana, R., Gonzalez-Arenzana, L., Garijo, P., Lopez, R., Santamaria, P., & Gutierrez, A. R. (2021, Sep). Selection Process of a Mixed Inoculum of Non-Saccharomyces Yeasts Isolated in the DOCa. Rioja. *Fermentation-Basel, 7*(3). https://doi.org/10.3390/fermentation7030148

Farquhar, D., & Clingleffer, P. (2001). Evaluation of the quality performance of the clones of Pinot Noir. 11th Australian Wine Industry Technical Conference.

Ferreira da Silva, P., Lima, J. C., Freitas, A. A., Shimizu, K., Maçanita, A. L., & Quina, F. H. (2005, 2005/08/01). Charge-Transfer Complexation as a General Phenomenon in the Copigmentation of Anthocyanins. *The Journal of Physical Chemistry A, 109*(32), 7329-7338. https://doi.org/10.1021/jp052106s

Ferrer-Gallego, R., Goncalves, R., Rivas-Gonzalo, J. C., Escribano-Bailon, M. T., & de Freitas, V. (2012, Nov 15). Interaction of phenolic compounds with bovine serum albumin (BSA) and alpha-amylase and their relationship to astringency perception. *Food Chemistry, 135*(2), 651-658. https://doi.org/10.1016/j.foodchem.2012.04.123

Forino, M., Picariello, L., Lopatriello, A., Moio, L., & Gambuti, A. (2020). New insights into the chemical bases of wine color evolution and stability: the key role of acetaldehyde. *European Food Research & Technology, 246*(4), 733-743. https://doi.org/10.1007/s00217-020-03442-x

Frey, A. S. (2015). *LC/MS method development for the separation of anthocyanins and anthocyanin-derived pigments in red wines* Lincoln University].

Fu, L. H., Zheng, L., & Yan, X. (2015). Contribution of Monomeric Anthocyanins to the Color of Young Red Wine: Statistical and Experimental Approaches. *Journal of Food Science, 80*(12), C2751-C2758. https://doi.org/10.1111/1750-3841.13155

Fulcrand, H., Benabdeljalil, C., Rigaud, J., Cheynier, V., & Moutounet, M. (1998). A new class of wine pigments generated by reaction between pyruvic acid and grape anthocyanins. *Phytochemistry, 47*(7), 1401-1407. https://doi.org/10.1016/S0031-9422(97)00772-3

Fulcrand, H., & Cheynier, V. (2004, Aug 22). Overview of wine tannin structures and their formation pathways. *Abstracts of Papers of the American Chemical Society, 228*, U245-U245. <Go to ISI>://WOS:000223712801108

Fulcrand, H., Doco, T., Es-Safi, N.-E., Cheynier, V., & Moutounet, M. (1996). Study of the acetaldehyde induced polymerisation of flavan-3-ols by liquid chromatography-ion spray mass spectrometry. *Journal of Chromatography A, 752*(1), 85-91. https://doi.org/10.1016/S0021-9673(96)00485-2

Fulcrand, H., Duenas, M., Salas, E., & Cheynier, V. (2006). Phenolic reactions during winemaking and ageing. *American Journal of Enology and Viticulture, 57*(3), 289-297. <Go to ISI>://WOS:000240934100006

Galland, S., Mora, N., Abert-Vian, M., Rakotomanomana, N., & Dangles, O. (2007, 2007/09/01). Chemical Synthesis of Hydroxycinnamic Acid Glucosides and Evaluation of Their Ability To Stabilize Natural Colors via Anthocyanin Copigmentation. *Journal of Agricultural and Food Chemistry, 55*(18), 7573-7579. https://doi.org/10.1021/jf071205v

Gambacorta, G., Antonacci, D., Pati, S., la Gatta, M., Faccia, M., Coletta, A., & La Notte, E. (2011, 2011/12/01). Influence of winemaking technologies on phenolic composition of Italian red wines. *European Food Research and Technology, 233*(6), 1057-1066. https://doi.org/10.1007/s00217-011-1613-7

Gambuti, A., Picariello, L., Rinaldi, A., & Moio, L. (2018, Mar 15). Evolution of Sangiovese Wines With Varied Tannin and Anthocyanin Ratios During Oxidative Ageing. *Frontiers in Chemistry, 6*. https://doi.org/10.3389/fchem.2018.00063

García-Ruiz, A., Bartolomé, B., Martínez-Rodríguez, A. J., Pueyo, E., Martín-Álvarez, P. J., & Moreno-Arribas, M. V. (2008). Potential of phenolic compounds for controlling lactic acid bacteria growth in wine. *Food Control, 19*(9), 835-841. https://doi.org/10.1016/j.foodcont.2007.08.018

Geldenhuys, L., Oberholster, A., & du Toit, W. J. (2012). Monitoring the Effect of Micro-oxygenation before Malolactic Fermentation on South African Pinotage Red Wine with Different Colour and Phenolic Analyses. *South African Journal of Enology and Viticulture, 33*(2), 150-160. <Go to ISI>://WOS:000317941900003

Gómez-Alonso, S., García-Romero, E., & Hermosín-Gutiérrez, I. (2007). HPLC analysis of diverse grape and wine phenolics using direct injection and multidetection by DAD and fluorescence. *Journal of Food Composition and Analysis $V 20*(7), 618-626.

Gomez-Plaza, E., Gil-Munoz, R., Lopez-Roca, J. M., Martinez-Cutillas, A., & Fernandez-Fernandez, J. I. (2001). Phenolic compounds and color stability of red wines: Effect of skin maceration time. *American Journal of Enology and Viticulture, 52*(3), 266-270. <Go to ISI>://WOS:000172980100016

Gomez-Plaza, E., Olmos, O., & Bautista-Ortin, A. B. (2016, Aug 1). Tannin profile of different Monastrell wines and its relation to projected market prices. *Food Chemistry, 204*, 506-512. https://doi.org/10.1016/j.foodchem.2016.02.124

Gómez-Plaza, E., Olmos, O., & Bautista-Ortín, A. B. (2016). Tannin profile of different Monastrell wines and its relation to projected market prices. *Food Chemistry, 204*, 506-512. https://doi.org/10.1016/j.foodchem.2016.02.124

González-Manzano, S., Dueñas, M., Rivas-Gonzalo, J. C., Escribano-Bailón, M. T., & Santos-Buelga, C. (2009, 2009/05/15/). Studies on the copigmentation between anthocyanins and flavan-3-ols and their influence in the colour expression of red wine. *Food Chemistry, 114*(2), 649-656. https://doi.org/https://doi.org/10.1016/j.foodchem.2008.10.002

González-Muñoz, B., Garrido-Vargas, F., Pavez, C., Osorio, F., Chen, J., Bordeu, E., O'Brien, J. A., & Brossard, N. (2022). Wine astringency: more than just tannin–protein interactions. *Journal of the Science of Food and Agriculture, 102*(5), 1771-1781. https://doi.org/https://doi.org/10.1002/jsfa.11672

González-Neves, G., Gil, G., Favre, G., Baldi, C., Hernández, N., & Traverso, S. (2016, 11/01/). Influence of Winemaking Procedure and Grape Variety on the Colour and Composition of Young Red Wines. *South African Journal of Enology and Viticulture; Vol 34, No 1 (2013)DO - 10.21548/34-1-1089*. http://www.journals.ac.za/index.php/sajev/article/view/1089/398

Gordillo, B., Rivero, F. J., Jara-Palacios, M. J., Gonzalez-Miret, M. L., & Heredia, F. J. (2021, Jun 1). Impact of a double post-fermentative maceration with ripe and overripe seeds on the phenolic composition and color stability of Syrah red wines from warm climate. *Food Chemistry, 346*. https://doi.org/10.1016/j.foodchem.2020.128919

Grabber, J. H., Zeller, W. E., & Mueller-Harvey, I. (2013, Mar 20). Acetone enhances the direct analysis of procyanidin- and prodelphinidin-based condensed tannins in lotus species by the butanol-HCl-iron assay. *J Agric Food Chem, 61*(11), 2669-2678. https://doi.org/10.1021/jf304158m

Grainger, K. (2009). Wine quality: tasting and selection (Vol. 8). John Wiley & Sons.

Guadalupe, Z., Soldevilla, A., Saenz-Navajas, M. P., & Ayestaran, B. (2006, Apr 21). Analysis of polymeric phenolics in red wines using different techniques combined with gel permeation chromatography fractionation. *Journal of Chromatography A, 1112*(1-2), 112-120. https://doi.org/10.1016/j.chroma.2005.11.100

Guaita, M., & Bosso, A. (2019). Polyphenolic characterization of grape skins and seeds of four Italian red cultivars at harvest and after fermentative maceration. *Foods, 8*(9), 395. https://doi.org/10.3390/foods8090395

Gutierrez-Escobar, R., Aliano-Gonzalez, M. J., & Cantos-Villar, E. (2021, Feb). Wine Polyphenol Content and Its Influence on Wine Quality and Properties: A Review. *Molecules, 26*(3). https://doi.org/10.3390/molecules26030718

Han, G., Dai, L., Sun, Y., Li, C., Ruan, S., Li, J., & Xu, Y. (2021, 2021/11/01/). Determina tion of the age of dry red wine by multivariate techniques using color parameters and pigments. *Food Control, 129*, 108253. https://doi.org/https://doi.org/10.1016/j.foodcont.2021.108253

Harbertson, J. F., Kennedy, J. A., & Adams, D. O. (2002). Tannin in Skins and Seeds of Cabernet Sauvignon, Syrah, and Pinot noir Berries during Ripening. *American Journal of Enology and Viticulture, 53*(1), 54. http://www.ajevonline.org/content/53/1/54.abstract

Harrison, R. (2018). Practical interventions that influence the sensory attributes of red wines related to the phenolic composition of grapes: a review. *International Journal of Food Science & Technology, 53*(1), 3-18. https://doi.org/doi:10.1111/ijfs.13480

Hayasaka, Y., & Kennedy, J. A. (2003). Mass spectrometric evidence for the formation of pigmented polymers in red wine. *Australian Journal of Grape and Wine Research, 9*(3), 210-220. https://doi.org/10.1111/j.1755-0238.2003.tb00272.x

He, F., Liang, N.-N., Mu, L., Pan, Q.-H., Wang, J., Reeves, M. J., & Duan, C.-Q. (2012). Anthocyanins and their variation in red wines II. Anthocyanin derived pigments and their color evolution. *Molecules, 17*(2), 1483-1519. https://doi.org/10.3390/molecules17021483

He, F., Pan, Q.-H., Shi, Y., & Duan, C.-Q. (2008). Chemical synthesis of proanthocyanidins in vitro and their reactions in ageing wines. *Molecules, 13*(12), 3007-3032.

He, J., Oliveira, J., Silva, A. M., Mateus, N., & De Freitas, V. (2010, Aug 11). Oxovitisins: a new class of neutral pyranone-anthocyanin derivatives in red wines. *J Agric Food Chem, 58*(15), 8814-8819. https://doi.org/10.1021/jf101408q

Heras-Roger, J., Alonso-Alonso, O., Gallo-Montesdeoca, A., Diaz-Romero, C., & Darias-Martin, J. (2016, Jun). Influence of copigmentation and phenolic composition on wine color. *Journal of Food*

Science and Technology-Mysore, 53(6), 2540-2547. https://doi.org/10.1007/s13197-016-2210-3

Herderich, M. J., & Smith, P. A. (2008, 2005/07/01). Analysis of grape and wine tannins: Methods, applications and challenges. *Australian Journal of Grape and Wine Research, 11*(2), 205-214. https://doi.org/10.1111/j.1755-0238.2005.tb00288.x

Hermosín‐Gutiérrez, I., Gómez‐Alonso, S., Pérez‐Navarro, J., Kurt, A., Colak, N., Akpınar, E., Hayirlioglu‐Ayaz, S., & Ayaz, F. A. (2020). Vitis vinifera Turkish grape cultivar Karaerik. Part I: anthocyanin composition, and identification of a newly found anthocyanin. *J Sci Food Agric, 100*(3), 1301-1310. https://doi.org/10.1002/jsfa.10145

Hoshino, T., Matsumoto, U., Goto, T., & Harada, N. (1982, 1982/01/01/). Evidence for the self-association of anthocyanins IV. PMR spectroscopic evidence for the vertical stacking of anthocyanin molecules. *Tetrahedron Letters, 23*(4), 433-436. https://doi.org/https://doi.org/10.1016/S0040-4039(00)86852-5

Huang, R., & Xu, C. M. (2021, Jan). An overview of the perception and mitigation of astringency associated with phenolic compounds. *Comprehensive Reviews in Food Science and Food Safety, 20*(1), 1036-1074. https://doi.org/10.1111/1541-4337.12679

Hufnagel, J. C., & Hofmann, T. (2008, Feb 27). Orosensory-directed identification of astringent mouthfeel and bitter-tasting compounds in red wine. *Journal of Agricultural and Food Chemistry, 56*(4), 1376-1386. https://doi.org/10.1021/jfO73031n

Jeandet, P., Bessis, R., Sbaghi, M., Meunier, P., & Trollat, P. (1995). Resveratrol Content of Wines of Different Ages: Relationship With Fungal Disease Pressure in the Vineyard. *American Journal of Enology and Viticulture, 46*(1), 1-4. https://www.ajevonline.org/content/ajev/46/1/1.full.pdf

Jeffery, D. W., Mercurio, M. D., Herderich, M. J., Hayasaka, Y., & Smith, P. A. (2008, 2008/04/01). Rapid Isolation of Red Wine Polymeric Polyphenols by Solid-Phase Extraction. *Journal of Agricultural and Food Chemistry, 56*(8), 2571-2580. https://doi.org/10.1021/jf073478w

Jiang, X. H., Lu, Y. Y., & Liu, S. Q. (2020, Mar). Effects of Different Yeasts on Physicochemical and Oenological Properties of Red Dragon Fruit Wine Fermented with Saccharomyces cerevisiae, Torulaspora delbrueckii and Lachancea thermotolerans. *Microorganisms, 8*(3). https://doi.org/10.3390/microorganisms8030315

Jorgensen, E. M., Marin, A. B., & Kennedy, J. A. (2004). Analysis of the Oxidative Degradation of Proanthocyanidins under Basic Conditions. *J. Agric. Food Chem, 52*(8), 2292-2296. https://doi.org/10.1021/jf035311i

Ju, Y. L., Yang, Yue, X. F., He, R., Deng, S. L., Yang, X., Liu, X., & Fang, Y. L. (2021, Oct 30). The condensed tannin chemistry and astringency properties of fifteen Vitis davidii Foex grapes and wines. *Food Chemistry-X, 11*, 100125. https://doi.org/10.1016/j.fochx.2021.100125

Karaoğlan, S. N. Y., Çelik, Z. D., Darici, M., Kelebek, H., Erten, H., Işçi, B., Altindişli, A., & Cabaroglu, T. (2015). Effect of terroir on the phenolic compounds of Muscat of Bornova Wines from 3 different sub-regions of Aegean, Turkey. *BIO Web of Conferences, 5*, 2017. https://doi.org/10.1051/bioconf/20150502017

Kassara, S., & Kennedy, J. A. (2011, 2011/08//). Relationship between red wine grade and phenolics. 2. Tannin composition and size. *Journal of Agricultural and Food Chemistry, 59*(15), 8409-8412. https://doi.org/10.1021/jf201054p

Kennedy, J. A. (2008). Grape and wine phenolics: Observations and recent findings. *Ciencia e investigación agraria, 35*(2), 107-120. https://doi.org/10.4067/S0718-16202008000200001

Kennedy, J. A., & Jones, G. P. (2001, 2001/04/01). Analysis of Proanthocyanidin Cleavage Products Following Acid-Catalysis in the Presence of Excess Phloroglucinol. *Journal of Agricultural and Food Chemistry, 49*(4), 1740-1746. https://doi.org/10.1021/jf001030o

Kennedy, J. A., & Taylor, A. W. (2003, 2003/05/02/). Analysis of proanthocyanidins by high-performance gel permeation chromatography. *Journal of Chromatography A, 995*(1), 99-107. https://doi.org/https://doi.org/10.1016/S0021-9673(03)00420-5

Knight, S., Klaere, S., Fedrizzi, B., & Goddard, M. R. (2015). Regional microbial signatures positively correlate with differential wine phenotypes: Evidence for a microbial aspect to terroir. *Sci Rep, 5*(1), 14233-14233. https://doi.org/10.1038/srep14233

Kyraleou, M., Kallithraka, S., Theodorou, N., Teissedre, P.-L., Kotseridis, Y., & Koundouras, S. (2017). Changes in tannin composition of syrah grape skins and seeds during fruit ripening under contrasting water conditions. *Molecules, 22*(9), 1453-1418. https://doi.org/10.3390/molecules22091453

Lai, P. F. H., Hsu, P.-C., Liou, B.-K., Divate, R. D., Wang, P.-M., & Chung, Y.-C. (2020). Improved phenolic compositions and sensory attributes of red wines by saccharomyces cerevisiae mutant cm8 overproducing cell-wall mannoproteins. *Processes, 8*(11), 1-13. https://doi.org/10.3390/pr8111483

Laitila, J. E. (2021, Mar 15). Composition and evolution of oligomeric proanthocyanidin-malvidin glycoside adducts in commercial red wines. *Food Chem, 340*, 127905. https://doi.org/10.1016/j.foodchem.2020.127905

Laitila, J. E., & Salminen, J.-P. (2020, 2020/03/18). Relevance of the Concentrations and Sizes of Oligomeric Red Wine Pigments to the Color Intensity of Commercial Red Wines. *Journal of Agricultural and Food Chemistry, 68*(11), 3576-3584. https://doi.org/10.1021/acs.jafc.9b07941

Laitila, J. E., Suvanto, J., & Salminen, J. P. (2019, Oct 1). Liquid chromatography-tandem mass spectrometry reveals detailed chromatographic fingerprints of anthocyanins and anthocyanin adducts in red wine. *Food Chem, 294*, 138-151. https://doi.org/10.1016/j.foodchem.2019.02.136

Lambert, S. G., Asenstorfer, R. E., Williamson, N. M., Iland, P. G., & Jones, G. P. (2011). Copigmentation between malvidin-3-glucoside and some wine constituents and its importance to colour expression in red wine. *Food Chemistry, 125*(1), 106-115. https://doi.org/10.1016/j.foodchem.2010.08.045

Lampir, L., & Pavlousek, P. (2013, 01/01). Influence of Locality on Content of Phenolic Compounds in White Wines. *Czech Journal of Food Sciences, 31*, 619-626. https://doi.org/10.17221/337/2013-CJFS

Langsrud, Ø. (2003, 04/01). ANOVA for unbalanced data: Use type II instead of type III sums of squares. *Statistics and Computing, 13*, 163-167. https://doi.org/10.1023/A:1023260610025

Lee, D. F., Swinny, E. E., & Jones, G. P. (2004). NMR identification of ethyl-linked anthocyanin–flavanol pigments formed in model wine ferments. *Tetrahedron Letters, 45*(8), 1671-1674. https://doi.org/10.1016/j.tetlet.2003.12.110

Lemut, M. S., Trost, K., Sivilotti, P., & Vrhovsek, U. (2011). Pinot Noir grape colour related phenolics as affected by leaf removal treatments in the Vipava Valley. *Journal of Food Composition and Analysis, 24*(6), 777-784. https://doi.org/10.1016/j.jfca.2011.03.003

Leydet, Y., Gavara, R., Petrov, V., Diniz, A. M., Jorge Parola, A., Lima, J. C., & Pina, F. (2012, Nov). The effect of self-aggregation on the determination of the kinetic and thermodynamic constants of the network of chemical reactions in 3-glucoside anthocyanins. *Phytochemistry, 83*, 125-135. https://doi.org/10.1016/j.phytochem.2012.06.022

Li, S.-Y., & Duan, C.-Q. (2019, 2019/07/04). Astringency, bitterness and color changes in dry red wines before and during oak barrel ageing: An updated phenolic perspective review. *Critical Reviews in Food Science and Nutrition, 59*(12), 1840-1867. https://doi.org/10.1080/10408398.2018.1431762

Li, S.-Y., Liu, P.-T., Pan, Q.-H., Shi, Y., & Duan, C.-Q. (2015). Association Between Modification of Phenolic Profiling and Development of Wine Color during Alcohol Fermentation. *Journal of Food Science, 80*(4), C703-C710. https://doi.org/https://doi.org/10.1111/1750-3841.12833

Li, Z., Pan, Q. H., Jin, Z. M., He, J. J., Liang, N. N., & Duan, C. Q. (2009, Aug). Evolution of 49 Phenolic Compounds in Shortly-aged Red Wines Made from Cabernet Gernischt (Vitis vinifera L. cv.). *Food Science and Biotechnology, 18*(4), 1001-1012. <Go to ISI>://WOS:000269492000030

Longo, R., Pearson, W., Merry, A., Solomon, M., Nicolotti, L., Westmore, H., Dambergs, R., & Kerslake, F. (2020, Sep). Preliminary Study of Australian Pinot Noir Wines by Colour and Volatile Analyses, and the Pivot (c) Profile Method Using Wine Professionals. *Foods, 9*(9). https://doi.org/10.3390/foods9091142

Lorenzo, C., Pardo, F., Zalacain, A., Alonso, G. L., & Salinas, M. R. (2005, 2005/09/01). Effect of Red Grapes Co-winemaking in Polyphenols and Color of Wines. *Journal of Agricultural and Food Chemistry, 53*(19), 7609-7616. https://doi.org/10.1021/jf050848c

Lorrain, B., Ky, I., Pechamat, L., & Teissedre, P.-L. (2013). Evolution of Analysis of Polyhenols from Grapes, Wines, and Extracts. *Molecules, 18*(1). https://doi.org/10.3390/molecules18011076

Manso-Martinez, C., Saenz-Navajas, M. P., Menendez, C. M., & Hernandez, M. M. (2021, Jul). Wine quality and berry size: a case study with Tempranillo Tinto progenies. *Journal of the Science of Food and Agriculture, 101*(9), 3952-3960. https://doi.org/10.1002/jsfa.11035

Mateus, N., Oliveira, J., Haettich-Motta, M., & de Freitas, V. (2004, 1900/01/01). New Family of Bluish Pyranoanthocyanins. *Journal of Biomedicine and Biotechnology, 2004*, 860752. https://doi.org/10.1155/S1110724304404033

Mattivi, F., Vrhovsek, U., Masuero, D., & Trainotti, D. (2009). Differences in the amount and structure of extractable skin and seed tannins amongst red grape varieties. *Australian Journal of Grape and Wine Research, 15*(1), 27-35. https://doi.org/10.1111/j.1755-0238.2008.00027.x

Maza, M. A., Pereira, C., Martinez, J. M., Camargo, A., Alvarez, I., & Raso, J. (2020, Jul). PEF treatments of high specific energy permit the reduction of maceration time during vinification of Caladoc

and Grenache grapes. *Innovative Food Science & Emerging Technologies, 63*. https://doi.org/10.1016/j.ifset.2020.102375

McRae, J. M., Dambergs, R. G., Kassara, S., Parker, M., Jeffery, D. W., Herderich, M. J., & Smith, P. A. (2012, Oct 10). Phenolic Compositions of 50 and 30 Year Sequences of Australian Red Wines: The Impact of Wine Age. *Journal of Agricultural and Food Chemistry, 60*(40), 10093-10102. https://doi.org/10.1021/jf301571q

McRae, J. M., & Kennedy, J. A. (2011). Wine and grape tannin interactions with salivary proteins and their impact on astringency: A review of current research. *Molecules, 16*(3), 2348-2364. https://doi.org/10.3390/molecules16032348

McRae, J. M., Kirby, N., Mertens, H. D. T., Kassara, S., & Smith, P. A. (2014). Measuring the molecular dimensions of wine tannins: comparison of small-angle X-ray scattering, gel-permeation chromatography and mean degree of polymerization. *Journal of Agricultural and Food Chemistry, 62*(29), 7216. https://doi.org/10.1021/jf405308m

McRae, J. M., Schulkin, A., Kassara, S., Holt, H. E., & Smith, P. A. (2013, 2013/01/23). Sensory Properties of Wine Tannin Fractions: Implications for In-Mouth Sensory Properties. *Journal of Agricultural and Food Chemistry, 61*(3), 719-727. https://doi.org/10.1021/jf304239n

Mercurio, M. D., Dambergs, R. G., Herderich, M. J., & Smith, P. A. (2007, 2007/06/01). High Throughput Analysis of Red Wine and Grape PhenolicsAdaptation and Validation of Methyl Cellulose Precipitable Tannin Assay and Modified Somers Color Assay to a Rapid 96 Well Plate Format. *Journal of Agricultural and Food Chemistry, 55*(12), 4651-4657. https://doi.org/10.1021/jf063674n

Merkyte, V., Longo, E., Windisch, G., & Boselli, E. (2020, Dec). Phenolic Compounds as Markers of Wine Quality and Authenticity. *Foods, 9*(12). https://doi.org/10.3390/foods9121785

Minnaar, P. P., du Plessis, H. W., Jolly, N. P., van der Rijst, M., & du Toit, M. (2019). Non-Saccharomyces yeast and lactic acid bacteria in Co-inoculated fermentations with two Saccharomyces cerevisiae yeast strains: A strategy to improve the phenolic content of Syrah wine. *Food Chemistry: X, 4*, 100070-100070. https://doi.org/10.1016/j.fochx.2019.100070

Minnaar, P. P., du Plessis, H. W., Paulsen, V., Ntushelo, N., Jolly, N. P., & du Toit, M. (2017). Saccharomyces cerevisiae, Non-Saccharomyces Yeasts and Lactic Acid Bacteria in Sequential Fermentations: Effect on Phenolics and Sensory Attributes of South African Syrah Wines. *S. Afr. J. Enol. Vitic, 38*(2), 237-244. https://doi.org/10.21548/38-2-1621

Miyagusuku-Cruzado, G., Garcia-Cano, I., Rocha-Mendoza, D., Jimenez-Flores, R., & Giusti, M. M. (2020, Jul). Monitoring Hydroxycinnamic Acid Decarboxylation by Lactic Acid Bacteria Using High-Throughput UV-Vis Spectroscopy. *Molecules, 25*(14). https://doi.org/10.3390/molecules25143142

Moharrami, S., & Hashempour, H. (2021, Aug 5). Comparative study of low-voltage electric field-induced, ultrasound-assisted and maceration extraction of phenolic acids. *Journal of Pharmaceutical and Biomedical Analysis, 202*. https://doi.org/10.1016/j.jpba.2021.114149

Monagas, M., BartolomÉ, B., & GÓMez-CordovÉS, C. (2005). Updated Knowledge About the Presence of Phenolic Compounds in Wine. *Crit Rev Food Sci Nutr, 45*(2), 85-118. https://doi.org/10.1080/10408690490911710

Monagas, M., Martín-Álvarez, P. J., Bartolomé, B., & Gómez-Cordovés, C. (2006). Statistical interpretation of the color parameters of red wines in function of their phenolic composition during ageing in bottle. *European Food Research & Technology, 222*(5-6), 702-709. https://doi.org/10.1007/s00217-005-0037-7

Morata, A., Calderón, F., González, M. C., Gómez-Cordovés, M. C., & Suárez, J. A. (2007, 2007/01/01/). Formation of the highly stable pyranoanthocyanins (vitisins A and B) in red wines by the addition of pyruvic acid and acetaldehyde. *Food Chemistry, 100*(3), 1144-1152. https://doi.org/https://doi.org/10.1016/j.foodchem.2005.11.024

Mouls, L., & Fulcrand, H. (2012a). UPLC-ESI-MS study of the oxidation markers released from tannin depolymerization: toward a better characterization of the tannin evolution over food and beverage processing. *J. Mass Spectrom, 47*(11), 1450-1457. https://doi.org/10.1002/jms.3098

Mouls, L., & Fulcrand, H. (2012b). UPLC‐ESI‐MS study of the oxidation markers released from tannin depolymerization: toward a better characterization of the tannin evolution over food and beverage processing. *Journal of Mass Spectrometry, 47*(11), 1450-1457. https://doi.org/10.1002/jms.3098

Mouls, L., & Fulcrand, H. (2015). Identification of new oxidation markers of grape-condensed tannins by UPLC–MS analysis after chemical depolymerization. *Tetrahedron, 71*(20), 3012-3019. https://doi.org/10.1016/j.tet.2015.01.038

Muñoz García, R., Simancas, R. O., Díaz-Maroto, M. C., Alañón Pardo, M. E., & Pérez-Coello, M. S. (2021). Effect of microwave maceration and SO_2 free vinification on volatile composition of red wines. *Foods, 10*(6), 1164. https://doi.org/10.3390/foods10061164

Nave, F., Bras, N., Cruz, L., Teixeira, N., Mateus, N., Ramos, M., Di Meo, F., Trouillas, P., Dangles, O., & Freitas, V. (2012, 11/06). The Influence of a Flavan-3-ol Substituent on the Affinity of Anthocyanins (Pigments) Toward Vinylcatechin Dimers and Procyanidins (Copigments). *The journal of physical chemistry. B, 116*. https://doi.org/10.1021/jp307782y

New Zealand Winegrower Inc. (2022). *Annual Report 2022*. https://www.nzwine.com/media/22749/nzw-annual-report-2022.pdf

Nikfardjam, M. P., Wagner, B., & Pickering, G. (2015). Polyphenol Characterization of Red Wine from Germany's Wurttemberg Region. *Mitteilungen Klosterneuburg, 65*(4), 237-249. <Go to ISI>://WOS:000368212900003

Oberholster, A., Elmendorf, B. L., Lerno, L. A., King, E. S., Heymann, H., Brenneman, C. E., & Boulton, R. B. (2015, Apr 15). Barrel maturation, oak alternatives and micro-oxygenation: Influence on red wine ageing and quality. *Food Chemistry, 173*, 1250-1258. https://doi.org/10.1016/j.foodchem.2014.10.043

Oliveira, J., de Freitas, V., & Mateus, N. (2009). A novel synthetic pathway to vitisin B compounds. *Tetrahedron Letters, 50*(27), 3933-3935. https://doi.org/10.1016/j.tetlet.2009.04.072

Parpinello, G. P., Versari, A., Chinnici, F., & Galassi, S. (2009, 2009/12/01/). Relationship among sensory descriptors, consumer preference and color parameters of Italian Novello red wines. *Food Research International, 42*(10), 1389-1395. https://doi.org/https://doi.org/10.1016/j.foodres.2009.07.005

Parr, W. V., Grose, C., Hedderley, D., Maraboli, M. M., Masters, O., Araujo, L. D., & Valentin, D. (2020, Nov). Perception of quality and complexity in wine and their links to varietal typicality: An

investigation involving Pinot noir wine and professional tasters. *Food Research International, 137*. https://doi.org/10.1016/j.foodres.2020.109423

Pati, S., Losito, I., Gambacorta, G., La Notte, E., Palmisano, F., & Zambonin, P. G. (2006, Jul). Simultaneous separation and identification of oligomeric procyanidins and anthocyanin-derived pigments in raw red wine by HPLC-UV-ESI-MSn. *J Mass Spectrom, 41*(7), 861-871. https://doi.org/10.1002/jms.1044

Pavez, C., Gonzalez-Munoz, B., O'Brien, J. A., Laurie, V. F., Osorio, F., Nunez, E., Vega, R. E., Bordeu, E., & Brossard, N. (2022, Jan 15). Red wine astringency: Correlations between chemical and sensory features. *Lwt-Food Science and Technology, 154*. https://doi.org/10.1016/j.lwt.2021.112656

Perestrelo, R., Bordiga, M., Locatelli, M., Silva, C., & Câmara, J. S. (2020, 2020/03/01/). Polyphenols, biogenic amines and amino acids patterns in Verdelho wines according to vintage. *Microchemical Journal, 153*, 104383. https://doi.org/https://doi.org/10.1016/j.microc.2019.104383

Pérez-Navarro, J., Izquierdo-Cañas, P. M., Mena-Morales, A., Martínez-Gascueña, J., Chacón-Vozmediano, J. L., García-Romero, E., Hermosín-Gutiérrez, I., & Gómez-Alonso, S. (2019, 2019/10/15/). Phenolic compounds profile of different berry parts from novel Vitis vinifera L. red grape genotypes and Tempranillo using HPLC-DAD-ESI-MS/MS: A varietal differentiation tool. *Food Chemistry, 295*, 350-360. https://doi.org/https://doi.org/10.1016/j.foodchem.2019.05.137

Perez-Prieto, L. J., De la Hera-Orts, M. L., Lopez-Roca, J. M., Fernandez-Fernandez, J. I., & Gomez-Plaza, E. (2003, Nov). Oak-matured wines: influence of the characteristics of the barrel on wine colour and sensory characteristics. *Journal of the Science of Food and Agriculture, 83*(14), 1445-1450. https://doi.org/10.1002/jsfa.1551

Petrozziello, M., Torchio, F., Piano, F., Giacosa, S., Ugliano, M., Bosso, A., & Rolle, L. (2018, Apr 27). Impact of Increasing Levels of Oxygen Consumption on the Evolution of Color, Phenolic, and Volatile Compounds of Nebbiolo Wines. *Frontiers in Chemistry, 6*. https://doi.org/10.3389/fchem.2018.00137

Picariello, L., Slaghenaufi, D., & Ugliano, M. (2020, Apr). Fermentative and post-fermentative oxygenation of Corvina red wine: influence on phenolic and volatile composition, colour and wine oxidative response. *Journal of the Science of Food and Agriculture, 100*(6), 2522-2533. https://doi.org/10.1002/jsfa.10278

Pinasseau, L., Verbaere, A., Roques, M., Meudec, E., Vallverdú-Queralt, A., Terrier, N., Boulet, J. C., Cheynier, V., & Sommerer, N. (2016). A Fast and Robust UHPLC-MRM-MS Method to Characterize and Quantify Grape Skin Tannins after Chemical Depolymerization. *Molecules (Basel, Switzerland), 21*(10), <xocs:firstpage xmlns:xocs=""/>. https://doi.org/10.3390/molecules21101409

Piñeiro, Z., Cantos-Villar, E., Palma, M., & Puertas, B. (2011, Nov 9). Direct liquid chromatography method for the simultaneous quantification of hydroxytyrosol and tyrosol in red wines. *J Agric Food Chem, 59*(21), 11683-11689. https://doi.org/10.1021/jf202254t

Piombino, P., Pittari, E., Gambuti, A., Curioni, A., Giacosa, S., Mattivi, F., Parpinello, G. P., Rolle, L., Ugliano, M., & Moio, L. (2020). Preliminary sensory characterisation of the diverse astringency of single cultivar Italian red wines and correlation of sub‐qualities with chemical composition.

Australian Journal of Grape and Wine Research, 26(3), 233-246. https://doi.org/10.1111/ajgw.12431

Pittari, E., Moio, L., & Piombino, P. (2021, Feb). Interactions between Polyphenols and Volatile Compounds in Wine: A Literature Review on Physicochemical and Sensory Insights. *Applied Sciences-Basel, 11*(3). https://doi.org/10.3390/app11031157

Poncet-Legrand, C., Cabane, B., Bautista-Ortín, A.-B., Carrillo, S., Fulcrand, H., Pérez, J., & Vernhet, A. (2010, 2010/09//). Tannin oxidation: intra- versus intermolecular reactions. *Biomacromolecules, 11*(9), 2376-2386. https://doi.org/10.1021/bm100515e

Poncet-Legrand, C., Cabane, B., Bautista-Ortín, A. B., Carrillo, S., Fulcrand, H., Pérez, J., & Vernhet, A. (2010, Sep 13). Tannin oxidation: intra- versus intermolecular reactions. *Biomacromolecules, 11*(9), 2376-2386. https://doi.org/10.1021/bm100515e

Poncet-Legrand, C., Gautier, C., Cheynier, V., & Imberty, A. (2007, Oct 31). Interactions between flavan-3-ols and poly(L-proline) studied by isothermal titration calorimetry: Effect of the tannin structure. *Journal of Agricultural and Food Chemistry, 55*(22), 9235-9240. https://doi.org/10.1021/jf071297o

Preys, S., Mazerolles, G., Courcoux, P., Samson, A., Fischer, U., Hanafi, M., Bertrand, D., & Cheynier, V. (2006). Relationship between polyphenolic composition and some sensory properties in red wines using multiway analyses. *Analytica Chimica Acta, 563*(1), 126-136. https://doi.org/10.1016/j.aca.2005.10.082

Price, S. F., Breen, P. J., Valladao, M., & Watson, B. T. (1995a). Cluster Sun Exposure and Quercetin in Pinot-Noir Grapes and Wine. *American Journal of Enology and Viticulture, 46*(2), 187-194. <Go to ISI>://WOS:A1995RD29800006

Price, S. F., Breen, P. J., Valladao, M., & Watson, B. T. (1995b). Cluster Sun Exposure and Quercetin in Pinot noir Grapes and Wine. *American Journal of Enology and Viticulture, 46*(2), 187-194. https://www.ajevonline.org/content/ajev/46/2/187.full.pdf

Remy-Tanneau, S., Le Guernevé, C., Meudec, E., & Cheynier, V. (2003, Jun 4). Characterization of a colorless anthocyanin-flavan-3-ol dimer containing both carbon-carbon and ether interflavanoid linkages by NMR and mass spectrometry. *J Agric Food Chem, 51*(12), 3592-3597. https://doi.org/10.1021/jf021227b

Rivas, E. G.-P., Alcalde-Eon, C., Santos-Buelga, C., Rivas-Gonzalo, J. C., & Escribano-Bailón, M. T. (2006). Behaviour and characterisation of the colour during red wine making and maturation. *Analytica Chimica Acta, 563*(1), 215-222. https://doi.org/10.1016/j.aca.2005.11.044

Robinson, J. (2016). *Understanding Wines: Explaining Style and Quality*. Wine [and] Spirit Education Trust. https://books.google.co.nz/books?id=BSO_swEACAAJ

Roullier-Gall, C., Lucio, M., Noret, L., Schmitt-Kopplin, P., & Gougeon, R. D. (2014). How subtle is the "terroir" effect? Chemistry-related signatures of two "climats de Bourgogne". *Plos One, 9*(5), e97615. https://doi.org/10.1371/journal.pone.0097615

Ruiz-Rodriguez, A., Palma, M., & Barroso, C. G. (2021, May). Influence of Temperature during Pre-Fermentative Maceration and Alcoholic Fermentation on the Phenolic Composition of 'Cabernet Sauvignon' Wines. *Foods, 10*(5). https://doi.org/10.3390/foods10051053

Rustioni, L., Bedgood, D. R., Failla, O., Prenzler, P. D., & Robards, K. (2012, 2012/06/15/). Copigmentation and anti-copigmentation in grape extracts studied by spectrophotometry and post-column-reaction HPLC. *Food Chemistry, 132*(4), 2194-2201. https://doi.org/https://doi.org/10.1016/j.foodchem.2011.12.058

Sabel, A., Bredefeld, S., Schlander, M., & Claus, H. (2017). Wine phenolic compounds: Antimicrobial properties against yeasts, lactic acid and acetic acid bacteria. *Beverages (Basel), 3*(3), 29. https://doi.org/10.3390/beverages3030029

Sáenz-López, R., Fernández-Zurbano, P., & Tena, M. T. (2004, 2004/10/15/). Analysis of aged red wine pigments by capillary zone electrophoresis. *Journal of Chromatography A, 1052*(1), 191-197. https://doi.org/https://doi.org/10.1016/j.chroma.2004.08.007

Saenz-Navajas, M. P., Echavarri, F., Ferreira, V., & Fernandez-Zurbano, P. (2011, May). Pigment composition and color parameters of commercial Spanish red wine samples: linkage to quality perception. *European Food Research and Technology, 232*(5), 877-887. https://doi.org/10.1007/s00217-011-1456-2

Salas, E., Atanasova, V., Poncet-Legrand, C., Meudec, E., Mazauric, J. P., & Cheynier, V. (2004, Jun 18). Demonstration of the occurrence of flavanol-anthocyanin adducts in wine and in model solutions. *Analytica Chimica Acta, 513*(1), 325-332. https://doi.org/10.1016/j.aca.2003.11.084

Salas, E., Fulcrand, H., Meudec, E., & Cheynier, V. (2003, Dec 31). Reactions of anthocyanins and tannins in model solutions. *Journal of Agricultural and Food Chemistry, 51*(27), 7951-7961. https://doi.org/10.1021/jf0345402

Salminen, J.-P. (2018, 2018/09/05). Two-Dimensional Tannin Fingerprints by Liquid Chromatography Tandem Mass Spectrometry Offer a New Dimension to Plant Tannin Analyses and Help To Visualize the Tannin Diversity in Plants. *Journal of Agricultural and Food Chemistry, 66*(35), 9162-9171. https://doi.org/10.1021/acs.jafc.8b02115

Sanchez-Iglesias, M., Gonzalez-Sanjose, M. L., Perez-Magarino, S., Ortega-Heras, M., & Gonzalez-Huerta, C. (2009, Dec 23). Effect of Micro-Oxygenation and Wood Type on the Phenolic Composition and Color of an Aged Red Wine. *Journal of Agricultural and Food Chemistry, 57*(24), 11498-11509. https://doi.org/10.1021/jf902173d

Sanz, M., de Simon, B. F., Esteruelas, E., Munoz, A. M., Cadahia, E., Hernandez, T., Estrella, I., & Pinto, E. (2011, Apr 13). Effect of Toasting Intensity at Cooperage on Phenolic Compounds in Acacia (Robinia pseudoacacia) Heartwood. *Journal of Agricultural and Food Chemistry, 59*(7), 3135-3145. https://doi.org/10.1021/jf1042932

Schmidtke, L. M., Clark, A. C., & Scollary, G. R. (2011). Micro-Oxygenation of Red Wine: Techniques, Applications, and Outcomes. *Critical Reviews in Food Science and Nutrition, 51*(2), 115-131. https://doi.org/10.1080/10408390903434548

Seguin, G. (1986, 1986/08/01). 'Terroirs' and pedology of wine growing. *Experientia, 42*(8), 861-873. https://doi.org/10.1007/BF01941763

Sener, H. (2018). Effect of Temperature and Duration of Maceration on Colour and Sensory Properties of Red Wine - A Review. *South African Journal of Enology and Viticulture, 39*(2), 227-234. https://doi.org/10.21548/39-2-3160

Setford, P. C., Jeffery, D. W., Grbin, P. R., & Muhlack, R. A. (2017). Factors affecting extraction and evolution of phenolic compounds during red wine maceration and the role of process

modelling. *Trends in Food Science & Technology, 69*, 106-117. https://doi.org/10.1016/j.tifs.2017.09.005

Silva, I., Campos, F. M., Hogg, T., & Couto, J. A. (2011). Wine phenolic compounds influence the production of volatile phenols by wine ‐ related lactic acid bacteria. *J Appl Microbiol, 111*(2), 360-370. https://doi.org/10.1111/j.1365-2672.2011.05052.x

Singleton, V. L. (1992). Tannins and the Qualities of Wines. In R. W. Hemingway & P. E. Laks (Eds.), *Plant Polyphenols: Synthesis, Properties, Significance* (pp. 859-880). Springer US. https://doi.org/10.1007/978-1-4615-3476-1_51

Smith, P. A., McRae, J. M., & Bindon, K. A. (2015). Impact of winemaking practices on the concentration and composition of tannins in red wine. *Australian Journal of Grape and Wine Research, 21*(S1), 601-614. https://doi.org/10.1111/ajgw.12188

Somers, T. C. (1971, 1971/09/01/). The polymeric nature of wine pigments. *Phytochemistry, 10*(9), 2175-2186. https://doi.org/https://doi.org/10.1016/S0031-9422(00)97215-7

Somers, T. C., & Evans, M. E. (1974). Wine quality: correlations with colour density and anthocyanin equilibria in a group of young red wines. *Wine quality: correlations with colour density and anthocyanin equilibria in a group of young red wines., 25*(11), 1369-1379.

Somers, T. C., & Evans, M. E. (1977). Spectral evaluation of young red wines: Anthocyanin equilibria, total phenolics, free and molecular SO 2 , "chemical age". *Journal of the Science of Food and Agriculture, 28*(3), 279-287. https://doi.org/10.1002/jsfa.2740280311

Somers, T. C., & Vérette, E. (1988). Phenolic Composition of Natural Wine Types. In H.-F. Linskens & J. F. Jackson (Eds.), *Wine Analysis* (pp. 219-257). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-83340-3_8

Song, J., Smart, R., Wang, H., Dambergs, B., Sparrow, A., & Qian, M. C. (2015). Effect of grape bunch sunlight exposure and UV radiation on phenolics and volatile composition of Vitis vinifera L. cv. Pinot noir wine. *Food Chemistry, 173*(C), 424-431. https://doi.org/10.1016/j.foodchem.2014.09.150

Spayd, S. E., Tarara, J. M., Mee, D. L., & Ferguson, J. C. (2002). Separation of Sunlight and Temperature Effects on the Composition of Vitis vinifera cv. Merlot Berries. *American Journal of Enology and Viticulture, 53*(3), 171. http://www.ajevonline.org/content/53/3/171.abstract

Stark, T., Wollmann, N., Wenker, K., Lösch, S., Glabasnia, A., & Hofmann, T. (2010, 2010/05/26). Matrix-Calibrated LC-MS/MS Quantitation and Sensory Evaluation of Oak Ellagitannins and Their Transformation Products in Red Wines. *Journal of Agricultural and Food Chemistry, 58*(10), 6360-6369. https://doi.org/10.1021/jf100884y

Stoj, A., Kapusta, I., & Domagala, D. (2020, Mar 2). Classification of Red Wines Produced from Zweigelt and Rondo Grape Varieties Based on the Analysis of Phenolic Compounds by UPLC-PDA-MS/MS. *Molecules, 25*(6). https://doi.org/10.3390/molecules25061342

Suprun, A. R., Dubrovina, A. S., Tyunin, A. P., & Kiselev, K. V. (2021, Apr). Profile of Stilbenes and Other Phenolics in Fanagoria White and Red Russian Wines. *Metabolites, 11*(4). https://doi.org/10.3390/metabo11040231

Swiegers, J. H., Bartowsky, E. J., Henschke, P. A., & Pretorius, I. S. (2005). Yeast and bacterial modulation of wine aroma and flavour. *Australian Journal of Grape and Wine Research, 11*(2), 139-173. https://doi.org/DOI 10.1111/j.1755-0238.2005.tb00285.x

Trouillas, P., Sancho Garcia, J., Freitas, V., Gierschner, J., Otyepka, M., & Dangles, O. (2016, 03/09). Stabilizing and Modulating Color by Copigmentation: Insights from Theory and Experiment. *Chemical Reviews, 116*. https://doi.org/10.1021/acs.chemrev.5b00507

Tsutomu, H. (1992, 1992/02/01/). Self-association of flavylium cations of anthocyanidin 3,5-diglucosides studied by circular dichroism and 1H NMR. *Phytochemistry, 31*(2), 647-653. https://doi.org/https://doi.org/10.1016/0031-9422(92)90053-S

Urvieta, R., Jones, G., Buscema, F., Bottini, R., & Fontana, A. (2021). Terroir and vintage discrimination of Malbec wines based on phenolic composition across multiple sites in Mendoza, Argentina. *Sci Rep, 11*(1), 2863-2863. https://doi.org/10.1038/s41598-021-82306-0

Uzkuc, N. M. C., Bayhan, A., & Toklucu, A. K. (2021, Nov 8). Phenolics and color components of young Cabernet Sauvignon wines: effect of spontaneous fermentation and bottle storage. *European Food Research and Technology*. https://doi.org/10.1007/s00217-021-03884-x

Valentin, D., Parr, W. V., Peyron, D., Grose, C., & Ballester, J. (2016). Colour as a driver of Pinot noir wine quality judgments: An investigation involving French and New Zealand wine professionals. *Food Quality and Preference, 48*(PA), 251-261. https://doi.org/10.1016/j.foodqual.2015.10.003

Vallverdu-Queralt, A., Meudec, E., Eder, M., Lamuela-Raventos, R. M., Sommerer, N., & Cheynier, V. (2017, Jul 15). Targeted filtering reduces the complexity of UHPLC-Orbitrap-HRMS data to decipher polyphenol polymerization. *Food Chemistry, 227*, 255-263. https://doi.org/10.1016/j.foodchem.2017.01.106

Vernhet, A., Dubascoux, S., Cabane, B., Fulcrand, H., Dubreucq, E., & Poncet-Legrand, C. (2011). Characterization of oxidized tannins: comparison of depolymerization methods, asymmetric flow field-flow fractionation and small-angle X-ray scattering. *Analytical and Bioanalytical Chemistry, 401*(5), 1563-1573. https://doi.org/10.1007/s00216-011-5076-2

Vestergaard, M., & Ingmer, H. (2019, Jun). Antibacterial and antifungal properties of resveratrol. *Int J Antimicrob Agents, 53*(6), 716-723. https://doi.org/10.1016/j.ijantimicag.2019.02.015

Vidal, S., Francis, L., Guyot, S., Marnet, N., Kwiatkowski, M., Gawel, R., Cheynier, V., & Waters, E. J. (2003). The mouth ‐ feel properties of grape and apple proanthocyanidins in a wine ‐ like medium. *Journal of the Science of Food and Agriculture, 83*(6), 564-573. https://doi.org/10.1002/jsfa.1394

Vidal, S., Francis, L., Noble, A., Kwiatkowski, M., Cheynier, V., & Waters, E. (2004). Taste and mouth-feel properties of different types of tannin-like polyphenolic compounds and anthocyanins in wine. *Analytica Chimica Acta, 513*(1), 57-65. https://doi.org/10.1016/j.aca.2003.10.017

Viñas, P., Campillo, N., Martínez-Castillo, N., & Hernández-Córdoba, M. (2009, 2009/02/27/). Solid-phase microextraction on-fiber derivatization for the analysis of some polyphenols in wine and grapes using gas chromatography–mass spectrometry. *Journal of Chromatography A, 1216*(9), 1279-1284. https://doi.org/https://doi.org/10.1016/j.chroma.2008.12.058

Waterhouse, A., Sacks, G., & Jeffery, D. (2016). *Understanding Wine Chemistry*.

Waterhouse, A. L. (2002). Wine phenolics. *Alcohol and Wine in Health and Disease, 957*, 21-36. https://doi.org/DOI 10.1111/j.1749-6632.2002.tb02903.x

Watrelot, A. A., & Norton, E. L. (2020, May). Chemistry and Reactivity of Tannins in Vitis spp.: A Review. *Molecules, 25*(9). https://doi.org/10.3390/molecules25092110

Weber, F., Greve, K., Durner, D., Fischer, U., & Winterhalter, P. (2013). Sensory and Chemical Characterization of Phenolic Polymers from Red Wine Obtained by Gel Permeation Chromatography [10.5344/ajev.2012.12074]. *American Journal of Enology and Viticulture, 64*(1), 15. http://www.ajevonline.org/content/64/1/15.abstract

Wei, L. (2014). Effects of aspects of terroir on the phenolic composition of New Zealand Pinot noir wines.

Willemse, C. M., Stander, M. A., Vestner, J., Tredoux, A. G. J., & de Villiers, A. (2015, Dec 15). Comprehensive Two-Dimensional Hydrophilic Interaction Chromatography (HILIC) x Reversed-Phase Liquid Chromatography Coupled to High-Resolution Mass Spectrometry (RP-LC-UV-MS) Analysis of Anthocyanins and Derived Pigments in Red Wine. *Analytical Chemistry, 87*(24), 12006-12015. https://doi.org/10.1021/acs.analchem.5b03615

Wimalasiri, P. M. (2020). *The effect of grape stem inclusion fermentation on Pinot noir wine composition : A thesis submitted in partial fulfilment of the requirements for the Degree of Master of Science at Lincoln University* Lincoln University].

Wollmann, N., & Hofmann, T. (2013, 2013/03/06). Compositional and Sensory Characterization of Red Wine Polymers. *Journal of Agricultural and Food Chemistry, 61*(9), 2045-2061. https://doi.org/10.1021/jf3052576

Yang, Y., Deed, R. C., Araujo, L. D., Waterhouse, A. L., & Kilmartin, P. A. (2021a, Sep 3). Effect of microoxygenation applied before and after malolactic fermentation on monomeric phenolics and tannin composition of Pinot Noir wine. *Australian Journal of Grape and Wine Research*. https://doi.org/10.1111/ajgw.12520

Yang, Y., Deed, R. C., Araujo, L. D., Waterhouse, A. L., & Kilmartin, P. A. (2021b, Jul 18). Effect of microoxygenation on acetaldehyde, yeast and colour before and after malolactic fermentation on Pinot Noir wine. *Australian Journal of Grape and Wine Research*. https://doi.org/10.1111/ajgw.12512

Yang, Y., Deed, R. C., Araujo, L. D., Waterhouse, A. L., & Kilmartin, P. A. (2022). Effect of microoxygenation on acetaldehyde, yeast and colour before and after malolactic fermentation on Pinot Noir wine. *Australian Journal of Grape and Wine Research, 28*(1), 50-60. https://doi.org/10.1111/ajgw.12512

Yilmaz, Y., & Toledo, R. T. (2004, Jan 28). Major flavonoids in grape seeds and skins: Antioxidant capacity of catechin, epicatechin, and gallic acid. *Journal of Agricultural and Food Chemistry, 52*(2), 255-260. https://doi.org/10.1021/jf030117h

Zeller, W. E. (2019). Activity, Purification, and Analysis of Condensed Tannins: Current State of Affairs and Future Endeavors. *Crop Science, 59*(3), 886-904. https://doi.org/https://doi.org/10.2135/cropsci2018.05.0323

Zeng, L., Teissedre, P. L., & Jourdes, M. (2016, Jan 15). Structures of polymeric pigments in red wine and their derived quantification markers revealed by high-resolution quadrupole time-of-flight

mass spectrometry. *Rapid Commun Mass Spectrom, 30*(1), 81-88. https://doi.org/10.1002/rcm.7416

Zhang, P. Z., Ma, W., Meng, Y. Q., Zhang, Y. F., Jin, G., & Fang, Z. X. (2021, Jul). Wine phenolic profile altered by yeast: Mechanisms and influences. *Comprehensive Reviews in Food Science and Food Safety, 20*(4), 3579-3619. https://doi.org/10.1111/1541-4337.12788

Zhao, X., He, F., Zhang, X. K., Shi, Y., & Duan, C. Q. (2022, May 1). Impact of three phenolic copigments on the stability and color evolution of five basic anthocyanins in model wine systems. *Food Chem, 375*, 131670. https://doi.org/10.1016/j.foodchem.2021.131670