Linking the phenolic potential of grape berries with polyphenol distribution during wine making: A Sauvignon Blanc case study

by

Davin Lance Williams

Thesis presented in partial fulfilment of the requirements for the degree of
Master of Science

at
Stellenbosch University
The Institute for Wine Biotechnology, Faculty of AgriSciences

Supervisor: Prof Melané Vivier
Co-supervisor: Dr Anscha Zietsman

April 2019

Stellenbosch University https://scholar.sun.ac.za

Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: April 2019

Copyright © 2019 Stellenbosch University
All rights reserved

Stellenbosch University https://scholar.sun.ac.za

Summary

The focus of this study was the phenolic potentials of grape and wine matrices of Sauvignon Blanc produced in either a high or low light microclimate. Phenolic compounds are important in grapes due to their protective function against environmental stress and fungal infection. In ripe grapes, polyphenols are unequally distributed as the skin is rich in flavonols, the seeds in flavan-3-ols, whereas the pulp contains low quantities of hydroxycinnamic acids. The polyphenol content of grapes can be altered through viticultural treatments such as manipulating the light microclimate of the bunches during development. Maintenance of a high light microclimate typically enhances polyphenol accumulation, thus increasing the phenolic potential of the grapes. The polyphenols that are present within the grapes are extracted to the derived juice and wine during winemaking where these compounds could influence colour as well as the sensory perception of wine. The extraction of phenolic compounds into the juice can be enhanced by modulating the winemaking steps, such as incorporating extended pre-fermentative skin contact. This modulation is mostly excluded in white wine-making due to potential negative implications of a high polyphenol content on white wine properties. In general, much less information on polyphenols is available for white grapes and wines.

The aim of this study was to profile and quantify the phenolic potentials of Sauvignon Blanc grapes and to “follow” the compounds throughout the wine-making process, in all the grape and wine matrices, including the waste products. Pomace and juice sediments generated during winemaking and discarded as wastes can be considered as “traps” for phenolic compounds that accumulate in them. These waste matrices, when analysed alongside the juice and wine matrices could provide valuable information on the fate of polyphenols throughout the wine-making process.

The approach in this study was to contrast Sauvignon Blanc grapes and wines that were exposed to two microclimates that differed in terms of the light exposure in the bunch zone. The study benefitted from a fully characterised model vineyard site where grapes from a high light (HL) and low light (LL) microclimate could be sourced. The first step was to profile and quantify the polyphenolic content and composition of the whole grapes, as well as their skins, pulps and seeds. The results confirmed previous findings that the berries from the HL environment accumulated significantly more polyphenols and that it was mostly the skins and to a lesser degree the pulp that responded to increased exposure by producing certain polyphenols. The flavonols found in the skins were strongly upregulated by the high light microclimate. The results confirmed that the two microclimates yielded berries with distinctly different phenolic potentials (a high phenolic potential, HP from the HL microclimate and a low phenolic potential (LP from the LL microclimate). In addition to the flavonols, certain hydroxycinnamic acids and flavan-3-ols could be quantified in this study.

Stellenbosch University https://scholar.sun.ac.za

The next objective was to compare a standard white-wine making procedure (as the control) with modulated wine-making steps chosen to allow enhanced extraction of the polyphenols from the grape matrices of the HP and LP grapes. Enhanced extraction was modulated by implementing either pre-fermentative skin contact, fermentation in contact with the juice sediment, or a combination of these two treatments in the making of the wines. In addition to profiling and measuring the polyphenolic compounds in the juices, wines and waste matrices, the impact of the treatments on the sensorial quality of the wine was also evaluated from a mouthfeel perspective.

Stellenbosch University https://scholar.sun.ac.za

Polyphenol transference occurred only partially from the grapes to the juice as no flavonols nor flavan-3-ols originating from the seeds were detected in the latter. During juice processing, total polyphenol levels also decreased considerably from the free run to the enzyme clarified juice as both hydroxycinnamic acids and flavan-3-ol concentrations decreased. This decrease was primarily linked to a decrease in catechin and caftaric acid which were the most abundant polyphenols in the juice matrix. Analysis of the corresponding sediment revealed that a considerable quantity of already extracted polyphenols settled out of suspension after enzyme clarification of the juice. Analysis of the pomace revealed that a fraction of polyphenols were also not extracted from the berry and remained in the pomace regardless of treatment, but the pomace was also considered as a phenolic trap due to flavonoid increases after pressing. The phenolic composition of the pomace also correlated with the fresh grape skin but contained lower quantities of polyphenols. The increase in total polyphenol content of the juices, which included hydroxycinnamic acids and flavan-3-ols, through pre-fermentative skin contact was in agreement with literature. The phenolic compounds that were increased in the juice were also higher in the corresponding sediments but did not clearly reflect in the pomace. The analysis method used to determine the cell wall composition of the pomace did not indicate significant alteration during the juice processing steps but the juice data revealed that sufficient deconstruction must have occurred to allow extraction of more phenolic compounds.

In the standard and skin contact clarified juice made from high phenolic potential grapes, only the concentrations of catechin and caffeic were elevated in comparison to the low phenolic potential juice. High phenolic potential grapes in combination with skin contact yielded juices with higher total polyphenol content but mainly due to elevated levels of catechin.

The phenolic profiles of the standard and skin contact wines correlated with the phenolic profiles of their preceding juices. Sediment contact during fermentation increased the total polyphenol content of all wines, specifically the flavan-3-ols, and more so in the high phenolic potential wine. The phenolic potential along with treatments induced perceptible differences on the sensorial characteristics of the wines. Standard high phenolic potential wines were less bitter than its low

Stellenbosch University https://scholar.sun.ac.za

phenolic potential counterparts. Skin contact increased wine sweetness whereas sediment contact increased bitterness.

This study provided new insight into the transference of polyphenols during white wine making as well as the potential implications of phenolic potential of grapes and additional wine making treatments on transference and the organoleptic properties of the wine.

Stellenbosch University https://scholar.sun.ac.za

Stellenbosch University https://scholar.sun.ac.za

Opsomming

Die fokus van hierdie studie was die fenoliese potensiaal van Sauvignon Blanc druif- en wyn matrikse wat in hoë of lae lig mikroklimate geproduseer was. In druiwe funksioneer fenole as beskermingsagente wat weerstand teen omgewingsstres en swaminfeksies bied. Fenole is oneweredig versprei in die ryp druiwekorrel: die doppe bevat hoë vlakke van flavonole, die sade bevat flavan-3-ole en die pulp het lae vlakke van hidroksiekaneelsure. Die fenoliese vlakke van die druiwe kan deur wingerdboukundige behandelinge verander word, byvoorbeeld deur die lig mikroklimaat waarin die druifkorrel ontwikkel te manipuleer. Die handhaweing van ‘n hoë lig mikroklimaat verhoog fenoliese opeenhoping, sodoende ook die fenoliese potensiaal van die druiwe. Die fenole in die druiwe word na die sap en wyn ge-ekstraheer tydens die wynmaak proses, en beïnvloed die kleur asook sensoriese persepsie van die wyn. Die ekstraksie kan verhoog word deur die aanpas van wynmaak stappe, soos die insluiting van verlengde dopkontak voor fermentasie. Hierdie modulerende stap word meestal uit die witwynmaak prosedure uitgesluit as gevolg van die moontlike negatiewe gevolge wat tipies verbind word met hoë vlakke van fenole in witwyn. Oor die algemeen is daar minder inligting beskikbaar rakende fenole in wit druiwe in vergelyking met dit wat bekend is vir rooi druiwe.

Die doel van hierdie studie was om die fenoliese profiel en -potensiaal van Sauvignon Blanc druiwe te bepaal en om hierdie verbindings te volg deur die wynmaak proses, in al die druif- en wyn matrikse, asook in die afvalmateriaal. Druifprosesserings afval soos doppe en sap sediment wat gedurende wynmaak gegenereer en gewoonlik weggegooi word, kan as fenoliese “lokvalle” beskou word vir die verbindings wat daarin akkumuleer. Wanneer hierdie afvalmatrikse saam met die sap en wyn geanaliseer word, kan waardevolle inligting rakende die lot van fenoliese verbindings gedurende die wynmaak proses ingewin word.

Die aanslag van hierdie studie was om Sauvignon Blanc druiwe and wyn vanaf twee mikroklimate, wat verskil in terme van ligblootselling, te vergelyk. Hierdie studie het gebaat by ‘n ten volle gekaraktariseerde “model” wingerd waar bewys is dat hoë lig (HL) en lae lig (LL) mikroklimaat-druiwe gelewer kan word. Die eerse stap was om die profiel van die fenoliese verbindings in die heelkorrels, asook in die doppe, pulp en pitte te kwantifiseer. Die uitslag dat druiwe vanaf die HL mikroklimaat meer fenole geakkumuleer het en dat dit meerendeels die doppe en tot ‘n mindere mate die pulp was wat geaffekteer was, het resulte van vorige studies bevestig. Die flavonool opbou in die doppe was sterk opgereguleer deur die HL mikroklimaat. Die uitslag het bevetsig dat die twee mikroklimate druifkorrrels geproduseer het met duidelik onderkeibare fenoliese potensiale (‘n hoë fenoliese potensiaal, vanaf die HL mikroklimaat; en ‘n lae fenoliese potensiaal, vanaf die LL mikroklimaat).

Stellenbosch University https://scholar.sun.ac.za

Bykomend tot die flavonole, kon sekere hidroksiekaneelsure en flavan-3-ole akkuraat gemeet word in hierdie studie.

Die volgende doelwit was om ‘n standaard witwynmaak prosedure (kontrole) te vergelyk met gemoduleerde wynmaak prosedures, wat spesifiek geskies was om verhoogde fenoliese ekstraksie vanuit die HP en LP druiwe toe te laat. Dopkontak voor fermentasie, sediment kontak gedurende fermentasie, óf ‘n kombinasie van die twee is uitgetoets om ekstraksie te verhoog. Tesame met die kwantifisering en bepaling van die fenoliese profiel van die sap-, wyn- en afvalmatrikse, is die impak van hierdie behandelinge op die sensoriese kwaliteit van die wyn in terme van die mondgevoel, ook bepaal.

Stellenbosch University https://scholar.sun.ac.za

Polifenoliese oordrag vanaf die druiwe na die sap het slegs gedeeltelik plaasgevind, want geen flavonole of flavan-3-ole (oorspronklik afkomstig vanaf die pitte) was teenwoordig in die sap nie. Gedurende die verwerking van die sap was daar ‘n afname in totale polifenoliese vlakke vanaf die afloopsap na die ensiem-verhelderde sap, aangesien beide hidroksiekaneelsure en flavan-3-ole verminder het. Hierdie verlaging was hoofsaaklik veroorsaak deur ‘n verlaging in katikiene (“catechins”) en kofarsuur (“caftaric acid”), wat die vollopste fenoliese verbindings in die sap was. Die ooreenstemmende sediment matriks het onthul dat ‘n groot hoeveelheid ge-ekstraheerde fenole, in die sediment uitsak na ensiem behandeling van die sap. Die ontleding van die druiwedopafval het aangedui dat ‘n fraksie fenole nie vanuit die druif ge-ekstraheer was nie en in die druiwedopafval agter gebly het ongeag of dopkontak toegepas is. Die druiwedopafval kan dus as ‘n fenoliese lokval beskou word aangesien die flavonoiëd konsentrasie toegeneem het na die pers stap. Die polifenoliese samestelling van die druiwedopafval het ooreenkomste met die vars druiwedoppe getoon, maar het laer vlakke van polifenole gehad. Die toename in totale polifenoliese inhoud van die sap (wat hidroksiekaneelsure en flavan-3-ole insluit) as gevolg van dopkontak het vorige bevindings in literatuur bevestig. Die fenoliese verbindings wat in die sap toegeneem het, was ook hoër in die ooreenstemmende sediment, maar het nie ooreenkomstig in die druiwedopafval gereflekteer nie. Die analitiese metode wat gebruik was om die selwandsamestelling van die druiwedopafval te bepaal, het nie noemenswaardige veranderinge gedurende sap verwerking aangedui nie, maar die sap data het bewys dat selwand dekonstruksie wel tot ‘n mate moes plaasgevind het om uitloging van meer fenole toe te laat.

In die standaard- en dopkontak verhelderde sap, gemaak van LP druiwe, het slegs die konsentrasie van katikiene en kafeïensuur verhoog in vergelyking met die LP sap. HP druiwe in kombinasie met dopkontak het sappe gelewer met hoër totale fenoliese vlakke, hoofsaaklik as gevolg van hoër katikienvlakke.

Stellenbosch University https://scholar.sun.ac.za

Die fenoliese profiel van die standaard en dopkontakwyne het ooreengestem met die fenoliese profiel van hul ooreenkomstige sappe. Sediment kontak gedurende fermentasie het ’n toename in totale fenoliese vlakke van alle wyne, veral flavan-3-ole veroorsaak, en wel tot ‘n groter mate in die HP wyne. Die fenoliese potensiaal, tesame met die behandelinge het ook opmerkbare verskille in die sensoriese eienskappe van die wyne veroorsaak. Standaard HP wyne was minder bitter in vergelyking met die standaard LP wyne. Dop kontak het oor die algemeen wyne soeter gemaak, in teenstelling met die verhoging in bitterheid wat versoorsaak was deur sediment kontak.

Hierdie studie het nuwe insigte gebring in ons verstaan van die verspreiding van polifenole gedurende die produksie van witwyn asook die moontlike impak wat die fenoliese potensiaal van die druiwe en gemoduleerde wynmaak behandelinge op die fenoliese verspreiding en die sensoriese eienskappe van wyn maak.

Stellenbosch University https://scholar.sun.ac.za

Stellenbosch University https://scholar.sun.ac.za

This thesis is dedicated to my Mother for affording me the opportunities to get where I am today

Stellenbosch University https://scholar.sun.ac.za

Biographical sketch

Davin Lance Williams was born in Stellenbosch, South Africa, on 9 December 1993. He attended Lückhoff High School, where he matriculated in 2011. In 2012, Davin enrolled for a BSc degree in Human Life Sciences in at the University of Stellenbosch and obtained his degree in 2015. In 2016, he enrolled for a BSc Honours degree in Wine Biotechnology at the Institute for Wine Biotechnology at Stellenbosch University, after which he continued with an MSc degree.

Stellenbosch University https://scholar.sun.ac.za

Stellenbosch University https://scholar.sun.ac.za

Acknowledgements

I wish to express my sincere gratitude and appreciation to the following persons and institutions:

Stellenbosch University https://scholar.sun.ac.za

- Prof Melané Vivier, who as supervisor, provided useful advice and insight during discussions and for her input, patience and support in during the writing of this thesis.

- Dr Anscha Zietsman, who as co-supervisor, provided guidance, valuable advice and showed remarkable patience during the experimental and writing of this thesis. Above all, thank you for encouragement.

- Miss Isabel Greyling, for her invaluable contribution to the logistic preparation and execution of the experimental work of this study.

- Dr Philip Young, for his advice during discussions and input with data analysis.

- Mrs Anke Berry and Dr Chandré Honth, for their assistance with execution of harvest, winemaking and experimental work.

- Dr Hans Eyeghe-Bickong, for his assistance with chemical analysis.

- The Institute for Wine Biotechnology for granting me this opportunity.

- The University of Stellenbosch, The National Research Foundation and Winetech for financial support.

- My mother and sister, for their support and encouragement throughout my studies.

Stellenbosch University https://scholar.sun.ac.za

Preface

This thesis is presented as a compilation of 6 chapters. Each chapter is introduced separately and is written according to the style of the journal *South African Journal of Enology and Viticulture*.

Stellenbosch University https://scholar.sun.ac.za

Chapter 1 **General Introduction and project aims**

Chapter 2 **Literature review**
White grape polyphenols and the factors that influence their levels in the berry, juice and wine matrices.

Chapter 3 **Research results**
Comparison of polyphenol levels and distribution patterns in the tissues of ripe Sauvignon blanc berries obtained from high and low light microclimates

Chapter 4 **Research results**
The distribution of polyphenols from grapes with a low and high phenolic potential to juice and wine during wine making

Chapter 5 **Research results**
Linking polyphenol distribution during wine making with the fractions lost in the waste matrices and the berry cell wall composition.

Chapter 4 **General discussion and conclusions**

Stellenbosch University https://scholar.sun.ac.za

Table of Contents

Stellenbosch University https://scholar.sun.ac.za

Stellenbosch University https://scholar.sun.ac.za

Stellenbosch University https://scholar.sun.ac.za

Stellenbosch University https://scholar.sun.ac.za

Stellenbosch University https://scholar.sun.ac.za

Stellenbosch University https://scholar.sun.ac.za

Chapter 1

Stellenbosch University https://scholar.sun.ac.za

General introduction and project aims

Stellenbosch University https://scholar.sun.ac.za

Chapter 1- General introduction and project aims

1.1. Introduction

Stellenbosch University https://scholar.sun.ac.za

Polyphenols are a complex group of secondary metabolites that accumulate predominantly within vacuoles of the skin and seed cells of grapes during berry development and ripening (Kennedy et al. 2000; Kennedy et al. 2001; Ivanova et al. 2010). These compounds have several biological functions in the plant body and most are involved in plant and tissue protection against stressful environmental conditions (Downey et al. 2004; Fontes et al. 2011), or in response against infection (Del Rio et al. 2004). Plant polyphenols have also received significant attention due to their inherent antioxidant properties and are often promoted for their potential human health benefits, for example their role in preventing diseases such as atherosclerosis and coronary artery disease (Darias-Martín et al. 2000; Davalos et al. 2005).

The polyphenol composition of grapes is cultivar dependant (Rodríguez Montealegre et al. 2006) and subject to multiple regulatory impacts. Biosynthesis of polyphenols occurs via a branching pathway that leads to multiple compound groups that all form part of polyphenols (Adams 2006; Conde et al. 2007). The biosynthetic pathway is the same for red and white grape varieties with some specific mutations in the regulatory genes of the last committed step of anthocyanin formation in white grapes, leading to the lack of anthocyanin pigments in the grapes of white cultivars (Boss et al. 1996). The regulation of this pathway has been shown to be linked to developmental control, as well as changes in environmental stress responses (Downey et al. 2006; Koyama et al. 2012).

Phenols are subdivided into two main groups based on their distinctive chemical structures: flavonoids and non-flavonoids. Flavonoids is further divided into flavan-3-ols, flavonols and anthocyanins; and non-flavonoids into hydroxycinnamic acids, hydroxybenzoic acids and stilbenes (Fulcrand et al. 2006; Conde et al. 2007; Fontes et al. 2011). These compounds are chemically characterised by a phenyl ring in the backbone with multiple hydrocarbon substitution possibilities. Phenol structures are present in monomeric and polymeric forms (tannins). Polymeric polyphenols are synthesized during berry development, but may also form through polymerization of monomers (Kennedy et al. 2000; Downey et al. 2003; Conde et al. 2007). The distribution patterns of the different polyphenols in grape berries has confirmed that at the ripe stage the skins are rich in flavonols and anthocyanins (red berries only); the seeds contain the highest levels of flavan-3-ols; whereas low quantities of hydroxycinnamic acids occur in pulp tissues (Rodríguez Montealegre et al. 2006; Di Lecce et al. 2014; Dragana et al. 2016).

The polyphenols that are present within grapes are transferred to the derived juice and wine during winemaking when the grape cells are disrupted (Ristic et al. 2006). The polyphenol classes have been extensively studied for their roles in perceived wine quality, particularly in red wines. The translation of phenolic compounds from the grapes into the must can be enhanced through the application of various

Stellenbosch University https://scholar.sun.ac.za

winemaking techniques (Hernanz et al. 2007; Patel et al. 2010; Cejudo-Bastante et al. 2011). For example, pre-fermentative skin contact has been shown to increase the polyphenol concentration of wine, explained by the fact that skins contain a substantial amount of the total extractable polyphenols.

Stellenbosch University https://scholar.sun.ac.za

Polyphenols are reactive compounds which can polymerize with other phenols, or interact with the berry's structural cell wall polymers during winemaking. The grape skin cell wall consists predominantly of complex polysaccharides such as pectin, cellulose and hemicellulose (Lecas and Brillouet 1994; Ortega-Regules et al. 2008; Arnous and Meyer 2009). Proanthocyanidins in particular, have high binding affinity to both soluble and insoluble pectin within the juice and wine matrices. Bindon et al. (2016) reported precipitation of the pectin-proanthocyanidin complex and a decrease in soluble proanthocyanidins in a model solution (citrate buffer at pH 3.4). The binding affinity of polyphenols to grape cell wall polysaccharides and associated proteins, could therefore influence subsequent polyphenol extraction during winemaking. Binding can occur both during polyphenol extraction, or afterwards to soluble cell wall polymers present within the must or wine (Bindon et al. 2010a; Bindon et al. 2016). The extent of phenol and cell wall polymer interaction will be influenced by steps like extended skin contact, which would concurrently increase their abundance (and therefore their chance to interact) in the juice and wine matrices (Bindon and Smith, 2013). By forming complexes, the bio-availability, or functionality of phenols may be affected (Fulcrand et al. 2006; Bindon et al. 2010b; Bautista-Ortín et al. 2015; Bindon et al. 2016). It is now becoming increasingly clear that it is not only the polyphenolic content and profile, but also the type and potential for matrix interactions in the juices and wines that will determine the overall impact of these compounds on perceived wine quality impact factors.

In white grape cultivars, the most common polyphenols include caffeic acid, caftaric acid, catechin, epicatechin, procyanidin and quercetin (Rodríguez Montealegre et al. 2006; Di Lecce et al. 2014; Dragana et al. 2016), with the noticeable absence of anthocyanins (Bourziex et al. 1983). Polyphenols in white wine making has received much less attention and significant scope still exist to provide scientific confirmation and quantified data to support/test some of the major assumptions, generalised facts and/or sparse data regarding the potential impacts of increased polyphenolic content in white grapes and wines. Increases in polyphenols in grapes and wine could occur through either climatic impacts, viticultural treatments and/or wine-making steps. In some white cultivars, bunch exposure is used to increase fruity aromas over more herbaceous aromas of less exposed grapes (Suklje et al. 2014). Grape bunch exposure to sunlight will however also increase grape berry polyphenols (Berli et al. 2011; Gregan et al. 2011; Šebela et al. 2017). Few studies have placed focus on whether this increase in berry phenolics translates to an increase in juice and/or wine phenolics. Ristic et al. (2006) found wines made from extremely shaded red grapes to be lower in total tannins and anthocyanins. Polyphenols are not primarily targeted for extraction from the berries since white wine-making techniques are usually carefully considered to not promote abundance of these compounds in the wines. One line of thought is that although (some) white cultivars could benefit from skin contact to

Stellenbosch University https://scholar.sun.ac.za

extract more favourable aroma and flavour precursors from the skin, it also poses risks that could outweigh the benefits since it may lead to juices and wines being more susceptible to oxidation reactions impacting colour (Cheynier et al. 1989; Cejudo-Bastante et al. 2011; Ferreira-lima et al. 2016) and unwanted sensory and mouthfeel properties linked to increased pyrazines or phenolic content (Aleixandre-Tudo et al. 2015; Sokolowsky et al. 2015).

Stellenbosch University https://scholar.sun.ac.za

1.2. Project aims and objectives

Against this background, this study aims to provide insight into the polyphenolic content and profiles of Sauvignon Blanc grapes and wines. Particular focus will be placed on the transitioning of the compounds from the grapes to the wines and the ultimate impacts on the sensorial qualities of the wines, if increased polyphenolic extraction would occur.

The proposed study will be conducted as part of a "Wine-as-a-system" analysis of Sauvignon Blanc produced from a low light (LL) and high light (HL) microclimate where both the phenolic potential (this study) and the aromatic potential (the MSc study of Ms Isabel Greyling) of the grapes and wines will be studied.

Such an approach to study the polyphenolic compounds from grapes to wines, throughout all the matrices of the wine-making process (grape tissues, juice, pomace, sediment and wines) could provide important and novel insights. By characterising each winemaking matrix, it could lead to insight into the potential "fate" of a specific group of compounds. Reports of treatments that increase the polyphenol concentration of juices and wines are available (Gomez-Miguez et al. 2007; Hernanz et al. 2007; Patel et al. 2010; Di Lecce et al. 2013; Ferreira-lima et al. 2016), and the presence of residual polyphenols in pomace has also been confirmed (Kammerer et al. 2004; Rossello and Teissedre 2013), but previous work, to our knowledge, did not have an integrated analysis of all matrices, to account for polyphenol losses or gains. The pomace and juice sediments will therefore be included in the analyses to achieve a more comprehensive understanding of changes in polyphenol composition from one matrix to the next. The resources available to this study and the specific objectives are contextualised below.

The study has the advantage of prior work in a Sauvignon Blanc vineyard in the Elgin valley of South Africa, a high altitude and cool climate area (Young et al. 2016; Du Plessis 2017; Honeth 2018). This vineyard has been validated as a model vineyard according to field-omics principles (Alexanderson et al., 2014) and will also be used in this study to sample grapes from. In this vineyard, over multiple seasons, two distinct wine styles were achieved by creating high light (HL) and low light (LL) bunch microclimates through early leaf removal. The characterisation of the microclimates confirmed that leaf removal did not affect the bunch or berry temperatures, but caused light exposure to be consistently changed in the bunch zone, leading to more exposed grapes in the HL environment. (Young et al, 2016). Molecular and metabolite profiling of the berries during all growth stages showed that while berry size and the sugars and organic acids were minimally

Stellenbosch University https://scholar.sun.ac.za

affected, the major impacts were on amino acids, as well as secondary metabolites such as pigments and volatiles compounds and/or precursors (Young et al. 2016; Du Plessis 2017). Moreover, the phenolic composition of the LL versus HL berries was significantly different, with the more exposed grapes accumulating more phenolic compounds. The increased phenolic content and profiles were also supported by observed changes in the gene expression patterns of the enzyme-encoding genes of the phenylpropanoid pathway between the LL and HL berries (Du Plessis 2017). Similar results have been reported from other experiments where light exposure was modulated (Downey et al. 2004; Gregan et al. 2011; Koyama et al. 2012). From this model vineyard (Suklje et al. 2014 and Honeth 2018), as well as from other similar studies (Ristic et al. 2010; Oliveira et al. 2012), it has been concluded that the modulated light exposure also influence the wine styles of the Sauvignon Blanc. Grapes from the LL environments typically produced more herbaceous and green Sauvignon Blanc styles, in contrast to more thiol-driven, aromatic styles achieved from grapes obtained from the HL environments. Furthermore, HL environments delivered wine that were perceived as bitter (Suklje et al, 2014) and bitterness in white wine has been connected to high catechin concentrations (Gawel et al 2013).

Stellenbosch University https://scholar.sun.ac.za

This highly characterised vineyard therefore provides the ability to study grape berries from the same vineyard that have been extensively characterised in terms of their metabolic profiles. For the purpose of this study, the LL microclimate is expected to provide grapes with lower “phenolic potential”, compared to the higher “phenolic potential” of the berries from the HL microclimates. Phenolic potential in this context refers to the question: “What is the polyphenolic content, profile of compounds, and their distribution patterns in the berry tissues (skin, pulp and seed)”? Given the fact that the metabolic potential of grapes is “fixed” at harvest, the harvested berries holds the maximum phenolic potential that could be available for extraction during the wine-making steps. To link the grape potential to the wine matrix potentials, the typical white-wine making steps could be modulated to stimulate increased extractability of polyphenolic compounds from grape skins and pomace.

The approach would rely on the ability to implement analytical methods to extract and measure a variety of polyphenols in the tissues from the grapes, as well as the wine matrices such as juice, pomace, wines and sediments. Given the known interactions between berry cell wall components and polyphenols, the inclusion of profiling tools such as comprehensive microarray polymer profiling (CoMPP) to also assess the structural disintegration of the berry cell walls throughout wine-processing could provide evidence of the modulating effects of the cell walls by trapping polyphenols. The experiments were planned to also allow sensorial analyses on all the wines.

The following objectives were formulated:

1. Quantify and profile the polyphenolic content of grapes harvested from the LL and HL microclimates from the Sauvignon Blanc model vineyard to characterise (and confirm) their polyphenol potentials and

Stellenbosch University https://scholar.sun.ac.za

compare the distribution patterns of the compounds between the different berry tissues (skin, pulp and seeds) of LL and HL berries.

2. Implement an experimental layout to test the impacts of cold, pre-fermentative skin contact and/or sediment contact during fermentation as modulated wine-making steps to potentially increase extractability of polyphenols into the juice and wine matrices. Follow the outcomes by determining the polyphenolic contents and profiles of the pomace, juice, sediment and wines, as well as the sensorial profiles of the wines for mouthfeel and astringency (as factors typically linked to increased phenolic content).

Stellenbosch University https://scholar.sun.ac.za

3. Investigate potential polyphenol interactions with the cell wall material and grape proteins during wine-making by relating the polyphenolic contents and profiles of the typically discarded fractions (pomace and juice sediment) to the disintegrated berry cell wall structures, as determined by cell wall profiling.

Objectives 1-3 are addressed in chapters 3-5 of the thesis. The thesis furthermore includes a concise literature review presented in chapter 2 and is concluded in chapter 6 with general insights and concluding remarks.

1.3. References

Adams DO (2006) Phenolics and Ripening in Grape Berries. Am J Enol Vitic 3:249–256.

Aleixandre-Tudo J, Weightman C, Panzeri V, Nieuwoudt HH, Toit WJ (2015) Effect of Skin Contact Before and During Alcoholic Fermentation on the Chemical and Sensory Profile of South African Chenin. South African J Enol Vitic 36:366–377.

Arnous A, Meyer AS (2009) Quantitative prediction of cell wall polysaccharide composition in grape (vitis vinifera l.) and apple (malus domestica) skins from acid hydrolysis monosaccharide profiles. J Agric Food Chem 57:3611–3619. doi: 10.1021/jf900780r

Bautista-Ortín AB, Ruiz-García Y, Marín F, Molero N, Apolinar-Valiente R, Gómez-Plaza E (2015) Remarkable proanthocyanidin adsorption properties of monastrell pomace cell wall material highlight its potential use as an alternative fining agent in red wine production. J Agric Food Chem 63:620–633. doi: 10.1021/jf503659y

Berli FJ, Fanzone M, Piccoli P (2011) Solar UV-B and ABA Are Involved in Phenol Metabolism of Vitis vinifera L . Increasing Biosynthesis of Berry Skin Polyphenols. J Agric Food Chem 4874–4884. doi: 10.1021/jf200040z

Bindon KA, Li S, Kassara S, Smith PA (2016) Retention of Proanthocyanidin in Wine-like Solution Is Conferred by a Dynamic Interaction between Soluble and Insoluble Grape Cell Wall Components. J Agric Food Chem 64:8406–8419. doi: 10.1021/acs.jafc.6b02900

Bindon KA, Smith PA (2013) Comparison of the affinity and selectivity of insoluble fibres and commercial proteins for wine proanthocyanidins. Food Chem 136:917–928. doi: 10.1016/j.foodchem.2012.08.016

Bindon KA, Smith PA, Holt H, Kennedy JA (2010a) Interaction between Grape-Derived Proanthocyanidins and Cell Wall Material . 2 . Implications for Vinification. J Agric Food Chem 10736–10746. doi:

Stellenbosch University https://scholar.sun.ac.za

10.1021/jf1022274

Bindon KA, Smith PA, Kennedy JA (2010b) Interaction between Grape-Derived Proanthocyanidins and Cell Wall Material . 1 . Effect on Proanthocyanidin Composition and Molecular Mass. J Agric Food Chem 2520–2528. doi: 10.1021/jf9037453

Stellenbosch University https://scholar.sun.ac.za

Boss. K. P, Davies. C, Robinson, S. P (1996) Expression of anthocyanin biosynthesis pathway genes in red and white grapes. Plant molecular biology. 32 (2): 565-569.

Bourzeix. M, Heredia. N, Kovac. V (1983) Richness of different varieties of total phenolic and anthocyanins. Progressive Agriculture and Viticulture. 17: 532-428

Cejudo-Bastante M, Castro-Vazquez L, Hermosín-Gutierrez MP-C (2011) Combined Effects of Prefermentative Skin Maceration and Oxygen Addition of Must on Color-Related Phenolics , Volatile Composition , n White Wine and Sensory Characteristics of Air e. J Agric Food Chem 12171–12182. doi: 10.1021/jf202679y

Cheynier V, Rigaud J, Souquet J-MM, Barillere JM, Moutounet M (1989) Effect of Pomace Contact and Hyperoxidation on the Phenolic Composition and Quality of Grenache and Chardonnay Wines. Am J Enol Vitic 40:36–42.

Conde C, Silva P, Fontes N, Dias ACP, Tavares RM, Sousa MJ, Agasse A, Delrot S, Gerós H (2007) Biochemical Changes throughout Grape Berry Development and Fruit and Wine Quality. Food Chem 1:1–22.

Darias-Martín JJ, Rodríguez O, Díaz E, Lamuela-Raventós RM (2000) Effect of skin contact on the antioxidant phenolics in white wine. Food Chem 71:483–487. doi: 10.1016/S0308-8146(00)00177-1

Davalos A, Bartolome B, Gomez-Corsoves C (2005) Food Chemistry Antioxidant properties of commercial grape juices and vinegars. J Food Chem 93:325–330. doi: 10.1016/j.foodchem.2004.09.030

Del Rio J, Gomez P, Baidez A, Fuster Ma, Ortuno A, Frias V (2004) Phenolic compounds have a role in the defence mechanism protecting grapevine against the fungi involved in Petri disease. Phytophysiology 87–94.

Di Lecce G, Arranz S, Jáuregui O, Tresserra-rimbau A, Quifer-rada P, Lamuela-raventós RM (2014) Phenolic profiling of the skin , pulp and seeds of Albariño grapes using hybrid quadrupole time-of-flight and triple-quadrupole mass spectrometry. Food Chem 145:874–882. doi: 10.1016/j.foodchem.2013.08.115

Di Lecce G, Boselli E, D'Ignazi G, Frega NG (2013) LWT - Food Science and Technology Evolution of phenolics and glutathione in Verdicchio wine obtained with maceration under reductive conditions. LWT - Food Sci Technol 53:54–60. doi: 10.1016/j.lwt.2013.03.006

Downey MO, Dokoozlian NK, Krstic MP (2006) Cultural Practice and Environmental Impacts on the Flavonoid Composition of Grapes and Wine : A Review of Recent Research. Am J Enol Vitic 3:257–268.

Downey MO, Harvey JS, Robinson SP (2003) Analysis of tannins in seeds and skins of Shiraz grapes throughout berry development. Aust J Grape Wine Res 15–27.

Downey MO, Harvey JS, Robinson SP, Industry CP, Unit H, Box PO, Osmond G (2004) The effect of bunch shading on berry development and flavonoid accumulation in Shiraz grapes. Aust J Grape Wine Res 55–73.

Dragana C, Davidovic SM, Todic SR, Bešlic ZS, Tešic L (2016) Identification and quantification of phenolic compounds in berry skin , pulp , and seeds in 13 grapevine varieties grown in Serbia. 211:243–252.

Du Plessis K (2017) The evaluation of the impact of microclimatic factors on grapevine berries in a vineyard

Stellenbosch University https://scholar.sun.ac.za

setting through molecular profiling. Thesis, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

Ferreira-lima NE, Burin VM, Caliari V (2016) Impact of Pressing Conditions on the Phenolic Composition , Radical Scavenging Activity and Glutathione Content of Brazilian Vitis vinifera White Wines and Evolution During Bottle Ageing. Food Biotechnol Technol 944–957. doi: 10.1007/s11947-016-1680-7

Fontes N, Delrot S, Gualtar C De, Functional G (2011) Grape Berry Vacuole: A Complex and Heterogeneous Membrane System Specialized in the Accumulation of Solutes. Am J Enol Vitic. doi: 10.5344/ajev.2011.10125

Fulcrand H, Salas E, Cheynier V (2006) Phenolic Reactions during Winemaking and Aging. 3:289–297.

Gomez-Miguez JM, Gonzalez-Miret LM, Hernanz D, Fernandez AM, Vicario IM, Heredia FJ (2007) Effects of prefermentative skin contact conditions on colour and phenolic content of white wines. J Food Eng 78:238–245. doi: 10.1016/j.jfoodeng.2005.09.021

Gregan SM, Wargent JJ, Liu L, Shinkle J, Hofmann R, Winfield C, Trough M, Jordan B (2011) Effects of solar ultraviolet radiation and canopy manipulation on the biochemical composition of Sauvignon Blanc grapes. Aust J Grape Wine Res. doi: 10.1111/j.1755-0238.2012.00192.x

Hernanz D, Recamales ÁF, González-Miret ML, Gómez-Míguez MJ, Vicario IM, Heredia FJ (2007) Phenolic composition of white wines with a prefermentative maceration at experimental and industrial scale. J Food Eng 80:327–335. doi: 10.1016/j.jfoodeng.2006.06.006

Honeth. C (2018) Evaluating the effects of UVB radiation on field grown grape berry metabolites under high and low light microclimates and the consequent impacts on the resulting wines. Thesis, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

Ivanova V, Stefova M, Chinnici F (2010) Determination of the polyphenol contents in Macedonian grapes and wines by standardized spectrophotometric methods. J Serbian Chem Soc 75:45–59. doi: 10.2298/JSC1001045I

Kammerer D, CLaus A, Carle R, Schieber A (2004) Polyphenol Screening of Pomace from Red and White Grape Varieties (Vitis vinifera L .) by HPLC-DAD-MS / MS. J Agric Food Chem 52:27–30.

Kennedy JA, Hayasaka Y, Waters EJ, Jones GP (2001) Composition of Grape Skin Proanthocyanidins at Different Stages of Berry Development. 5348–5355.

Kennedy JA, Matthews MA, Waterhouse AL (2000) Changes in grape seed polyphenols during fruit ripening. 55:77–85.

Koyama K, Ikeda H, Poudel PR, Goto-yamamoto N (2012) Phytochemistry Light quality affects flavonoid biosynthesis in young berries of Cabernet Sauvignon grape. Phytochemistry 78:54–64. doi: 10.1016/j.phytochem.2012.02.026

Lecas M, Brillouet J-M (1994) Cell wall compostion of grape berry skins. 35:1241–1243.

Oliveira M, Vilanova M, Diago P, Genisheva Z, Tardaguila J (2012) Early leaf removal impact on volatile composition of Tempranillo wines. 935–942. doi: 10.1002/jsfa.4673

Ortega-Regules A, Ros-garcía JM, Bautista-ortín BA, Lopez-Roca JM, Gómez-plaza E (2008) Differences in morphology and composition of skin and pulp cell walls from grapes (Vitis vinifera L .): technological implications. Eur Food Res Technol 223–231. doi: 10.1007/s00217-007-0714-9

Patel P, Herbst-Johnstone M, Lee SA, Gardner RC, Weaver R, Nicolau L, Kilmartin PA (2010) Influence of Juice Pressing Conditions on Polyphenols , Antioxidants , and Varietal Aroma of Sauvignon blanc

Stellenbosch University https://scholar.sun.ac.za

Microferments. Agric Food Chem 58:7280–7288. doi: 10.1021/jf100200e

Ristic R, Bindon K, Francis LI, Herderich MJ, Iland PG (2010) Flavonoids and C 13 -norisoprenoids in Vitis vinifera L . cv . Shiraz : relationships between grape and wine composition , wine colour and wine sensory properties. doi: 10.1111/j.1755-0238.2010.00099.x

Stellenbosch University https://scholar.sun.ac.za

Ristic R, Downey MO, Iland PG, Bindon K, Francis L, Herderich M, Robinson S (2006) Exclusion of sunlight from Shiraz grapes alters wine colour ,. Aust J Grape Wine Res 53–65.

Rodríguez Montealegre R, Romero Peces R, Chacón Vozmediano JL, Martínez Gascueña J, García Romero E (2006) Phenolic compounds in skins and seeds of ten grape Vitis vinifera varieties grown in a warm climate. J Food Compos Anal 19:687–693. doi: 10.1016/j.jfca.2005.05.003

Rossello C, Teissedre P (2013) Characterization of Polyphenols and Antioxidant Potential of White Grape Pomace Byproducts (Vitis vinifera L.). doi: 10.1021/jf403168k

Šebela D, Turóczy Z, Olejníčková J, Kumšta M, Sotolář R (2017) Effect of Ambient Sunlight Intensity on the Temporal Phenolic Profiles of Vitis Vinifera L . Cv . Chardonnay During the Ripening Season – A Field Study. South African J Enol Vitic 38:94–102.

Sokolowsky M, Rosenberger A, Fischer U (2015) Sensory impact of skin contact on white wines characterized by descriptive analysis , time – intensity analysis and temporal dominance of sensations analysis. Food Qual Prefer 39:285–297. doi: 10.1016/j.foodqual.2014.07.002

Suklje K, Antalick G, Coetzee Z, Schmidtke LM, Basa Cesnic H, Brandt J, Toit WJ, Lisjak K, Deloire A (2014) Effect of leaf removal and ultraviolet radiation on the composition and sensory perception of Vitis vinifera L . cv . Sauvignon Blanc wine. Aust J Grape Wine Res 10:223–233. doi: 10.1111/ajgw.12083

Young PR, Eyeghe-bickong HA, Plessis K, Alexandersson E, Jacobson DA, Coetzee Z, Deloire A, Vivier MA (2016) Grapevine Plasticity in Response to an Altered Microclimate : Sauvignon Blanc Modulates Speci fi c Metabolites in Response to Increased Berry Exposure 1. 170:1235–1254. doi: 10.1104/pp.15.01775

Stellenbosch University https://scholar.sun.ac.za

Chapter 2

Stellenbosch University https://scholar.sun.ac.za

Literature review

White grape polyphenols and the factors that influence their levels in the berry, juice and wine matrices.

Stellenbosch University https://scholar.sun.ac.za

Chapter 2 - White grape polyphenols and the factors that influence their levels in the berry, juice and wine matrices.

2.1. Introduction

Stellenbosch University https://scholar.sun.ac.za

Non-volatile polyphenols are secondary metabolites that are produced during development and ripening of the grape berry. These compounds exert defensive functions against infection and environmental stresses (Del Rio et al. 2004; Gregan et al. 2011; Agati et al. 2013). A diverse family of non-volatile phenolic compounds exist which includes: phenolic acids, flavonoids, stilbenes, coumarins and lignans (Adams 2006; Garrido and Borges 2013).

Non-volatile polyphenols are unevenly distributed throughout white and red grape berry tissues where each tissue primarily accumulate a particular group in abundance. The environment in which grapes grow has an influence on its phenolic composition and viticultural practices, aimed at manipulating the growth environment (soil, water, nitrogen, light), have been established to modulate the levels of the compounds due to their quality impacts (Koundouras et al. 2006; Cavaliere et al. 2010; Gregan et al. 2011). The phenolic composition of grapes is important as these compounds are distributed to the juice and wine during wine making where they influence colour and sensory properties wine (Ristic et al. 2006; Cejudo-Bastante et al. 2011b). Bitterness and astringency are the main sensory attributes associated with phenolic compounds in wine (Arnold et al. 1980; Robichaud and Noble 1990; Brossaud et al. 2000). The phenolic content of juice and wine can also be manipulated by altering certain wine making parameters such as skin contact time (Gomez-Miguez et al. 2007; Hernanz et al. 2007; Cejudo-Bastante et al. 2011a), pressing pressure (Maggu et al. 2007; Patel et al. 2010; Ferreira-Lima et al. 2016) or juice clarification steps (Singleton et al. 1975; Liu et al. 1987; Coelho et al. 2018).

Polyphenol content of juice and wine can also be influenced by oxidation (Darias-Martín et al. 2000; Recamales et al. 2006; Gomez-Miguez et al. 2007), the addition of sulfur dioxide (Danilewicz et al. 2008; Coetzee et al. 2013), binding reactions to cell wall material (Hazak et al. 2005; Bindon et al. 2010a; Bindon et al. 2016) and proteins (Esteruelas et al. 2011; Van Sluyter et al. 2015), which affects their availability during wine making.

Polyphenols in white cultivars are not as well studied as they are in red cultivars. Red wine making entails skin contact during fermentation which enhances total polyphenol content, importantly anthocyanins and tannins which are crucial compounds for colour stability and sensory perception (Ristic et al. 2006; Paixão et al. 2007; Ristic et al. 2010). Although white wine has a lower polyphenol content, these compounds exert a noticeable effect on white wine quality and therefore require more research attention (Cejudo-Bastante et al. 2011a; Aleixandre-Tudo et al. 2015). This review will give an overview on the structural chemistry of grape polyphenols and their synthesis. The distribution of polyphenols in the grape tissues of white cultivars, their

Stellenbosch University https://scholar.sun.ac.za

composition in juice and wine and the factors that influence their availability during wine making will be the focal point.

2.2. Grape polyphenols: structure and chemistry

Stellenbosch University https://scholar.sun.ac.za

Polyphenols are chemical compounds characterized by the presence of a hydroxylated phenyl ring within the backbone. These compounds are divided into flavonoids and non-flavonoids based on their chemical structure and can be further subdivided based on their substitution patterns on the phenyl ring (Figure 2.1) (Moreno and Peinado 2012). In the following sections the chemical structure of the most prevalent polyphenolic compounds found in white and red grapes will be discussed.

2.2.1. Non-flavonoids

The non-flavonoid phenols are constituted by three groups: hydroxycinnamic acids, hydroxybenzoic acids and stilbenes. Hydroxycinnamic acids and hydroxybenzoic acids are collectively referred to as phenolic acids. These compounds generally occur either in their free or conjugated form.

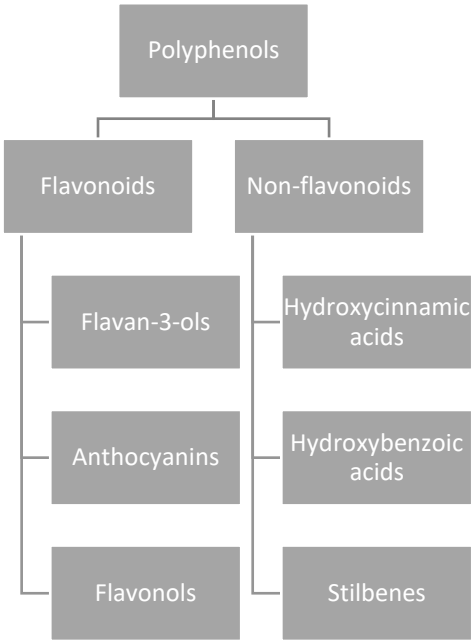


Figure 2.1: Classification of the major grape polyphenol classes.

2.2.1.1. Hydroxycinnamic acids

Hydroxycinnamic acids (HCAs) generally contain nine carbons (C_6-C_3) and may display *cis* or *trans* (most abundant) isomeric forms due to the presence of a double bond on the lateral side of the phenyl ring. HCAs are mainly present as tartaric acid esters, but may also be found as bound glycosides (Figures 2.2A and B) (Moreno and Peinado 2012). The most common HCAs in grapes include caftaric acid, coumaric acid, caffeic acid, coutaric acid and fertaric acid (Rodríguez Montealegre et al. 2006; Di Lecce et al. 2014; Dragana et al. 2016).

Stellenbosch University https://scholar.sun.ac.za

2.2.1.2. Hydroxybenzoic acids

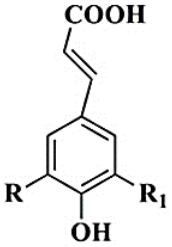
Hydroxybenzoic acids (HBA) consists of seven carbons (C_6-C_1) and are present as glycosylated forms or as esters in grapes (Figure 2.2C) (Moreno and Peinado 2012). The most common HBA in grapes include gallic acid, ellagic acid, syringic acid and vanillic acid (Di Lecce et al. 2014; Dragana et al. 2016).

Stellenbosch University https://scholar.sun.ac.za

2.2.1.3. Stilbenes

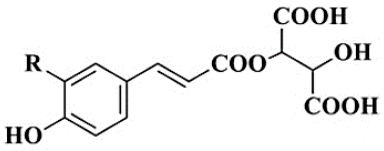
Stilbene, the least abundant class of non-flavonoids, are comprised of two phenyl rings joined by a two carbon bridge (Moreno and Peinado 2012) (Figure 2.2D). Resveratrol is the main stilbene in grapes and is commonly present as *cis*-resveratrol (Di Lecce et al. 2014; Dragana et al. 2016).

A - Hydroxycinnamic acids



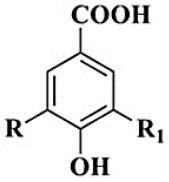
	R	R_1
Coumaric	H	H
Caffeic	OH	H
Coutaric	H	H
Caftaric	H	OH

B – Esterified hydroxycinnamic acids



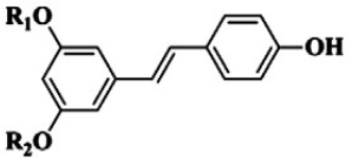
	R
Coumaroyltartaric acid (coutaric acid)	H
Caffeoyltartaric acid (caffeic acid)	OH
Feruloyltartaric acid (fertaric acid)	OCH_3

B - Hydroxybenzoic acids



	R	R_1
Gallic	OH	OH
Vanillic	OCH_3	H
Syringic	OCH_3	OCH_3
Salicylic	H	H

D – Stilbenes



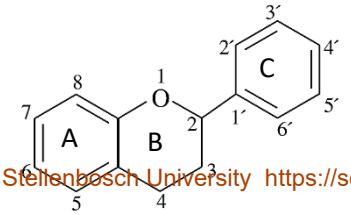
	R	R_2
Trans-resveratrol	H	H

Figure 2.2: The chemical structures of (A) hydroxycinnamic acids, (B) the esterified form of hydroxycinnamic acids, (C) hydroxybenzoic acids and (D) stilbenes commonly found in grapes. R and R1 may be substituted by an H, OH or OCH3 to form a different non-flavonoid specific to a class (Garrido and Borges 2013).

2.2.2. Flavonoids

Flavonoids have a C_6-C_3-C_6 structure and is subdivided into flavonols, flavan-3-ols and anthocyanins (Figure 2.1). These compounds are the most abundant polyphenols in grapes and contain two phenyl rings (A and B), which are connected to a heterocycle (C) (Figure 2.3).

Stellenbosch University https://scholar.sun.ac.za



Stellenbosch University https://scholar.sun.ac.za

Figure 2.3: The basic backbone of a flavonoid compound (Fulcrand et al. 2006).

2.2.2.1. Flavan-3-ols

The monomeric flavan-3-ol or flavanol forms a large group of different isoforms of catechin. Catechin contains two asymmetric carbons which allows the formation of four different isomers: (+/-) catechin and (+/-) epicatechin (Figure 2.4). The R1 group on the heterocycle can be substituted with a hydroxyl group (OH) to give rise to an additional four isomers (+/-) gallocatechin and (+/-) epigallocatechin. This hydroxyl group (on R1) may also be esterified with gallic acid to form epicatechin gallate (Moreno and Peinado 2012).

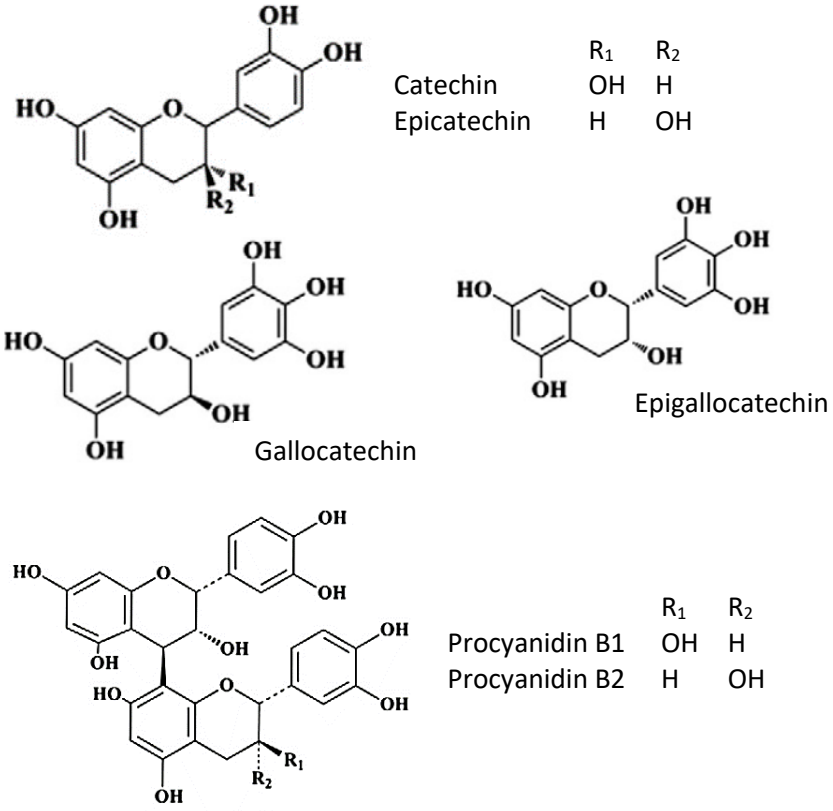


Figure 2.4: The flavan-3-ol subgroups present in grapes (Garrido and Borges 2013).

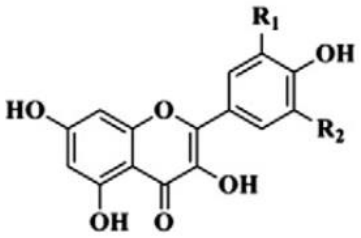
Flavan-3-ol monomers tend to form oligomers and polymers which are referred to as procyanidins or tannins respectively. The degree of polymerization dictates whether a flavan-3-ol is a procyanidin or tannin (Figure 2.4). Procyanidins are found as dimers (type-A and type-B) or trimers (type-C and type-D). In order for a flavan-3-ol to be considered as a tannin it should at least contain 10-12 flavan-3-ol monomeric subunits (Moreno and Peinado 2012).

Stellenbosch University https://scholar.sun.ac.za

2.2.2.2. Flavonols

The flavonols are yellow pigments and are present within the grapes in glycosylated form. Flavonols may also be conjugated with other sugars such as galactose, xylose and arabinose. The free forms of flavonols, aglycones, are present in wine as quercetin, kaempferol, myricetin and isorhamnetin (Figure 2.5) (Moreno and Peinado 2012).

Stellenbosch University https://scholar.sun.ac.za



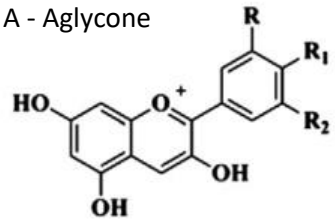
	R_1	R_2
Quercetin	OH	H
Kaempferol	H	H
Myricetin	OH	OH
Isorhamnetin	OCH_3	OH

Figure 2.5: The chemical structure of flavonols and their substitutive possibilities (Garrido and Borges 2013).

2.2.2.3. Anthocyanins

Anthocyanins are an abundant group of flavonoids and is responsible for the red colour of grapes. These phenolic compounds are mostly exclusively present in red cultivars (Bourziex et al 1983). An anthocyanin is an anthocyanidin bound to a sugar molecule via a glycosydic linkage where the glycosylated form is more stable than the aglycone (Morena and Peinado 2012). Six main anthocyanins have been identified in grape tissue which include delphinidin, cyanidin, malvidin, petunidin, peonidin and pelargonidin (Figure 2.6) (Kammerer et al. 2004; Romero-Cascales et al. 2005; Gomez-Alonso et al. 2007).

A - Aglycone



	R	R_1	R_2
Cyanidin	OH	OH	
Peonidin	OCH_3	OH	
Delphinidin	OH	OH	OH
Malvidin	H	OH	OCH_3
Petunidin	OCH_3	OH	OH
Pelargonidin	OCH_3	OH	H

B -Glycoside

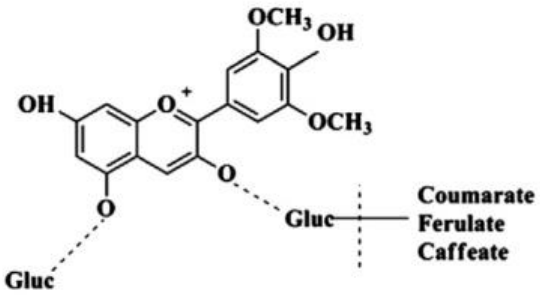


Figure 2.6: Anthocyanidins (A) and anthocyanins (B) commonly present in grapes (Garrido and Borges 2013).

Stellenbosch University https://scholar.sun.ac.za

2.3. Polyphenol biosynthesis

The biosynthesis of polyphenols occurs via two pathways: the phenylpropanoid pathway and the flavonoid pathway. The amino acid phenylalanine, a product of the shikimate pathway, serves as the precursor for the phenylpropanoid pathway while the resulting metabolites serve as the precursor for the flavonoid pathway (Figure 2.7) (Teixera et al. 2013).

Stellenbosch University https://scholar.sun.ac.za

Phenyl ammonia lyase (PAL) is the first enzyme involved in polyphenol biosynthesis and is responsible for the deamination of phenylalanine into a cinnamic acid. The cinnamic acid is subsequently hydroxylated to form *p*-coumaric acid by cinnamate-4hydroxylase (C4H). p-Coumaric acid is esterified by CoA-ligase (4CL), producing 4-coumaroyl-CoA. Hydroxylation of p-Coumaric acid may also occur to form caffeic acid which can be converted into ferulic acid by methylation. 4-Coumaroyl-CoA serves as a substrate for both the stilbene and flavonoid parts of the pathway (Adams 2006; Teixera et al. 2013).

Stilbene synthase (SS) and chalcone synthase (CHS) are responsible for the formation of stilbenes and flavonoids respectively. Both enzymes rely on the same substrate to generate their respective products (polyphenols). SS converts 4-coumaroyl-CoA to resveratrol through three condensation reactions where the terminal carboxyl group is removed prior to closure of the A ring. CHS combines three malonyl-CoAs with 4-coumaroyl-CoA to give tetrahydroxychalcone and carbon dioxide. Tertrahydroxychalcone serves as a substrate for the formation of all different classes of flavonoids (Adams 2006; Conde et al. 2007; Teixera et al. 2013). The enzyme glucose-flavonoids 3-*o*-glucosyl transferase (UFGT) is responsible for anthocyanin synthesis in red cultivars, but UFGT is not synthesized in white cultivars due to mutations in the promotor genes, VvMYBA1 and VvMYBA2 (Boss hjklet al 1996; Walker et al. 2007; Kobayashi 2009).

Stellenbosch University https://scholar.sun.ac.za

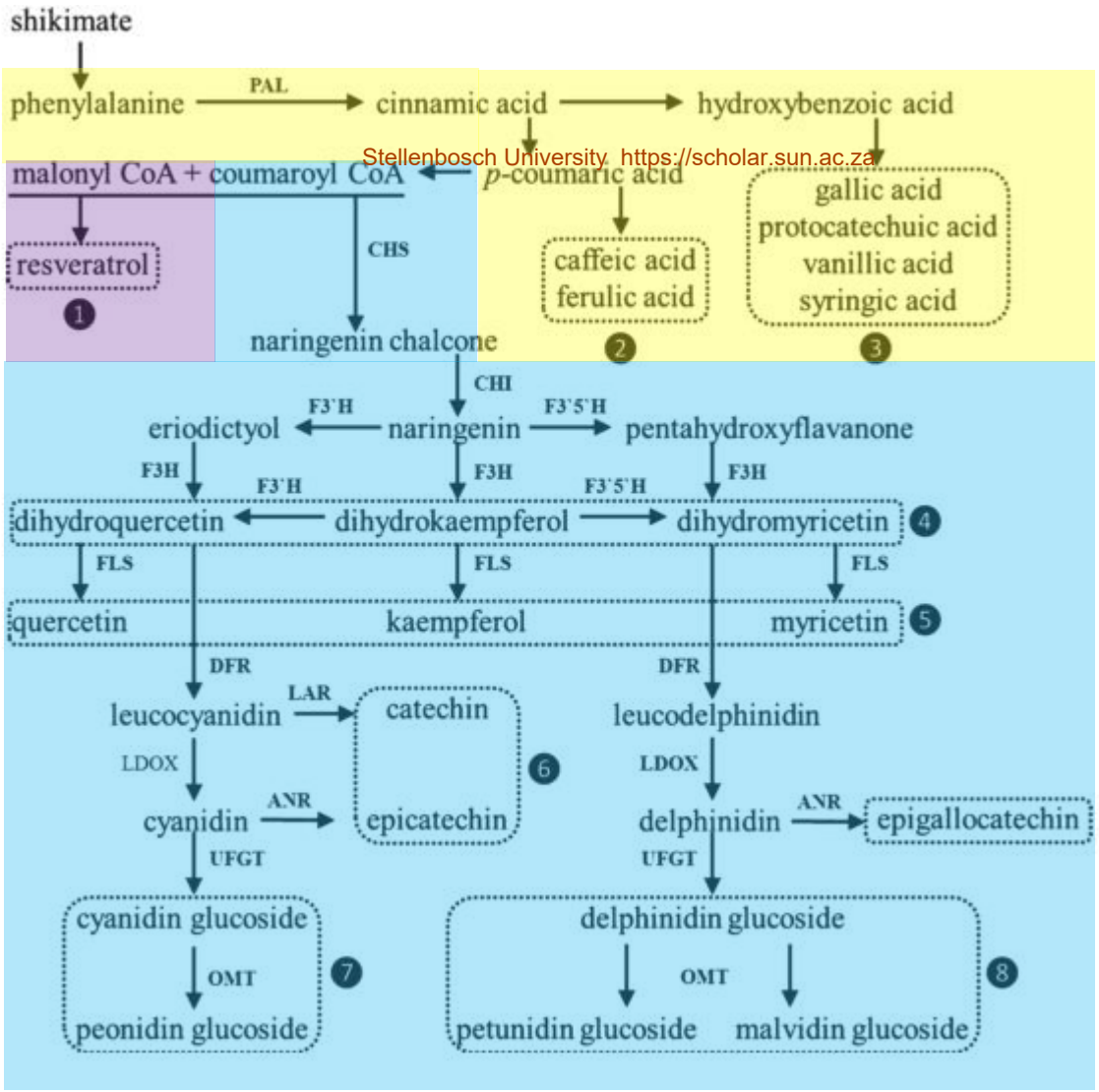


Figure 2.7: The biosynthesis pathway of non-volatile polyphenols in grapes. Individual compounds are outlined by dotted rectangles. The different parts of the pathway is coloured according to the compounds that are synthesized: hydroxycinnamic acids and hydroxybenzoic acids (yellow), stilbenes (purple) and flavonoids (blue). Subgroups are indicated by numbers: 1, stilbenes; 2, hydroxycinnamic acids; 3, hydroxybenzoic acids; 4, dihydroflavonols; 5, flavonols; 6, flavanols; 7, anthocyanins; 8, anthocyanins Abbreviations: phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), chalcone isomerase (CHI), flavonoid 3-hydroxylase (F3'H), flavonoid 3'5'-hydroxylase(F3'5'H), flavanone 3-hydroxylase (F3H), flavonol synthase (FLS), dihydroflavonol reductase (DFR), leucoanthocyanidin reductase (LAR), leucoantho- cyanidin dioxygenase (LDOX), anthocyanidin reductase (ANR), flavonoid 3-glucosyltransferase (UFGT), o-methyltransferase (OMT) (Berli et al. 2011).

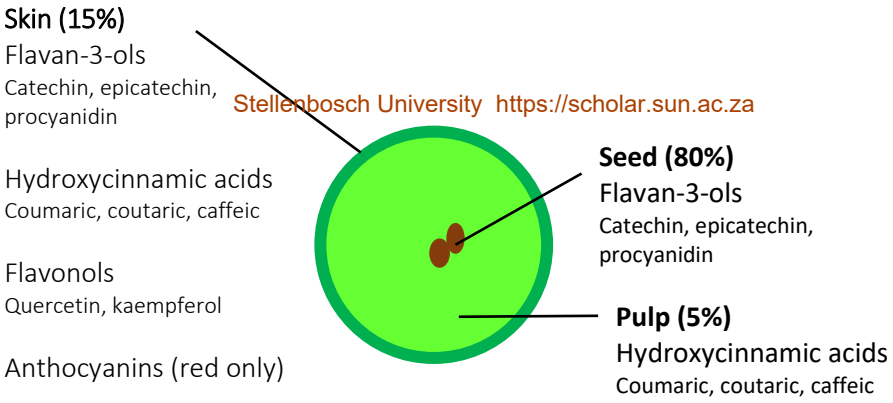
2.4. Polyphenols in white grapes, juice and wine

2.4.1. Grapes

Polyphenols are present throughout the grape tissues (skin, pulp and seed) and function as protectors against environmental stress and pathogen infection (Del Rio et al. 2004; Guidoni et al. 2008; Gregan et al. 2011;

Stellenbosch University https://scholar.sun.ac.za

Agati et al. 2013). The different phenolic classes are not evenly distribution throughout the grape tissues as specific polyphenols are more concentrated in particular tissues (Figure 2.8).



Stellenbosch University https://scholar.sun.ac.za

Figure 2.8: The distribution of phenolic groups throughout white, including red grape tissues. The diagram shows the main phenolic groups that each tissue accumulates (Rodríguez Montealegre et al. 2006; Di Lecce et al. 2014; Dragana et al. 2016).

Flavonoids are the most abundant polyphenol group in grapes. Flavan-3-ols are synthesized before *véraison* and decrease during ripening whereas flavonols increase after *véraison* (Downey et al. 2003; Berli et al. 2011; Šebela et al. 2017). The pulp contains the lowest concentration of flavan-3-ols with traces (0.2-2 µg/g fresh tissue) of catechin and epicatechin (Di Lecce et al. 2014; Dragana et al. 2016). In contrast, the seeds contain the highest concentration of flavan-3-ols with totals ranging from 478-1390-µg/g fresh weight (FW) for white cultivars (Table 2.1). The flavan-3-ols found in the seeds include catechin (present at the highest concentration), epicatechin, procyanidins and tannins with a degree of polymerization of up to 30 subunits (Kennedy et al. 2000; Alonso Borbalan et al. 2003; Di Lecce et al. 2014; Dragana et al. 2016). Compared to the seeds, the skin contains significantly lower concentrations of flavan-3-ols ranging between 21-96 µg/g fresh tissue (Rodríguez Montealegre et al. 2006; Di Lecce et al. 2014; Dragana et al. 2016). The individual flavan-3-ols present within the skin include catechin, epicatechin, dimeric and trimeric procyanidins (Freitas and Glories 1999; Rodríguez Montealegre et al. 2006; Di Lecce et al. 2014). Literature provides scarce evidence of tannins in the skin of white cultivars, whereas Downey et al. (2003) and Downey et al. (2004) reported tannins with a degree of polymerization up to 10 subunits in Shiraz skins (red cultivars).

High molecular weight polyphenols typically elutes in a non-resolved peak at the end of an HPLC run, which is generally accepted to indicate the presence of tannins (Di Lecce et al. 2014; Aleixandre-Tudo et al. 2018). These non-resolved peaks are reported as intact tannins which only provides an estimation of the total tannin content and not the degree of polymerization of the different fragments (Kennedy and Waterhouse 2000). In order to quantify and determine the degree of tannin polymerization, samples have to be purified (e.g. gel chromatography) followed by acid hydrolysis (e.g. phloroglucinolysis) (Matthews et al. 1997; Kennedy and Jones 2001; Downey et al. 2003).

Flavonols are primarily located within the skin of white grapes in glycosylated form. Total flavonol concentrations range between 30-170 µg/g fresh weight and commonly include quercetin, kaempferol,

Stellenbosch University https://scholar.sun.ac.za

isorhamnetin, myricetin and rutin in their glycosylated forms (glucoside, glucuronide, pentoside, etc). Quercetin is the most abundant flavonol in grape skins. These compounds are absent from the pulp whereas only trace amounts are present in the seeds (Rodríguez Montealegre et al. 2006; Di Lecce et al. 2014; Dragana et al. 2016). Flavonols have been proposed to protect against ultraviolet (UV) radiation (Teixera et al. 2013)

Stellenbosch University https://scholar.sun.ac.za

which justifies their concentration in the skin as it is the first line of defence against external stressors (Pinelo et al. 2006). It is important to note that concentration values for the same cultivar could differ between studies, as different extraction techniques and solvents are often used which would influence polyphenol extractability and therefore the reported levels (Su et al. 2014; Bosso et al. 2016).

Hydroxycinnamic acids (HCAs) are the second most abundant polyphenol in white grapes. The HCAs are synthesized before *véraison* where after synthesis decreases and concentrations remain constant (Berli et al. 2011; Šebela et al. 2017). The skin and pulp contain the highest concentrations of HCAs, with totals ranging between 12-20 µg/g FW and 2-6 µg/g FW respectively (Di Lecce et al. 2014; Dragana et al. 2016). The most abundant HCAs in grapes include caftaric acid, caffeic acid, coumaric acid and coutaric acid. Hydroxybenzoic acids (HBAs) and stilbenes are present in trace amounts within the skin of white grapes whereas these compounds are absent from the pulp. The seeds contain the highest concentration of HBAs (gallic acid, ellagic acid, gentisic acid) with totals reaching up to 100 µg/g FW (Rodríguez Montealegre et al. 2006; Di Lecce et al. 2014; Dragana et al. 2016).

Table 2.1: The phenolic compounds that accumulate in the tissue of white and red grapes (µg/g fresh weight) (Alonso Borbalan et al. 2003; Rodríguez Montealegre et al. 2006; Di Lecce et al. 2014; Dragana et al. 2016; Šebela et al. 2017).

	White grapes			Red grapes		
Compound	**Skin**	**Seed**	**Pulp**	**Skin**	**Seed**	**Pulp**
Hydroxycinnamic acids	12-38	Traces	2-6	2-18	Traces	Traces
Hydroxybenzoic acids	1-7	40-100	Traces	1-16	120-240	Traces
Flavonols	30-170	5-20	Traces	25-200	2-30	Traces
Flavan-3-ols	21-96	478-1390	Traces	14-96	80-2200	Traces

2.4.2. Juice

During white wine making, three sequential juice fractions are generated: free run, pressed and clarified juice (Table 2.2). The phenolic composition of the juices is relatively constant, but the concentration of individual polyphenols changes during wine making. The free run juice is generated after crushing and destemming, and contains HCA concentrations between 24-90 mg/L with caftaric acid being the most abundant. Flavonoid concentrations are low (0.4-3 mg/L) within free run juice with mostly catechin, epicatechin and quercetin-glucoside being present (Somers et al. 1987; Maggu et al. 2007; Patel et al. 2010; Ferreira-Lima et al. 2016). Pressed juice fractions have a similar total polyphenol composition as free run juice, with HCAs being more abundant than flavonoids. The HCAs concentrations in pressed juice is between 10-80 mg/L (Maggu et al.

Stellenbosch University https://scholar.sun.ac.za

2007; Patel et al. 2010) and flavonoids are present between 4-11 mg/L (Fuleki and Ricardo-Da-Silva 2003; Darias-Martín et al. 2004; Ferreira-Lima et al. 2016). Caftaric acid is also the most abundant polyphenol in pressed juice. Traces of dimeric procyanidins appear after pressing (Fuleki and Ricardo-Da-Silva 2003) which are not present after crushing. During pressing, grape cells are disrupted and compounds in the skin are

Stellenbosch University https://scholar.sun.ac.za

essentially forced into the juice which correlates with an increase in flavonoids and HCAs. A study by Honeth (2018) contradicts an increase in total flavonoid and HCA concentrations after pressing as these compounds were found at a lower concentration in the pressed juice compared to free run juice. Clarified juice have the same polyphenol composition as the preceding free run and pressed juice – HCAs concentration are higher than flavonoids. The total HCA concentration of clarified juice varies between 45-60 mg/L and flavonoids 4-11 mg/L (Hernanz et al. 2007; Cejudo-Bastante et al. 2011b; Coelho et al. 2018). Different methods exist (pectinolytic enzymes, bentonite, centrifugation and natural cold settling) for juice clarification prior to alcoholic fermentation, but it has been shown that the degree of juice clarity does not significantly influence the polyphenol concentrations (Singleton et al. 1975; Liu et al. 1987; Coelho et al. 2018).

2.4.3. Wine

The total polyphenol concentration of white wine ranges between 40-95 mg/L (Table 2.2) (Hernanz et al. 2007; Cejudo-Bastante et al. 2011b; Ferreira-Lima et al. 2016; Coelho et al. 2018). HCAs is the most abundant phenolic group found in white wine (25-50 mg/L) with caftaric acid or caffeic acid being present at the highest concentrations. Wine can have a higher concentration of HCAs than its preceding juice as hydroxycinnamic acid esters (e.g. caffeoyl tartaric acid, *p*-coumaroyl tartaric acid) are hydrolysed during fermentation, liberating their precursors (e.g. caffeic acid, coumaric acid) (Somers et al. 1987; Kallithraka et al. 2009; Di Lecce et al. 2013). Flavonoids in white wine are commonly present between 8-30 mg/L (Darias-Martín et al. 2000; Kallithraka et al. 2001; Recamales et al. 2006; Gomez-Miguez et al. 2007) where catechin is the dominant flavonoid. Young white wine contains procyanidin dimers and trimers (6-10 mg/L) which are generally not detected in the preceding juice (Kallithraka et al. 2001; Cosme et al. 2008; Aleixandre-Tudo et al. 2015). It has been suggested that the difference in the tannin content of red and white wines are partly due to the binding of anthocyanins (found only in red wines) to tannins that leads to their retention in solution (Singleton and Trousdale 1992). Flavonols in wine may be present as aglycones and glycosides compared to its presence only in glycosidic form in the grapes (Cejudo-Bastante et al. 2011b; Ferreira-Lima et al. 2016).

Stellenbosch University https://scholar.sun.ac.za

Table 2.2: The phenolic compounds present in white grape juice and wine (mg/L) (Gil-Minoz et al. 1999; Minussi et al. 2003; Gomez-Miguez et al. 2007; Hernanz et al. 2007; Cejudo-Bastante et al. 2011a; Ferreira-Lima et al. 2016; da Silva Padilha et al. 2017)

Phenolic group	Juice		Wine	
	White	Red	White	Red
Hydroxycinnamic acids	45-60	230-340	25-50	122-200
Hydroxybenzoic acids	Traces	5-22	Traces	16-28
Flavonols	Traces	Traces	Traces	10-30
Flavan-3-ols	4-11	250-370	8-30	180-310

Stellenbosch University https://scholar.sun.ac.za

Flavan-3-ols have been shown to make the biggest contribution towards the bitterness and astringency of wine, where the perceived intensity is enhanced with concentration (Arnold et al. 1980; Robichaud and Noble 1990; Brossaud et al. 2000). On the other hand HCAs such as caffeic acid and coumaric acid (commonly present in white wine), have been shown not to affect the quality of wine, nor the perception of bitterness and astringency (Verette et al. 1988). It is noteworthy to mention than HCAs can be perceived as both bitter and astringent at high concentrations (2 g/L) (Ferrer-Gallego et al. 2014). However, these concentrations are unrealistically high and would not be present in white wine at this quantity. Monomeric, oligomeric and polymeric flavan-3-ols contribute noticeably to wine astringency and bitterness. Robichaud and Noble (1990) spiked base white wine with different concentrations of catechin, gallic acid and grape seed tannin, and could relate catechin and gallic acid levels to bitterness and tannin to astringency. Similar results were reported by Arnold et al. (1980) and Brossaud et al. (2000) where the degree of flavan-3-ol polymerization was positively correlated to astringency and negatively correlated to bitterness. Other parameters such as pH and ethanol concentration also influence the perception of bitterness and astringency. A low pH and high ethanol concentration have been shown to increase the intensity at which bitterness and astringency is perceived in wine (Fischer and Noble 1994; Teissedre and Glories 2008; Gawel et al. 2017).

2.5. Impact of environmental factors on grape berry polyphenols

Several environmental factors influence polyphenol synthesis within the grape berry. Polyphenol synthesis within the grape is upregulated when environmental condition become stressful e.g. under water deficit (irrigation), high temperatures, intense light exposure or a nitrogen deficit within the soil (Haselgrove et al. 2000; Koundouras et al. 2006; Cavaliere et al. 2010; Šebela et al. 2017). In this review the focus will be on the effect of light exposure on grape flavonols.

Stellenbosch University https://scholar.sun.ac.za

2.5.1. Light exposure as environmental factor

The effect of light exposure on grape polyphenols is arguably the most influential and studied environmental factor. Investigations into the effect of light exposure on polyphenol composition have taken approaches involving the installation of physical structures including UV exclusion sheets (Gregan et al. 2011; Song et al. 2015; Honeth 2018), shading nets (Bureau et al. 2000; Berli et al. 2011) and bags or boxes (Dokoozlian 1996; Downey et al. 2004; Koyama et al. 2012). Other studies investigated leaf removal as the treatment (Gregan et al. 2011; Song et al. 2015; Šebela et al. 2017) or sampling from different parts of the canopy where light exposure is the most prevalent (Haselgrove et al. 2000). Some of these studies also focused on the effect of timing of shading or exposure treatments during grape development on the impact of berry and polyphenol composition.

Stellenbosch University https://scholar.sun.ac.za

Shading treatments attenuated flavonol concentrations and to a lesser extent tannin composition and quantity in whole grapes (Dokoozlian 1996; Downey et al. 2004; Koyama et al. 2012). These studies highlighted that differences in flavan-3-ol (tannin) concentrations due to light exposure were more prevalent during the earlier ripening period, but became comparable at harvest, whereas flavonols were consistently lower throughout ripening as well as at harvest in low light grapes. Furthermore, the skin flavonol concentrations was most affected by decreased light exposure, whereas the seeds were unaffected. The gene that regulates flavonol synthesis (flavonol synthase, FLS) was significantly upregulated after *véraison* in grapes in response to light exposure, but was comparable to shaded grapes pre-*véraison* (Downey et al. 2004; Koyama et al. 2012). The results of Dokoozlian (1996) supported the gene expression data of Downey et al. (2004) and Koyama et al. (2012), where polyphenols increased in grapes that were shaded throughout development and were only exposed to light during ripening, compared to a completely shaded control.

The use of UV exclusion sheets has been proven as an effective approach to study the effect of light exposure (or light quality) on flavonoid synthesis. The results of Gregan et al. (2011) on Sauvignon blanc grapes revealed that screens which allowed UV transmission had higher flavonol concentrations (glycosylated; quercetin and kaempferol) compared to fully shaded grapes (no leaves removed). These results also revealed that UV-B was the main elicitor of flavonol synthesis, as sheets which allowed UV-B transmission increased flavonol concentrations which were comparable to fully exposed grapes (leaves removed). Sheets which only allowed UV-A transmission only marginally increased flavonol concentrations. Similar results were reported by Song et al. (2015) and Honeth (2018) where UV-B transmission increased polyphenol concentration which reflected in the juice. Honeth (2018) also reported increased HCAs concentrations in the juice from Sauvignon blanc grapes originating from high light microcLimates (light exposed). The employment of plastic sheets as shading method appears to be more insightful than boxed treatments as it provides additional information on which UV rays stimulate polyphenol synthesis as some sheets allow selective transmission. Furthermore, the effect of light exposure was found to be more prominent on flavonoid synthesis compared to HCAs, HBA and stilbenes.

Stellenbosch University https://scholar.sun.ac.za

The removal of leaves around the bunch zones is often performed as a means of balancing vegetative growth and fruit production and reducing the incidence of fungal infection (Guidoni et al. 2008; Mosetti et al. 2016). Leaf removal is also a targeted treatment to increase the phenolic levels in grapes through light exposure. Similar to previously mentioned results on light exposure treatments, grapes where the leaves had been removed, displayed higher concentrations of flavonols in Sauvignon blanc grapes (Gregan et al. 2011) and higher HCAs, stilbenes and catechins in Chardonnay (Šebela et al. 2017).

Stellenbosch University https://scholar.sun.ac.za

2.6. The effect of skin contact on the polyphenol composition of juice and wine

The grape skin is the main source of flavan-3-ols (González-Manzano et al. 2004) and aroma compounds such as thiols, monoterpenes, esters and higher alcohols (Cabaroglu et al. 1997; Chone et al. 2006; Patel et al. 2010). White wine making generally involves crushing and destemming of the grapes, pressing of the crushed grapes to extract the juice, clarification of the juice and fermentation of clarified juice. These sequential procedures are usually considered effective as sufficient polyphenol, flavour (Ough 1969; Darias-Martín et al. 2004) and aroma compounds (Cabaroglu et al. 1997; Patel et al. 2010) are extracted from the skins for the production of quality white wine. Application of additional wine making techniques such as pre-fermentative skin contact (Darias-Martín et al. 2000; Gomez-Miguez et al. 2007; Hernanz et al. 2007; Cejudo-Bastante et al. 2011a; Olejar et al. 2015), adjusting pressing parameters (Darias-Martín et al. 2004; Maggu et al. 2007; Patel et al. 2010; Ferreira-Lima et al. 2016) or altering the steps to clarify the juice (van Jaarsveld et al. 2005; Coelho et al. 2018) have been shown to increase polyphenols, aroma and flavour precursor within the juice and wine. Here we will focus specifically on the effect of pre-fermentative skin contact on the polyphenol content of white grape juice and wine.

Pre-fermentative skin contact, or otherwise referred to as cold maceration, involves leaving the crushed and destemmed grape skins in contact with the juice at controlled conditions (temperature and time) prior to pressing. It has been well established that skin contact increases the polyphenol concentrations in the juice (Gomez-Miguez et al. 2007; Hernanz et al. 2007; Patel et al. 2010; Cejudo-Bastante et al. 2011a). Polyphenols diffuse from the vacuoles of the skin cells once the cells have been ruptured (during crushing) and extended skin contact facilitates this diffusion (Romero-Cascales et al. 2005). Hernanz et al. (2007) and Jesus et al. (2011) reported significant increases for all measured HCAs (caftaric acid, coutaric acid, caffeic acid, fertaric acid), flavonols (quercetin, kaempferol, myrecitin) and flavan-3-ols (catechin, epicatechin and dimeric procyanidins) in the juice after skin contact. On the contrary, Maggu et al. (2007) reported caftaric acid and quercetin at lower concentrations in the skin contact juice, but only allowed one hour of contact time. Skin contact is time and temperature dependent and these parameters influence the amount of polyphenols that are extracted into the juice and not the composition. It has been established that polyphenol extraction is more effective when contact time is long at low temperature (24 h at 5^{o}C), compared

Stellenbosch University https://scholar.sun.ac.za

to short contact times at a higher temperature (12 h at 20°C) (Gomez-Miguez et al. 2007; Hernanz et al. 2007).

White wine resulting from juice subjected to skin contact generally has a higher polyphenol concentration. Darias-Martín et al. (2000), Hernanz et al. (2007), Di Lecce et al. (2013) and Olejar et al. (2015) reported significantly higher polyphenol concentrations, particularly caftaric acid and catechin, in wines with skin contact. Not all polyphenol increases in the juice are subsequently reflected in the wine. Coutaric acid (Gomez-Miguez et al. 2007), catechin (Cejudo-Bastante et al. 2011a) and caftaric acid levels (Aleixandre-Tudo et al. 2015) have been reported to be lower in wine with skin contact.

Skin contact during fermentation is less common in white wine preparation than pre-fermentative skin contact. Fermentative skin contact involves initiating fermentation (adding yeast) to the crushed and destemmed must and pressing after alcoholic fermentation. This technique is standard for red wine making and enhances polyphenol extraction from the skins into the must. Singleton et al. (1975), Di Lecce et al. (2014) and Aleixandre-Tudo et al. (2015) reported significantly higher concentrations of HCAs and flavonoids in wine with fermentative skin contact compared to wines with pre-fermentative skin contact. The wines from Aleixandre-Tudo et al. (2015) was described as being bitter, astringent and sour, potentially due to the high catechin and polymeric flavan-3-ols recorded.

2.7. Polyphenol reactions that influence their availability in juice and wine

Polyphenols are highly reactive compounds. This reactivity relates to the phenyl ring in the backbone where the activated carbons are nucleophilic sites and promotes electrophilic substitution (Fulcrand et al. 2006). Phenols with the strongest nucleophilic properties are those with three hydroxyl groups equally distributed on the phenyl ring (Figure 2.4, catechin and epicatechin), as found in the A ring in the backbone of flavonoids (Figure 2.3). The adjacent B-ring is a electrophile and the position where interflavan bonds form during polyphenol polymerization (Adams 2006; Fulcrand et al. 2006). During wine making and aging, polyphenol concentrations fluctuate as a result of interactions with other polyphenols (Adams 2006; Fulcrand et al. 2006), grape derived metabolites (Maggu et al. 2007; Di Lecce et al. 2013; Ferreira-Lima et al. 2016; Tian et al. 2017) and supporting structures such as cell wall polysaccharides (Ortega-Regules et al. 2006; Bindon et al. 2010a; Bindon et al. 2010b), chemical constituents (sulfur dioxide) added by the winemaker (Danilewicz et al. 2008; Coetzee et al. 2013) and oxygen (Recamales et al. 2006; Cejudo-Bastante et al. 2011a). These parameters all influence the availability of unbound polyphenols which would in turn influence their effects on the sensory and colour attributes of wine.

Stellenbosch University https://scholar.sun.ac.za

2.7.1. Polyphenol changes during wine making

The structure of polyphenols in juice and wine can be modified through polymerization with other polyphenols or grape derived compounds, enzymatic and chemical reactions. These modifications influence the composition, concentration and bioactivity of polyphenols.

Stellenbosch University https://scholar.sun.ac.za

Enzymatic reactions that modify polyphenol composition in juice or wine can be divided into two groups: the first leads to oxidation of polyphenols (discussed later) and the second is hydrolysis of phenolic esters (Singleton et al. 1975; Somers et al. 1987; Danilewicz et al. 2008). A number of hydrolase enzymes are found in wine and are derived from yeast activity during fermentation. These enzymes function to cleave the glyosidic linkage of a phenolic ester, liberating the aglycone (Moreno and Peinado 2012). Somers et al. (1987) monitored changes in the concentration of hydroxycinnamic acid esters and aglycones during fermentation of Chardonnay juice. Aglycones (caftaric acid, caffeic acid, coumaric and ferulic acid) were not detected in the juice prior to fermentation, but were present at the end. It was speculated that enzymatic hydrolysis of the hydroxycinnamic acid esters (highest at the beginning of fermentation) occurred which was supported by a follow up experiment where treatment of wines with a commercial esterase confirmed hydrolysis (Somers et al. 1987). Similar increases in HCAs concentrations have been reported in Chardonnay (Cejudo-Bastante et al. 2011a; Cejudo-Bastante et al. 2011b) and Sauvignon blanc (Patel et al. 2010; Di Lecce et al. 2013).

The majority of polyphenol modification occurs during fermentation and aging. Somers et al. (1987) reported an increase in all aglycone HCAs during fermentation, whereas Di Lecce et al. (2014) reported a decrease in all HCAs. Di Lecce et al. (2013) also reported a decrease in flavonols, catechin and epicatechin, but an increase in procyanidins. Similarly to what happened in grape juice, polyphenol increases were assigned to hydrolysis of hydroxycinnamic acid esters and decreases to oxidation. In the case of procyanidin, Di Lecce et al. (2013) assigned the increase of this polymer and subsequent decrease of catechin and epicatechin to polymerization of these monomers. Based on these studies it is clear that HCAs are modified by different reactions which may be influenced by the cultivar (as witnessed in Chardonnay and Verdicchio).

During aging/storage, when wine making processes no longer play a role, polyphenol concentrations may fluctuate based on the reactivity of these compounds (Fulcrand et al. 2006). However, contradicting reports are found in literature for white cultivars (Chardonnay, Garganega, Manzoni, Airén, Verdicchio). Cejudo-Bastante et al. (2011b), Di Lecce et al. (2013) and Ferreira-Lima et al. (2016) reported increases in catechin and HCAs (caftaric acid, coutaric acid, coumaric acid ferulic acid). Cejudo-Bastante et al. (2011b) detected caffeic acid in aged wine which were not present in the juice. In contrast, (Gil-Minoz et al. 1999; Recamales et al. 2006; Kallithraka et al. 2009) reported decreases in catechin and all HCAs, except caffeic acid and coumaric acid. Ferreira-Lima et al. (2016), on the other hand, reported subtle increases in flavonols, but significantly higher concentrations of caftaric acid, caffeic acid and coumaric acid.

Stellenbosch University https://scholar.sun.ac.za

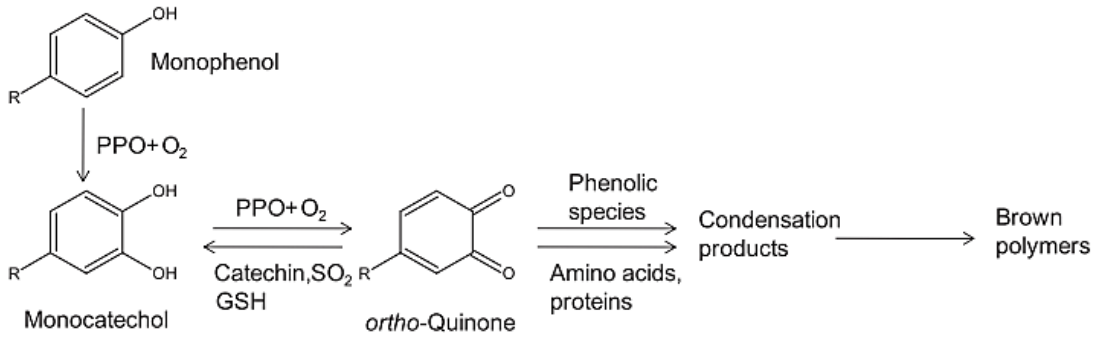
These studies suggested that the increase in HCAs was due to enzyme mediated hydrolysis of their ester precursors and decreases were due to oxidation reactions. Similarly, increases in catechin was a proposed result of acid mediated hydrolysis of polymeric flavan-3-ols whereas polymerization was proposed for decreases. Di Lecce et al. (2013) seemingly reported contradicting results as an increase in catechin and procyanidin dimers was simultaneously observed. Interflavan bonds have been shown to gradually hydrolyse over time in a mildly acidic wine-like solution producing intermediate carbocations (Vidal et al. 2002). Nucleophilic compounds such as monomeric flavan-3-ol, or other grape compounds in wine may attach to the intermediate carbocation, resulting in the formation of short oligomeric procyanidins and a reduction in high molecular weight proanthocyanidins (Vidal et al. 2002).

Stellenbosch University https://scholar.sun.ac.za

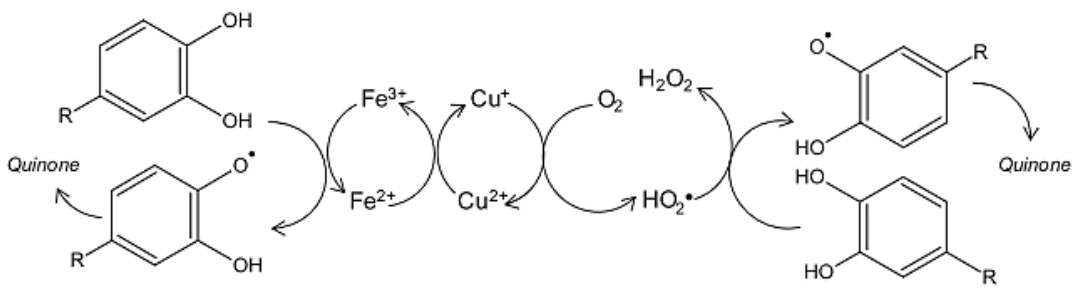
2.7.2. Polyphenol oxidation reactions in juice and wine

The structure of polyphenols plays an integral part in their susceptibility to oxidation. When two hydroxyl groups are present on adjacent carbons in the phenyl ring (Figure 2.2 and Figure 2.4), the phenol can readily oxidize into an *o*-quinone. The *o*-quinone is electrophilic and can react with other polyphenols to form dimers or polymers, or react with amino acid residues of proteins or with the cysteine residue of glutathione (Fulcrand et al. 2006), forming the so called grape reaction product (GRP). Polyphenol oxidation in juice and wine can occur via enzymatic oxidation which involves polyphenol oxidase and oxygen (Singleton et al. 1985), and chemical oxidation which is mediated by iron or copper and oxygen (Figure 2.8) (Danilewicz et al. 2008). Enzymatic oxidation primarily occurs in the juice, whereas chemical oxidation takes place in the wine where monomeric flavan-3-ols would be more susceptible (Oliveira et al. 2011). Oxidized polyphenols

A – Enzymatic oxidation



B – Chemical oxidation



Stellenbosch University https://scholar.sun.ac.za

(*o*-quinones) have the ability to polymerize with other quinones to form brown pigments (Fulcrand et al. 2006).

Figure 2.8: Enzymatic browning reaction in grape juice (A) and chemical oxidation reaction in wine (Danilewicz et al. 2008; Oliveira et al. 2011).

Stellenbosch University https://scholar.sun.ac.za

White wines and juices with higher polyphenol concentrations (HCAs, catechin and epicatechin) are more likely to turn brown compared to wines with lower concentrations (Cheynier et al. 1989; Darias-Martín et al. 2000; Gomez-Miguez et al. 2007; Ferreira-Lima et al. 2016). Oxidation also leads to a loss of varietal aroma where oxidized wines are typically described as having “farm-feed”, “hay” and “woody-like” aromas (Oliveira et al. 2011).

Multiple studies have reported polyphenol losses in juice and wine (storage included) and attributed it to oxidation reactions (Recamales et al. 2006; Gomez-Miguez et al. 2007; Kallithraka et al. 2009; Di Lecce et al. 2013; Ferreira-Lima et al. 2016). These losses were proposed to be due to oxidation of catechin, quercetin, epicatechin, caftaric acid, coutaric acid, coumaric acid and caffeic acid. However, none of these studies measured the formation of GRP. In contrast, Cheynier et al. (1989), Makris et al. (2003), Maggu et al. (2007), Patel et al. (2010), Coetzee et al. (2013) and Fracassetti et al. (2013) could definitively attribute polyphenol losses in juice and wine to oxidation as GRP was quantified and correlated with changes in glutathione. It is noteworthy to mention that the same polyphenols, present at lower levels, were found to be oxidized in the studies that measured GRP.

Sulfur dioxide (SO_2) is added to the must and juice as a precautionary measure against oxidation and as an antiseptic. It is commonly believed that SO_2 binds directly to oxygen which prevents oxidation of polyphenols to *o*-quinones. The work of Danilewicz et al. (2008) revealed that SO_2 mainly acts by reducing o-quinones back to its original form in a model solution and that direct reactions between SO_2 and oxygen were blocked by polyphenols. The use of benzenesulfonic acid as a nucleophile presented a competitive relationship with SO_2 to react with the *o*-quinone. A study by Fracassetti et al. (2013) monitored oxygen consumption of young Sauvignon blanc wines, and similar to Danilewicz et al. (2008), found oxygen consumption to increase proportional to the concentration of SO_2 and reducing substrate (glutathione). Wines were supplemented with glutathione, a nucleophile in its reduced form, and was also found to compete for reactions with the *o*-quinone, even in the presence of higher SO_2 levels. Similar observations were made by Coetzee et al. (2013) in Sauvignon blanc juice and wine. These results suggest a protective effect of glutathione when in its reduced form.

Wine making practices such as skin contact and pressing conditions allow ample time for oxygen to be introduced into the juice which would permit oxidation and the subsequent loss of polyphenols. As previously mentioned, wine making practices has the ability to increase the polyphenol concentrations of juices and wine, but it also contributes towards the susceptibility to oxidation. Skin contact and higher pressing

Stellenbosch University https://scholar.sun.ac.za

pressures also increased glutathione in the juice and wine and these higher levels were proposed to slow the browning process (Ferreira-Lima et al. 2016). According to Singleton et al. (1985), GRP cannot be oxidized further by polyphenol oxidase and does not contribute towards the formation of browning polymers.

Stellenbosch University https://scholar.sun.ac.za

2.7.3. The influence of grape cell walls on polyphenol availability

The grape skin cell wall consists of 30% neutral polysaccharides, (cellulose and hemicellulose), 20% acidic pectins and 15% proanthocyanidins, phenols and structural proteins (Lecas and Brillouet 1994; Chamorro et al. 2012;). Similar to other dicots, pectin is a major polysaccharide of the cell wall of *Vitis vinifera* berries and consists of a galacturonic acid rich backbone (Lecas and Brillouet 1994; Lyapkov et al. 2016). Three domains make up the pectin polysaccharide - homogalacturonan (HG), rhamnogalacturonan I (RGI) and rhamnogalacturonan II (RGII) (Arnous and Meyer 2009). Homogalacturonan is the most abundant pectic domain comprising 60% of the cell wall pectins and has a linear backbone of α (1→4) galacturonic acid residues (Lyapkov et al. 2016). Rhamnogalacturonan II is a complex and highly branched pectic polymer. The RG II backbone is constituted by seven to nine α (1→4) galacturonic acid residues substituted with as much as five side chains that consist of 12 different monosaccharides. RG-II can form dimers and are, together with arabinogalactan proteins, one of the main polysaccharides found in wine (Vidal et al 2003 and 2004). (Lyapkov et al. 2016). Rhamnogalacturonan I, which is also found in wine but at low quantities (Ayesteran et al 2004), contains a backbone with an alternating sequence of galacturonic acid and rhamnose residues. (Komalavilas and Mort 1989; Lyapkov et al. 2016). Cellulose and hemicellulose are comprised of neutral sugars and are associated through non-covalent, hydrogen bonds to form the cellulose-hemicellulose network. Cellulose is an insoluble polymer of unbranched β-1,4-D-glucan chains and functions as the main cell wall supporting structure. (Lyapkov et al. 2016). Hemicellulose contributes about 25% of the total primary cell wall polysaccharides and its backbone may consist of glucose or mannose (Lyapkov et al. 2016).

The grape skin cell wall structure consist of a hemicellulose-rich core that is strongly associated with RG-I, which is again coated with a pectin-rich layer consisting of highly esterified HG, arabinogalactan proteins and small quantities of RG-I that is generously substituted with arabinan side chains (Gao et al. 2016). Grape berry pulp cells have larger cells with thinner, more fragile cell walls than skin cells (Nunan et al. 1998). The most prominent compositional difference between the cell walls of the skin and pulp cells are the lower degree of methylesterification of the latter (Ortega-Regules et al. 2008).

The cell walls act as barriers to the diffusion of polyphenols from the vacuoles into the juice during wine making since polyphenols are mainly present in the vacuoles of cells of grape tissues. When the grape cell walls are disrupted the polyphenols that diffuse from the cell vacuoles may bind to the exposed cell wall materials (Bindon et al. 2010a; Bindon et al. 2016). This is one of the major reasons why juice and wine contain lower concentrations of polyphenols than the grapes from which it has been produced and is described as the extractability of phenolic compounds. Indeed, polyphenol extraction experiments done in a

Stellenbosch University https://scholar.sun.ac.za

model solution containing 12% ethanol revealed that only up to 23% of procyanidins could be extracted from grape skins (Fournand et al. 2006). Furthermore, Hernández-Hierro et al. (2014) proposed a correlation model between the composition of the skin cell wall structure of red cultivars and anthocyanins to establish the relationship between composition and extractability.

Stellenbosch University https://scholar.sun.ac.za

The mechanisms of the interaction between extracted flavan-3-ols and the cell wall polymers of the grape berry have been proposed to occur either through the formation of hydrogen bonds or hydrophobic interactions (Bourvellec et al. 2004). Hydrogen bonds form between hydroxyl groups of the B or C phenyl ring of tannins and the oxygen atoms of the glyosidic linkages that interconnect homogalacturonan of the cell wall polymers, or between the hydroxyl groups of tannins and acetyl groups of polymers (Figure 2.9). It was hypothesized that the strength of the cell wall and tannin interaction would be influenced by the structure and composition of both (Hanlin et al. 2010). A fraction of polyphenols was also covalently bound to cell wall polysaccharides which are only liberated through acid-catalysed cleavage (Lecas and Brillouet 1994; Downey et al. 2003; Fournand et al. 2006).

Polyphenols can be covalently bound to cell wall material though ester, ether or carbon-carbon bonds. It has been proposed that polyphenols bind to cell wall structures during transport and secretion from the Golgi vesicles to the cell wall matrix. These same Golgi vesicles also transport polyphenols to the cell vacuoles (Shahidi and Yeo 2016).

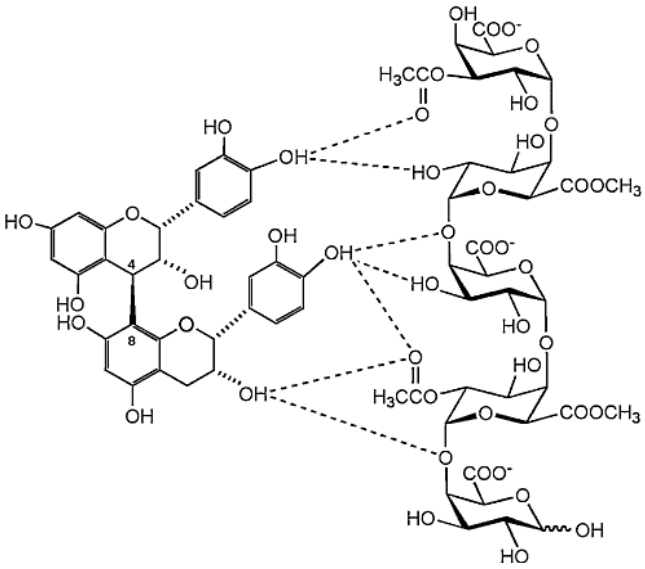


Figure 2.9: A diagram indicating hydrogen bond formation between the hydroxyl groups of a dimeric procyanidin and the oxygen atoms of the glyosidic linkages, hydroxyl and acetyl groups of homogalacturonan (Hanlin et al. 2010).

The polyphenol and grape cell wall interactions were investigated to determine the implications of this interaction for wine making, while others aim to utilize this effect to reduce the negative sensory attributes that polyphenols impart on wine (Fukui and Yokotsuka 2003; Cosme et al. 2008; Bindon and Smith 2013; Bindon et al. 2016). Most of the studies were performed on red grapes and wine.

Stellenbosch University https://scholar.sun.ac.za

Bindon et al. (2010b) investigated the affinity and selectivity of grape-derived cell wall material towards proanthocyanidins (containing anthocyanins in the polymer) in a model solution. The results indicated that cell wall material originating from the pulp had the highest affinity for proanthocyanidins by binding up to 47-57% of total seed and skin proanthocyanidins. The affinity of cell wall material from the skin and pulp was directly proportional to the degree of proanthocyanidin polymerization. Bindon et al. (2010a) also focused on how the interaction between proanthocyanidin and cell wall material during wine making will affect the concentration of the proanthocyanidins in the wine. Only 25% of the total berry proanthocyanidins was found in the wine, with a low degree of polymerization observed. It was concluded that proanthocyanidins with a high degree of polymerization were either not extracted from the berry or they were removed from the wine through interactions with cell wall materials. Bindon et al. (2010a) also reported that the inability of skin cell wall material to bind proanthocyanidins with a high degree of polymerization, to the same extent as the pulp, is due to its high proportion of pectin and lower cellulose and lignin content. This study, in addition to Renard et al. (2001) and Bindon et al. (2010b), confirmed proanthocyanidins to have the highest binding affinity for pectin, followed by xyloglucan. Changes in cell wall composition during ripening also affected the extent to which it would bind tannins. The cell wall composition of the pulp 40 days after *véraison* has been reported to have more than five times the binding capacity to the equivalent material at *véraison* (Hazak et al. 2005).

Stellenbosch University https://scholar.sun.ac.za

Extractable proanthocyanidins have also been found bound to soluble cell wall material in solution (Hazak et al. 2005). A commercial pectinase enzyme preparation have been shown to attenuate the ability of insoluble cell wall material to bind proanthocyanidins through depolymerization of the structure (Bindon et al. 2016). However, in the presence of soluble cell wall material (originated mainly from the pulp), the enzyme could not significantly decrease binding. The fraction containing soluble cell wall material did however also contain proteins and it was proposed as an alternative avenue for proanthocyanidin binding and precipitation (Bindon et al. 2016).

The interaction of proanthocyanidins with cell wall material has also been proposed as a potential use for a fining agent due to the bitter and astringent attributes that flavanol polymers could introduce to wine. Insoluble cell wall material from grape pulp, skin and pomace as well as apple pulp and pomace was shown to effectively remove proanthocyanidins from a model solution (Bindon and Smith 2013).

All of these studies only focused on the binding effect of polymeric flavanols. Further investigation is required on the likelihood of interaction between soluble or insoluble cell wall material and monomeric flavonoids and HCAs.

2.7.4. Polyphenol reactions with proteins and the formation of haze

Polyphenols form part of the non-protein compounds that play a role in haze formation in white wine (Van Sluyter et al. 2015). Polyphenol and protein reactions occur as early as the first step in wine making i.e. crushing and destemming (Tian et al. 2017). Once the juice flows, polyphenols and proteins are extracted

Stellenbosch University https://scholar.sun.ac.za

into the juice which allows opportunity for interaction. The mechanism of interaction involves binding of high molecular weight polyphenols (procyanidins and tannins) to protein, either through the formation of hydrogen bonds or hydrophobic interactions, to form a protein-polyphenol complex. These complexes cross-link with other protein-polyphenol complexes, forming larger aggregates (haze) visible to the naked eye (Van

Stellenbosch University https://scholar.sun.ac.za

Sluyter et al. 2015). The results of Tian et al. (2017) suggested an inverted relationship between total polyphenols and total proteins, chitinases and thaumatin-like proteins in Sauvignon blanc juice and wine. Protein concentrations within this study appeared to be decreased by the presence of polyphenols and not the other way around. A closer look into protein-polyphenol binding in a model solution revealed proteins to be a significant precipitant of proanthocyanidins. In this model solution, proanthocyanidin precipitates formed and the presence of proteins were only discovered following acid hydrolysis (Bindon et al. 2016). Furthermore, these results suggested that soluble protein could precipitate extracted flavan-3-ols polymers and therefore decrease their availability and thus influence wine properties. Esteruelas et al. (2011) also confirmed the presence of polyphenols in natural haze in Sauvignon blanc wine that could not be detected with high performance liquid chromatography, but were successfully identified and quantified using gas chromatography linked to mass spectrometry. The polyphenol concentration in wines before and after haze formation were comparable, but the levels in the haze (769 µg/g) were not negligible. Although protein binding favours polymeric polyphenols, it was established that monomeric flavonols (quercetin, catechin and epicatechin) and HCAs (caftaric acid, caffeic acid, coumaric acid, coutaric acid) also bind to protein. However, it is unclear whether these monomers can initiate aggregation, or whether they from part of the aggregates. These results suggest that polyphenols are already bound to soluble proteins in wine and that over time cross-linking occurs leading to the formation of haze. The formation of haze is sped up through an increase in temperature as the soluble protein structure unfolds, exposing its hydrophobic sites, which provides an opportunity for interaction with polyphenols (Van Sluyter et al. 2015).

2.8. Perspectives

Polyphenols are important compounds in wine due to their influence on wine colour and the perception of bitterness and astringency. The phenolic content of grapes and wine can be increased through manipulation of the light microcLimate in the vineyard or by altering pre-fermentative skin contact time respectively. Literature lacks information on the organoleptic properties of wine made from grapes with an enhanced polyphenol content. Further investigation is therefore required to provide insight into which phenolic groups are most affected in the wine matrix and the extent to which the organoleptic properties are influenced.

Polyphenols are reactive compounds and participate in multiple reactions with other compounds and structure which influence their levels in juice and wine. These reactions include oxidation, enzyme and pH mediated hydrolysis, binding to cell wall material and aggregating with proteins. Each of these reactions are generally solely investigated and it is unclear which has the biggest influence on wine polyphenol content.

Stellenbosch University https://scholar.sun.ac.za

With regards to cell wall binding, literature only focuses on red cultivars and high molecular weight flavan-3-ols, therefore the extent of interaction in white cultivars as well as monomeric polyphenols require attention.

2.9. References

Stellenbosch University https://scholar.sun.ac.za

Adams DO (2006) Phenolics and Ripening in Grape Berries. Am J Enol Vitic 3:249–256.

Agati G, Brunetti C, Di M, Ferrini F, Pollastri S, Tattini M (2013) Plant Physiology and Biochemistry Functional roles of fl avonoids in photoprotection : New evidence , lessons from the past. Plant Physiol Biochem 72:35–45. doi: 10.1016/j.plaphy.2013.03.014

Aleixandre-Tudo J, Weightman C, Panzeri V, Nieuwoudt HH, Toit WJ (2015) Effect of Skin Contact Before and During Alcoholic Fermentation on the Chemical and Sensory Profile of South African Chenin. South African J Enol Vitic 36:366–377.

Aleixandre-Tudo JL, Nieuwoudt H, Olivieri A, Luis J (2018) Phenolic profiling of grapes , fermenting samples and wines using UV- Visible spectroscopy with chemometrics.

Alonso Borbalan AM, Zorro L, Guillen DA, Garcia Barroso C (2003) Study of the polyphenol content of red and white grape varieties by liquid chromatography-mass spectrometry and its relationship to antioxidant power. J Chromatogr A 1012:31–38. doi: 10.1016/S0021-9673(03)01187-7

Arnold R, Noble A, Singleton V (1980) Bitterness and Astringency of Phenolic Fractions in Wine. J Agric Food Chem 675–678. doi: 10.1021/jf60229a026

Arnous A, Meyer AS (2009) Quantitative prediction of cell wall polysaccharide composition in grape (vitis vinifera l.) and apple (malus domestica) skins from acid hydrolysis monosaccharide profiles. J Agric Food Chem 57:3611–3619. doi: 10.1021/jf900780r

Berli FJ, Fanzone M, Piccoli P (2011) Solar UV-B and ABA Are Involved in Phenol Metabolism of Vitis vinifera L . Increasing Biosynthesis of Berry Skin Polyphenols. J Agric Food Chem 4874–4884. doi: 10.1021/jf200040z

Bindon KA, Li S, Kassara S, Smith PA (2016) Retention of Proanthocyanidin in Wine-like Solution Is Conferred by a Dynamic Interaction between Soluble and Insoluble Grape Cell Wall Components. J Agric Food Chem 64:8406–8419. doi: 10.1021/acs.jafc.6b02900

Bindon KA, Smith PA (2013) Comparison of the affinity and selectivity of insoluble fibres and commercial proteins for wine proanthocyanidins. Food Chem 136:917–928. doi: 10.1016/j.foodchem.2012.08.016

Bindon KA, Smith PA, Holt H, Kennedy JA (2010a) Interaction between Grape-Derived Proanthocyanidins and Cell Wall Material . 2 . Implications for Vinification. J Agric Food Chem 10736–10746. doi: 10.1021/jf1022274

Bindon KA, Smith PA, Kennedy JA (2010b) Interaction between Grape-Derived Proanthocyanidins and Cell Wall Material . 1 . Effect on Proanthocyanidin Composition and Molecular Mass. J Agric Food Chem 2520–2528. doi: 10.1021/jf9037453

Bosso A, Guaita M, Petrozziello M (2016) Influence of solvents on the composition of condensed tannins in grape pomace seed extracts. 207:162–169.

Boss. K. P, Davies. C, Robinson, S. P (1996) Expression of anthocyanin biosynthesis pathway genes in red and white grapes. Plant molecular biology. 32 (2): 565-569.

Bourvellec C Le, Guyot S, Renard CMGC (2004) Non-covalent interaction between procyanidins and apple cell wall material Part I . Effect of some environmental parameters. Biochem Biophys Acta 1672:192–

Stellenbosch University https://scholar.sun.ac.za

202. doi: 10.1016/j.bbagen.2004.04.001

Bourzeix. M, Heredia. N, Kovac. V (1983) Richness of different varieties of total phenolic and anthocyanins. Progressive Agriculture and Viticulture. 17: 532-428

Brossaud F, Cheynier V, Noble ANNC (2000) Bitterness and astringency of grape and wine polyphenols. 33–39.
Stellenbosch University https://scholar.sun.ac.za
Buffetto F (2014) Generation of new monoclonal antibodies to rhamnogalacturonan fragments. University of Nantes

Bureau SM, Baumes RL, Razungles AJ (2000) Effects of Vine or Bunch Shading on the Glycosylated Flavor Precursors in Grapes of Vitis vinifera L . Cv . Syrah. 1290–1297.

Cabaroglu T, Canbas a, Baumes R, Bayonove C, Lepoutre JP, Günata Z, Gu Z (1997) Aroma composition of a white wine of Vitis vinifera L. cv. Emir as affected by skin contact. J Food Sci 62:680–683. doi: 10.1111/j.1365-2621.1997.tb15434.x

Cavaliere C, Foglia P, Marini F, Samperi R, Antonacci D, Laganà A (2010) The interactive effects of irrigation , nitrogen fertilisation rate , delayed harvest and storage on the polyphenol content in red grape (Vitis vinifera) berries : A factorial experimental design. Food Chem 122:1176–1184. doi: 10.1016/j.foodchem.2010.03.112

Cejudo-Bastante M, Castro-Vazquez L, Hermosín-Gutierrez MP-C (2011a) Combined Effects of Prefermentative Skin Maceration and Oxygen Addition of Must on Color-Related Phenolics , Volatile Composition , n White Wine and Sensory Characteristics of Air e. J Agric Food Chem 12171–12182. doi: 10.1021/jf202679y

Cejudo-Bastante MJ, Hermosín-Gutierrez I, Castro-Vasquez LI, Perez-Coello M (2011b) Hyperoxygenation and Bottle Storage of Chardonnay White Wines : Effects on Color-Related Phenolics , Volatile Composition , and Sensory Characteristics. J Agric Food Chem 4171–4182. doi: 10.1021/jf104744q

Chamorro S, Viveros A, Alvarez I, Vega E, Brenes A (2012) Changes in polyphenol and polysaccharide content of grape seed extract and grape pomace after enzymatic treatment. Food Chem 133:308–314. doi: 10.1016/j.foodchem.2012.01.031

Cheynier V, Rigaud J, Souquet J-MM, Barillere JM, Moutounet M (1989) Effect of Pomace Contact and Hyperoxidation on the Phenolic Composition and Quality of Grenache and Chardonnay Wines. Am J Enol Vitic 40:36–42.

Chone X, Lavigne-Cruege V, Tominga T, van Leeuwen C, Castagnede C, Saucier C, Dubourdieu D (2006) Effect if vine nitrogen status on grape aromatic potential: Flavor precursors (S-cycteine conjugates), glutathione and phenolic content in Vitis vinifera L.cv, Sauvignon blanv grape juice. J Int Sci 1–6.

Coelho C, Julien P, Nikolantonaki M, Noret L, Magne M, Ballester J, Gougeon RD (2018) Molecular and Macromolecular Changes in Bottle-Aged White Wines Reflect Oxidative Evolution – Impact of Must Clarification and Bottle Closure. Front Chem 6:1–9. doi: 10.3389/fchem.2018.00095

Coetzee C, Lisjak K, Nicolau L (2013) Oxygen and sulfur dioxide additions to Sauvignon blanc must : effect on must and wine composition. 155–167. doi: 10.1002/ffj.3147

Conde C, Silva P, Fontes N, Dias ACP, Tavares RM, Sousa MJ, Agasse A, Delrot S, Gerós H (2007) Biochemical Changes throughout Grape Berry Development and Fruit and Wine Quality. Food Chem 1:1–22.

Cosme F, Ricardo-da-Silva, Laureano O (2008) Interactions between protein fining agents and proanthocyanidins in white wine. Food Chem 106:536–544. doi: 10.1016/j.foodchem.2007.06.038

da Silva Padilha CV, Aquino G, Eduardo M, Olinda A, Souza D, Elias G, Oliveira D De, Bordignon-luiz MT, Lima

Stellenbosch University https://scholar.sun.ac.za

S (2017) Rapid determination of flavonoids and phenolic acids in grape juices and wines by RP-HPLC / DAD : Method validation and characterization of commercial products of the new Brazilian varieties of grape. 228:106–115. doi: 10.1016/j.foodchem.2017.01.137

Danilewicz JC, Seccombe JT, Whelan J (2008) Mechanism of interaction of polyphenols, oxygen, and sulfur dioxide in model wine and wine. Am J Enol Vitic 59:128–136.
Stellenbosch University https://scholar.sun.ac.za
Darias-Martín JJ, Diaz-Gonzalez D, Diaz-romero C (2004) Influence of two pressing processes on the quality of must in white wine production. J Food Eng 63:335–340. doi: 10.1016/j.jfoodeng.2003.08.005

Darias-Martín JJ, Rodríguez O, Díaz E, Lamuela-Raventós RM (2000) Effect of skin contact on the antioxidant phenolics in white wine. Food Chem 71:483–487. doi: 10.1016/S0308-8146(00)00177-1

Del Rio J, Gomez P, Baidez A, Fuster Ma, Ortuno A, Frias V (2004) Phenolic compounds have a role in the defence mechanism protecting grapevine against the fungi involved in Petri disease. Phytophysiology 87–94.

Di Lecce G, Arranz S, Jáuregui O, Tresserra-rimbau A, Quifer-rada P, Lamuela-raventós RM (2014) Phenolic profiling of the skin , pulp and seeds of Albariño grapes using hybrid quadrupole time-of-flight and triple-quadrupole mass spectrometry. Food Chem 145:874–882. doi: 10.1016/j.foodchem.2013.08.115

Di Lecce G, Boselli E, D'Ignazi G, Frega NG (2013) LWT - Food Science and Technology Evolution of phenolics and glutathione in Verdicchio wine obtained with maceration under reductive conditions. LWT - Food Sci Technol 53:54–60. doi: 10.1016/j.lwt.2013.03.006

Dokoozlian NK (1996) Influence of Light on Grape Berry Growth and Composition Varies during Fruit Development. 121:869–874.

Downey MO, Harvey JS, Robinson SP (2003) Analysis of tannins in seeds and skins of Shiraz grapes throughout berry development. Aust J Grape Wine Res 15–27.

Downey MO, Harvey JS, Robinson SP, Industry CP, Unit H, Box PO, Osmond G (2004) The effect of bunch shading on berry development and flavonoid accumulation in Shiraz grapes. Aust J Grape Wine Res 55–73.

Dragana C, Davidovic SM, Todic SR, Bešlic ZS, Tešic L (2016) Identification and quantification of phenolic compounds in berry skin , pulp , and seeds in 13 grapevine varieties grown in Serbia. 211:243–252.

Esteruelas M, Kontoudakis N, Gil M, Fort MF, Canals JM, Zamora F (2011) Phenolic compounds present in natural haze protein of Sauvignon white wine. FRIN 44:77–83. doi: 10.1016/j.foodres.2010.11.010

Ferreira-Lima NE, Burin VM, Caliari V (2016) Impact of Pressing Conditions on the Phenolic Composition , Radical Scavenging Activity and Glutathione Content of Brazilian Vitis vinifera White Wines and Evolution During Bottle Ageing. Food Bioprocess Technol 944–957. doi: 10.1007/s11947-016-1680-7

Ferrer-Gallego R, Hernández-Hierro JM, Rivas-Gonzalo JC, Escribano-Bailón MT (2014) Sensory evaluation of bitterness and astringency sub-qualities of wine phenolic compounds : synergistic effect and modulation by aromas. Food Res Int 62:1100–1107. doi: 10.1016/j.foodres.2014.05.049

Fischer U, Noble a (1994) The Effect of Ethanol, Catechin Concentration, and pH on Sourness and Bitterness of Wine. Am J Enol Vitic 45:6–10.

Fournand D, Vicens A, Sifhoum L, Souquet J, Moutounet M, Cheynier V (2006) Accumulation and Extractability of Grape Skin Tannins and Anthocyanins at Different Advanced Physiological Stages. J Agric Food Chem. doi: 10.1021/jf061467h

Fracassetti D, Coetzee C, Vanzo A, Ballabio D, Toit WJ (2013) Oxygen Consumption in South African

Stellenbosch University https://scholar.sun.ac.za

Sauvignon Blanc Wines : Role of Glutathione , Sulphur Dioxide and Certain Phenolics. 34:156–169.

Freitas VAP De, Glories Y (1999) Concentration and compositional changes of procyanidins in grape seeds and skin of white Âfera varieties Vitis vinõ. 1606:1601–1606.

Fukui M, Yokotsuka K (2003) Content and Origin of Protein in White and Red Wines : Am J Enol Vitic 3:178–188.
Stellenbosch University https://scholar.sun.ac.za
Fulcrand H, Salas E, Cheynier V (2006) Phenolic Reactions during Winemaking and Aging. 3:289–297.

Fuleki T, Ricardo-Da-Silva (2003) Effects of Cultivar and Processing Method on the Contents of Catechins and Procyanidins in Grape Juice. J Agric Food Chem 640–646.

Gao Y (2016) Parameters involved in the enzymatic deconstruction of the Wine Grape cell wall matrix during winemaking.

Gao Y, Fangel JU, Willats WGT, Vivier MA, Moore JP (2016) Effect of Commercial Enzymes on Berry Cell Wall Deconstruction in the Context of Intravineyard Ripeness Variation under Winemaking Conditions. J Agric Food Chem 64:3862–3872. doi: 10.1021/acs.jafc.6b00917

Gao Y, Fangel JU, Willats WGT, Vivier MA, Moore JP (2015) Dissecting the polysaccharide-rich grape cell wall changes during winemaking using combined high-throughput and fractionation methods. Carbohydr Polym 133:567–577. doi: 10.1016/j.carbpol.2015.07.026

Garrido J, Borges F (2013) Wine and grape polyphenols - A chemical perspective. Food Res Int 54:1844–1858. doi: 10.1016/j.foodres.2013.08.002

Gawel R, Day M, van Sluyter S, Holt H, Waters E, Smith P (2017) Compositional Factors Affecting the Mouth-feel of White Wine. J Agric Food Chem 62:10008–10014.

Gil-Minoz R, Gomez-Plaza E, Martinez A, Lopez-Roca J (1999) Evolution of Phenolic Compounds during Wine Fermentation and Post-fermentation : Influence of Grape Temperature. J Food Compos Anal 272:259–272.

Gomez-Alonso S, Garcia-Romero E, Hermosın-Gutierrez I (2007) Analysis of diverse grape and wine phenolics using direct injection and multidetection by DAD and fluorescence. J Food Compos Anal 20:618–626. doi: 10.1016/j.jfca.2007.03.002

Gomez-Miguez JM, Gonzalez-Miret LM, Hernanz D, Fernandez AM, Vicario IM, Heredia FJ (2007) Effects of prefermentative skin contact conditions on colour and phenolic content of white wines. J Food Eng 78:238–245. doi: 10.1016/j.jfoodeng.2005.09.021

González-Manzano S, Rivas-Gonzalo JC, Santos-Buelga C (2004) Extraction of flavan-3-ols from grape seed and skin into wine using simulated maceration. Anal Chim Acta 513:283–289. doi: 10.1016/j.aca.2003.10.019

Gregan SM, Wargent JJ, Liu L, Shinkle J, Hofmann R, Winfield C, Trough M, Jordan B (2011) Effects of solar ultraviolet radiation and canopy manipulation on the biochemical composition of Sauvignon Blanc grapes. Aust J Grape Wine Res. doi: 10.1111/j.1755-0238.2012.00192.x

Guidoni S, Oggero G, Cravero S, Rabino M, Cravero MC, Balsari P (2008) Manual and mechanical leaf removal in the bunch zone (Vitis vinifera L cv., Barbera): Effects on berry composition , health, yield and wine quality in a warm temperature area. J Sci 49–58.

Hanlin RL, Hrmova M, Harbertson JF, Downey MO (2010) Review: Condensed tannin and grape cell wall interactions and their impact on tannin extractability into wine. Aust J Grape Wine Res 16:173–188. doi: 10.1111/j.1755-0238.2009.00068.x

Stellenbosch University https://scholar.sun.ac.za

Haselgrove L, Botting D, van Heeswijck PB, Dry PR, Ford C, Iland PG (2000) Canopy microcLimate and berry composition : The effect of bunch exposure on the phenolic composition of Vitis vinifera L cv . Shiraz grape berries. Aust J Grape Wine Res 6:141–149.

Hazak JC, Harbertson JF, Adams DO (2005) The Phenolic Components of Grape Berries in Relation to Wine Composition. 189–196.

Stellenbosch University https://scholar.sun.ac.za

Hernández-Hierro JM, Quijada-Morín N, Martínez-Lapuente L, Guadalupe Z, Ayestarán B, Rivas-Gonzalo JC, Escribano-Bailón MT (2014) Relationship between skin cell wall composition and anthocyanin extractability of Vitis vinifera L . cv . Tempranillo at different grape ripeness degree. Food Chem 146:41–47. doi: 10.1016/j.foodchem.2013.09.037

Hernanz D, Recamales ÁF, González-Miret ML, Gómez-Míguez MJ, Vicario IM, Heredia FJ (2007) Phenolic composition of white wines with a prefermentative maceration at experimental and industrial scale. J Food Eng 80:327–335. doi: 10.1016/j.jfoodeng.2006.06.006

Honeth. C (2018) Evaluating the effects of UVB radiation on field grown grape berry metabolites under high and low light microcLimates and the consequent impacts on the resulting wines. Thesis, University of Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

Kallithraka S, Arvanitoyannis IS, Kefalas P, El-zajouli A (2001) Instrumental and sensory analysis of Greek wines ; implementation of principal component analysis (PCA) for classication according to geographical origin.

Kallithraka S, Salacha MI, Tzourou I (2009) Changes in phenolic composition and antioxidant activity of white wine during bottle storage : Accelerated browning test versus bottle storage. Food Chem 113:500–505. doi: 10.1016/j.foodchem.2008.07.083

Kammerer D, CLaus A, Carle R, Schieber A (2004) Polyphenol Screening of Pomace from Red and White Grape Varieties (Vitis vinifera L .) by HPLC-DAD-MS / MS. J Agric Food Chem 52:27–30.

Kennedy JA, Jones GP (2001) Analysis of Proanthocyanidin Cleavage Products Following Acid-Catalysis in the Presence of Excess Phloroglucinol. J Agric Food Chem 1740–1746. doi: 10.1021/jf001030o

Kennedy JA, Matthews MA, Waterhouse AL (2000) Changes in grape seed polyphenols during fruit ripening. 55:77–85.

Kennedy JA, Waterhouse AL (2000) Analysis of pigmented high-molecular-mass grape phenolics using ion-pair , normal-phase high-performance liquid chromatography. J Chromatogr A 866:25–34.

Kobayashi S (2009) Regulation of Anthocyanin Biosynthesis in Grapes. J Japan Soc Jort Sci 78:387–393.

Komalavilas P, Mort AJ (1989) The acetylation at o-3 of galacturonic rhamnose-rich portion of pectins. 189:261–272.

Koundouras S, Marinos V, Gkoulioti A, Kotsteridis Y, van Leeuwen C (2006) Influence of Vineyard Location and Vine Water Status on Fruit Maturation of Nonirrigated Cv . Agiorgitiko (Vitis vinifera L .). Effects on Wine Phenolic and Aroma Components. J Agric Food Chem. doi: 10.1021/jf0605446

Koyama K, Ikeda H, Poudel PR, Goto-yamamoto N (2012) Phytochemistry Light quality affects flavonoid biosynthesis in young berries of Cabernet Sauvignon grape. Phytochemistry 78:54–64. doi: 10.1016/j.phytochem.2012.02.026

Lecas M, Brillouet J-M (1994) Cell wall compostion of grape berry skins. 35:1241–1243.

Liu J., Gallander JF, Wilker KL (1987) Effect of Juice Clarification on the Composition and Quality of Eastern US Table Wines. Am J Enol Vitic 38:147–150.

Stellenbosch University https://scholar.sun.ac.za

Lyapkov AA, Pestryakov A, De Souza Gomes A (2016) Polymerization.

Maggu M, Winz R, Kilmartin PA, Trought MCT, Trought M, Nicolau L (2007) Effect of Skin Contact and Pressure on the Composition of Sauvignon Blanc Must. J Agric Food Chem 55:10281–10288.

Makris DP, Psarra E, Kallithraka S, Kefalas P (2003) The effect of polyphenolic composition as related to antioxidant capacity in white wines. 36:805–814. doi: 10.1016/S0963-9969(03)00075-9

Stellenbosch University https://scholar.sun.ac.za

Matthews S, Mila I, Scalbert A, Pollet B, Lapierre C, Herve CLM, Rolando C, Donnelly DMX (1997) Method for Estimation of Proanthocyanidins Based on Their Acid Depolymerization in the Presence of Nucleophiles. J Agric Food Chem. doi: 10.1021/jf9607573

Minussi RC, Rossi M, Bologna L, Pastore M, Dura N, Rotilio D (2003) Phenolic compounds and total antioxidant potential of commercial wines. 82:409–416. doi: 10.1016/S0308-8146(02)00590-3

Moreno, J and Peinado, R (2012) Enological Chemistry. Elsevier Science and Technology. 53-76. doi: 10.1016/B978-0-12-388438-1.00005-4

Mosetti D, Herrera JC, Sabbatini P, Green A, Alberti G, Peterlunger E, Lisjak K, Castellarin S (2016) Impact of leaf removal after berry set on fruit composition and bunch rot in ' Sauvignon blanc ' Impact of leaf removal after berry set on fruit composition and bunch rot in ' Sauvignon blanc '. Vitis. doi: 10.5073/vitis.2016.55.57-64

Olejar KJ, Fedrizzi B, Kilmartin PA (2015) Influence of harvesting technique and maceration process on aroma and phenolic attributes of Sauvignon blanc wine. Food Chem 183:181–189. doi: 10.1016/j.foodchem.2015.03.040

Oliveira C, Silva Ferreira A, De Freitas V, Silva A (2011) Oxidation mechanisms occurring in wines. Food Res Int 44:1115–1126. doi: 10.1016/j.foodres.2011.03.050

Ortega-Regules A, Romero-Cascales I, Ros-Garcia J., Lopez-Roca J, Gomez-Plaza E (2006) A first approach towards the relationship between grape skin cell-wall composition and anthocyanin extractability. 563:26–32. doi: 10.1016/j.aca.2005.12.024

Ortega-Regules A, Ros-garcía JM, Bautista-ortín BA, Lopez-Roca JM, Gómez-plaza E (2008) Differences in morphology and composition of skin and pulp cell walls from grapes (Vitis vinifera L .): technological implications. Eur Food Res Technol 223–231. doi: 10.1007/s00217-007-0714-9

Ough CS (1969) Substances Extracted during Skin Contact with White Musts. I. General Wine Composition and Quality Changes with Contact Time. Am J Enol Vitic 20:93–100.

Paixão N, Perestrelo R, Marques JC, Câmara JS (2007) Relationship between antioxidant capacity and total phenolic content of red, rosé and white wines. Food Chem 105:204–214. doi: 10.1016/j.foodchem.2007.04.017

Patel P, Herbst-Johnstone M, Lee SA, Gardner RC, Weaver R, Nicolau L, Kilmartin PA (2010) Influence of Juice Pressing Conditions on Polyphenols , Antioxidants , and Varietal Aroma of Sauvignon blanc Microferments. Agric Food Chem 58:7280–7288. doi: 10.1021/jf100200e

Pinelo M, Arnous A, Meyer AS (2006) Upgrading of grape skins : Significance of structural components and extraction techniques for phenol release. doi: 10.1016/j.tifs.2006.05.003

Recamales F, Sayago A, Gonza ML, Hernanz D (2006) The effect of time and storage conditions on the phenolic composition and colour of white wine. 39:220–229. doi: 10.1016/j.foodres.2005.07.009

Renard, C.M., Baron, A., Guyot, S. and Drilleau, J. (2001) Inter- actions between apple cell walls and native apple polyphenols: quantification and some consequences. International Journal of Biological Macromolecules 29, 115–125

Stellenbosch University https://scholar.sun.ac.za

Ristic R, Bindon K, Francis LI, Herderich MJ, Iland PG (2010) Flavonoids and C 13 -norisoprenoids in Vitis vinifera L . cv . Shiraz : relationships between grape and wine composition , wine colour and wine sensory properties. doi: 10.1111/j.1755-0238.2010.00099.x

Ristic R, Downey MO, Iland PG, Bindon K, Francis L, Herderich M, Robinson S (2006) Exclusion of sunlight from Shiraz grapes alters wine colour , . Aust J Grape Wine Res 53–65.

Stellenbosch University https://scholar.sun.ac.za

Robichaud JL, Noble AC (1990) Astringency and Bitterness of Selected Phenolics in Wine. 343–353.

Rodríguez Montealegre R, Romero Peces R, Chacón Vozmediano JL, Martínez Gascueña J, García Romero E (2006) Phenolic compounds in skins and seeds of ten grape Vitis vinifera varieties grown in a warm cLimate. J Food Compos Anal 19:687–693. doi: 10.1016/j.jfca.2005.05.003

Romero-Cascales I, Ortega-Regules A, López-Roca JM, Fernández-Fernández JI, Gómez-Plaza E (2005) Differences in anthocyanin extractability from grapes to wines according to variety. Am J Enol Vitic 56:212–219.

Šebela D, Turóczy Z, Olejníčková J, Kumšta M, Sotolář R (2017) Effect of Ambient Sunlight Intensity on the Temporal Phenolic Profiles of Vitis Vinifera L . Cv . Chardonnay During the Ripening Season – A Field Study. South African J Enol Vitic 38:94–102.

Shahidi F, Yeo J (2016) Insoluble-Bound Phenolics in Food. doi: 10.3390/molecules21091216

Singleton V, Trousdale E (1992) Anthocyanin-tannin interactions explaining differences in the polymeric phenols between white and red wines. Am J Enol Vitic 41:63–70.

Singleton VL, Salgues M, Zaya J, Trousdale E (1985) Caftaric Acid Disappearance and Conversion to Products of Enzymic Oxidation in Grape Must and Wine. Am J Enol Vitic 36:50–56.

Singleton VL, Sieberhagen HA, Wet P De, Wyk CJ Van (1975) Composition and sensory qualities of wines prepared from the white grapes by fermentation with and without grape solids.

Somers TC, Verette E, Pocock KF (1987) Hydroxycinnamate Esters of Vitis vinifera : Changes during White Vinification , and Effects of Exogenous Enzymic Hydrolysis. 67–78.

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

Su D, Zhang R, Hou F, Zhang M, Guo J, Huang F, Deng Y (2014) Comparison of the free and bound phenolic profiles and cellular antioxidant activities of litchi pulp extracts from different solvents. 1–10.

Teissedre P, Glories Y (2008) Effect of pH , ethanol and acidity on astringency and bitterness of grape seed tannin oligomers in model wine solution. 19:286–291. doi: 10.1016/j.foodqual.2007.08.004

Teixera A, Jose E-D, Castellarin SD, Geros H (2013) Berry Phenolics of Grapevine under Challenging Environments. 18711–18739. doi: 10.3390/ijms140918711

Tian B, Harrison R, Morton J, Jaspers M, Hodge S, Grose C, Trought M (2017) Extraction of Pathogenesis-Related Proteins and Phenolics in Sauvignon Blanc as Affected by Grape Harvesting and Processing Conditions. Molecules. doi: 10.3390/molecules22071164

van Jaarsveld FP Van, Blom M, Hattingh S, Marais J (2005) Effect of Juice Turbidity and Yeast Lees Content on Brandy Base Wine and Unmatured Pot-still Brandy Quality. South African J Enol Vitic 26:116–130.

Van Sluyter SC, McRae JM, Falconer RJ, Smith PA, Bacic A, Waters EJ, Marangon M (2015) Wine protein haze: Mechanisms of formation and advances in prevention. J Agric Food Chem 63:4020–4030. doi: 10.1021/acs.jafc.5b00047

Stellenbosch University https://scholar.sun.ac.za

Verette E, Nobleb AC, Some TC (1988) Hydroxycinnamates of Vitis vinifera : Sensory Assessment in Relation to Bitterness in White Wines. 267–272.

Vidal S, Cartalade D, Souquet J-M, Fulcrand H, Cheynier V (2002) Changes in Proanthocyanidin Chain Length in Winelike Model. J Agric Food Chem 0–5. doi: 10.1021/jf011180e

Stellenbosch University https://scholar.sun.ac.za

Walker AR, Lee E, Bogs J, Mcdavid DAJ, Thomas MR, Robinson SP (2007) White grapes arose through the mutation of two similar and adjacent regulatory genes. Plant J 772–785. doi: 10.1111/j.1365-313X.2006.02997.x

Stellenbosch University https://scholar.sun.ac.za

Chapter 3

Stellenbosch University https://scholar.sun.ac.za

Research Results

Comparison of polyphenol levels and distribution patterns in the tissues of ripe Sauvignon blanc berries obtained from high and low light microclimates

Stellenbosch University https://scholar.sun.ac.za

Chapter 3 - Comparison of polyphenol levels and distribution patterns in the tissues of ripe Sauvignon blanc berries obtained from high and low light microclimates

Stellenbosch University https://scholar.sun.ac.za

3.1. Introduction

Polyphenols are secondary metabolites which accumulate within the grape berry during development and ripening (Downey et al. 2003; Downey et al. 2004). These compounds are a diverse group which contain a phenyl ring in the backbone with one or more hydroxyl groups attached to it (Fulcrand et al. 2006). Based on their chemical structure polyphenols are classified as flavonoids or non-flavonoids. Flavonoids are structurally more complex than non-flavonoids as it contains two polyhydroxylated phenyl rings, connected through a central pyran ring. Flavonoids are further divided into flavan-3-ols, anthocyanins and flavonols whereas the non-flavonoids are divided into hydroxycinnamic, hydroxybenzoic acids and stilbenes (Adams 2006; Kennedy et al. 2006; Garrido and Borges 2013).

Polyphenols are unevenly distributed throughout the berry tissues and in white cultivars approximately 80% is typically located within the seeds, 15% in the skin and 5% within the pulp (Di Lecce et al. 2014). The seeds predominantly consists, and are also the primary source, of flavan-3-ols, which include catechin, epicatechin and their polymeric form, procyanidins or referred to as condensed tannins (Kennedy et al. 2000; González-Manzano et al. 2004). The grape skin is rich in hydroxycinnamic acids (caftaric acid, coutaric acid, caffeic acid, ferulic acid) and is the primary source of flavonols (quercetin, myricetin, kaempferol, rutin) (Castillo-Munoz et al. 2010; Di Lecce et al. 2013). Although the bulk of the berry tissue is constituted by the pulp, it is not the main contributor to the total phenolic content of the berry since it contains lower levels of hydroxycinnamic acids and flavan-3-ols compared to the skin and seeds respectively (Pastrana-Bonilla et al. 2003; Di Lecce et al. 2014; Dragana et al. 2016).

Polyphenol distribution in Sauvignon Blanc grapes is similar to what is described above since distribution patterns have been found to be similar between different white and red cultivars, but with variations in the levels of individual compounds reported (Rodríguez Montealegre et al. 2006; Dragana et al. 2016).

At harvest, grapes have a fixed amount of polyphenols that can be extracted. This fixed amount determines the phenolic potential of the grapes which includes the sum and variety of free and bound polyphenols that would be available for extraction under wine making conditions. Polyphenol analysis on grape tissue, juice or wine is not performed according to a universal method, which complicates the comparison of phenolic compound levels (or phenolic potential) measured in different studies. Different solvent types (acetone, methanol, ethanol, ethyl acetate) have been shown to extract free polyphenols to varying degrees under cool conditions, with acetone being the most effective. Additionally, harsher extraction conditions (acidified methanol as solvent at 85^{o}C) has the ability to extract bound polyphenols originally not extracted under cool conditions (Su et al. 2014). The composition of extraction solvents also influences the amount of polyphenols that can be extracted. Combinations of methanol, acetone or ethanol

Stellenbosch University https://scholar.sun.ac.za

and water (1:1) were reported to be more effective at extracting polyphenols compared to the pure solvents (Agustin-Salazar et al. 2014; Bosso et al. 2016). The use of different analytical instruments may also impose limitations for accurate polyphenol identification and quantification (Kennedy and Waterhouse 2000; Di Lecce et al. 2014; Terblanche 2017). Arapitsas et al. (2018) recently reported ultra high pressure liquid-
Stellenbosch University https://scholar.sun.ac.za
chromatography (UHPLC) coupled with a tandem mass spectrometer (MS), operating in multiple reaction monitoring (MRM) mode, to be the gold standard for accurate detection of trace phenolic compounds. The method for polyphenol analysis is often altered depending on the compounds of interest (Somers et al. 1987; Castillo-Munoz et al. 2010; Gregan et al. 2011). Stilbenes are an example of phenolic group which is only detected when high performance liquid chromatography (HPLC) is coupled with MS (Kammerer et al. 2004; Di Lecce et al. 2013; Agustin-Salazar et al. 2014; Di Lecce et al. 2014). Similarly, the identification and quantification of high molecular weight tannins is quite problematic due to the inability of chromatography methods to separate these compounds (Kennedy and Waterhouse 2000).

The phenolic content of grapes can be altered as grapevine has shown to increase polyphenol accumulation in response to environmental stressors. With focus on light stress, leaf removal around the bunch zone during ripening creates a high light (HL) microclimate which can increase accumulation of polyphenol content of white and red grapes (Haselgrove et al. 2000; Downey et al. 2006; Šebela et al. 2017). Light exposure exerts the biggest effect on the grape skin flavonoid metabolism (Berli et al. 2011) where the genes involved in flavonoid synthesis are upregulated with increased exposure (Downey et al. 2004; Koyama et al. 2012; Du Plessis 2017). Flavonoids exert a protective effect against damaging ultraviolet radiation hence their upregulated synthesis during light stress (Gregan et al. 2011; Agati et al. 2013). Leaf removal also contributes towards the health of grape bunches as it reduces the incidence of fungal infection (Guidoni et al. 2008; Mosetti et al. 2016). The timing of leaf removal is important as it influences total soluble solids, pH, titratable acidity and methoxypyrazines, amongst other compounds of the berry (Bledsoe et al. 1988; Guidoni et al. 2008; Scheiner et al. 2010).

Sauvignon Banc is an excellent model cultivar to evaluate light stress due to its well described metabolite responses (Gregan et al. 2011; Young et al. 2016; Du Plessis 2017) and wine styles related (Suklje et al. 2014; Honeth 2018) to low and high light microclimates.

The aim of this study was to determine the “phenolic potential” of Sauvignon Blanc grapes harvested from low light (LL) and high light (HL) microclimates of a model vineyard (Young et al. 2016; du Plessis 2017; Honeth 2018) and determine the impact of the microclimates on polyphenols distribution in the skin, pulp and seeds.

Stellenbosch University https://scholar.sun.ac.za

3.2. Materials and methods

3.2.1. Viticultural treatments and management, and sampling within a field-omics workflow

A commercial *Vitis vinifera* 'Sauvignon Blanc' vineyard, established in 2004 was used as experimental site.
Stellenbosch University https://scholar.sun.ac.za
The details of the site, the management of the vineyard, the experimental design to generate and validate the experimental layout to obtain a high and low light microclimate were described in Young et al, 2016 (refer to Supplementary Figure S3.1 for a summary). The treatment involved total leaf and lateral shoot removal in the bunch/fruiting zone (corresponding to removal up to approximately 30–40 cm above the cordon) on the northeast-facing side of the canopy (i.e. the facet of the vine that received morning sunlight exposure in the Southern Hemisphere) at EL29 (Eichorn and Lorenz 1977), to create a high light microclimate (HL). The HL microclimate was maintained throughout the season, keeping the fruiting zone exposed through continuous lateral shoot removal. The canopy of the vines from the LL microclimate was not manipulated, which resulted in more shaded fruiting zones with reduced exposure. The leaf removal treatment was alternated down 13 adjacent vineyard rows, which included four panels per row and four vines per panel, thus creating a checkerboard plot layout. Grape bunches were harvest from the northeast facing side of the canopy and bunches from all 13 rows were pooled according to the treatment (HL or LL). There after three biological repeats were randomly formed per treatment. Berry samples were collected (n = 50 berries per sample) from each biological repeat. Samples were kept on ice in the vineyard prior to their partitioning into skin, pulp and seed in the laboratory. The portioned tissue was flash-frozen in liquid nitrogen stored at −80°C until further analysis.

3.2.2. Grape tissue preparation

Whole berries, skin, pulp and seeds from each biological repeat were kept separate at liquid nitrogen temperature and milled (Retch Mixer Mill MM 400, Germany) at 30 Hz for 1 min until a fine powder was obtained. Whole berries were milled without removing the seeds. The powder of each biological repeat was homogenized by collecting all of the powders in a single container and mixing. Tissue was milled until a 200 ml jar was filled for the whole berries, a 15 ml falcon tube for the seeds and a 50 ml falcon tube for the pulp and skin respectively. The dry weights of the whole berries, skin and pulp were determined by allowing moisture to evaporate at 80^{o}C until the reading stabilised.

3.2.3. Polyphenol extraction, identification and quantification

The individual phenolic acids and flavonoids of the whole berry (WB), skin, seeds and pulp were extracted using the method described in (Du Plessis 2017). A 70% (v/v) methanol (Merck, Germany) and water solvent was used as extraction reagent, with the pH adjusted to 1.5 using hydrochloric acid. Briefly, 200 mg of frozen, milled tissue was suspended in 1 mL acidified methanol. Samples were vortexed for 1 min and ultrasonified for 10 min followed by centrifugation at 14 000 rpm for 5 min. The supernatant was mixed 1:1 with p-

Stellenbosch University https://scholar.sun.ac.za

coumaric acid (Merck, Germany) (100 mg/L) as an internal standard, filtered (0.22 µm cellulose acetate membrane) and analysed using high-performance liquid chromatograph (HPLC). Three samples were prepared for each biological repeat.

Samples were directly injected into a high performance liquid chromatography instrument (HPLC, Agilent technologies, California, USA) equipped with a diode array detector (DAD) and fluorescence and UV-Vis detector (FLD) and controlled by ChemStation Rev. A. 10.02 software (Agilent technologies). A gradient of solvent A (15% phosphoric acid [Merck, Germany] in distilled water) and solvent B (80/20 [v/v] HCN [Merck, Germany]/solvent A) was applied to a Phenominex Prodigy ODS-2 column (4.6 x 150 nm, 3.5 µm) as follows: 0-68 min, 6.0-31% B; 68-73 min, 31-65% B; 73-78 min, 65% B; 78-83 min, 65-6% B and equilibrated for 10 min at 6.0% B. The flow rate was 1 ml/min at 40°C with a 20 µl injection volume and 93 min run time.

Stellenbosch University https://scholar.sun.ac.za

Phenolic compounds were identified by comparing the retention times with those of the standards. Detection was performed between 200 and 900 nm with the following quantification wavelengths: 280 nm (flavon-3-ols), 320 nm (phenolic acids) and 360 nm (flavonols). A serial dilution (0 – 250 mg/L) of standards: gallic acid, coumaric acid, caffeic acid, trans-caftaric acid, (+)-catechin, (-)-epicatechin was prepared to generate an equilibration curve. Structurally related reference compounds were used when standards were not available for quantification of a compound. Concentrations were normalised against the dry sample weight and expressed as µg/g dry berry weight (µg/g DW).

3.2.4. Statistical analysis

Factorial and One-way analysis of variance (ANOVA) was performed using Tibco Statistica (version 13, Oklahoma, USA) to determine the statistical significance of differences in polyphenol composition and concentration between tissue types or to analyse/compare the impacts of the microclimates on the polyphenols of the whole berry, skin, pulp and seeds. ANOVA results are reported as F-values and the highest -value correlates to the greatest significance. Fischer's least square difference (LSD) post hoc test was conducted to pinpoint which polyphenols responded statistically significant to a particular treatment. All significance differences were interpreted on a 5% significance level ($p<0.05$).

3.3. Results

3.3.1. Polyphenol levels in Sauvignon Blanc berry tissues from LL and HL microclimates

A total of fourteen polyphenols, belonging to the hydroxycinnamic acids (HCAs), flavan-3-ols and flavonols, were quantified in the berry tissues (Table 3.1 and Supplementary Tables S3.1-3.3). Certain phenolic groups, which included stilbenes and hydroxybenzoic acids, were not detected in any of the tissues with the analytical method used. The whole berries from the HL microclimate had a higher total polyphenol concentration compared to berries from the LL microclimate (Table 3.1 and Figure 3.1 A). The total concentrations of each phenolic group was also higher in grapes from the HL microclimate, with the most noticeable increase

Stellenbosch University https://scholar.sun.ac.za

observed for the flavonols (Table 3.1). By quantifying the total polyphenol concentration of these grapes, it provided an indication of the phenolic potential of the grapes, confirming that the HL microclimate had a higher phenolic potential compared to those from the LL microclimate. The data from the different tissue types were analysed further to investigate the distribution patterns.

Stellenbosch University https://scholar.sun.ac.za

Table 3.1: The total polyphenol concentrations of ripe Sauvignon Blanc berry tissues at harvest.

		Total Hydroxycinnamic acids	Total Flavan-3-ols	Total flavonols	Total Polyphenols
Whole berry	LL	402.4±16.7 d	792.6±85.6 d	14.1±2.7 d	1209.3±94.0 e
	HL	540.4±22.9 c	1058.6±84.1c	302.1±24.8 b	1901.1±117.4 d
	Population minimum	383.6	682.0	10.4	1076.2
	Population maximum	577.9	1181.0	336.0	2095.0
	Population average	471.4	925.6	158.1	1555.2
Skin	LL	617.5±40.8 b	180.3±14.7 f	85.4±14.7 c	883.2±40.8 f
	HL	851.1±76.6 a	229.3±10.3 e	1634.0±10.3 a	2714.4±76.6 c
	Population minimum	593.8	162.4	82.4	838.7
	Population maximum	892.5	237.7	1706.0	2836.3
	Population average	734.3	204.8	859.7	1798.8
Pulp	LL	275.9±22.0 f	N.D	N.D	275.9±22.0 h
	HL	344.1±21.5 e	N.D	N.D	344.1±21.5 g
	Population minimum	245.0	N.D	N.D	245.0
	Population maximum	366.5	N.D	N.D	366.5
	Population average	310.0	N.D	N.D	310.0
Seed	LL	51.63±1.7 g	8620.1±240.0 a	11.22±1.5 de	8682.9±243.3 b
	HL	51.06±2.5 g	8736.2±218.9 a	10.85±1.8 de	8798.1±233.2 a
	Population minimum	47.7	N.D	8.4	8213.1
	Population maximum	55.2	N.D	14.1	9155.2
	Population average	51.3	1818.5	11.0	8740.5

Abbreviations: LL, low light; HL, high light

Different letters indicate statistical significance (One-way ANOVA with Fisher's LSD post hoc test, $p<0.05$) and is relevant between the tissues within each phenolic group.

Phenolic groups were unevenly distributed throughout the berry tissues and was not affected by microclimate since distribution patterns were similar for LL and HL microclimates (Table 3.1), but contents were impacted. The seeds contained the highest concentration of flavan-3-ols whereas HCAs and flavonols were most abundant in the skin and the pulp only contained HCAs. The skin had the most diverse polyphenol composition as it contained noticeable concentrations of HCAs, flavan-3-ols and flavonols. The seeds contained trace amount of HCAs and flavonols relative to its total flavan-3-ol concentration. Figure 3.1 B is based on data from Table 3.1 and shows the distribution of phenolic groups throughout the berry tissues and the effect of the microclimates on distribution and content. The total polyphenol content of the skin was most affected by the HL microclimate as it had a 3-fold higher total polyphenol concentration due to increases in all phenolic groups. Flavonol accumulation was most significantly increased in the skin from the HL

Stellenbosch University https://scholar.sun.ac.za

microclimate. The total HCA concentration of the pulp was marginally increased by the HL microclimate whereas the seeds were unaffected.

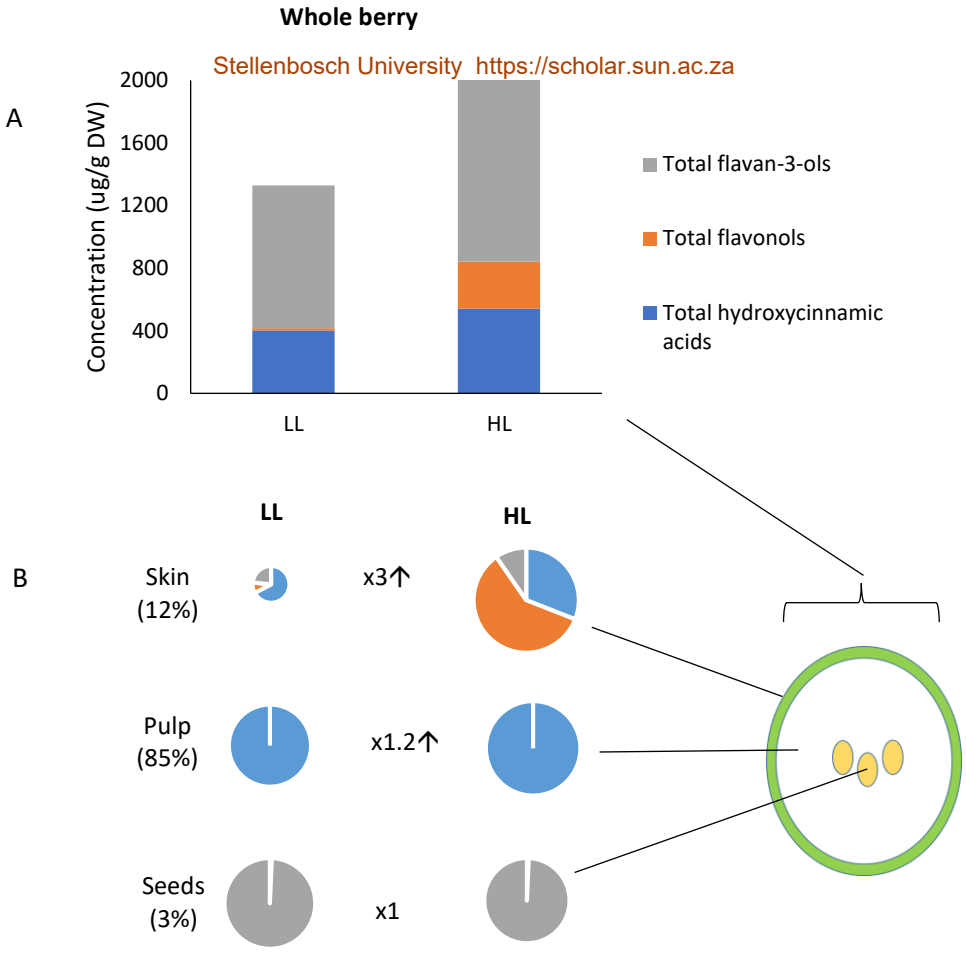


Figure 3.1: The composition of total polyphenol groups in the tissues of Sauvignon Blanc grapes (A) and whole berries (B) from a low light (LL) and high light (HL) microclimate. The proportion of each tissue is an indication of its total polyphenol concentration relative to the whole berry. The concentration of each phenolic group within a tissue was normalised to the percentage of the tissue that constitutes the whole berry. The arrows indicate how much the total concentration of the tissue from the HL microclimate differed from the LL microclimate.

3.3.2. Response of individual polyphenols to a high light microclimate

As previously mentioned – phenolic distribution was not affected by the microclimates. The distribution pattern of individual polyphenols throughout the grape tissues were similar in the LL and HL microclimates (Supplementary Tables S.1-S3.2). Caftaric acid was detected in all of the tissues with caffeic acid and coutaric acid being absent from the pulp and seed respectively. Of these HCAs, the skin had the highest concentration of each compound (Supplementary Table S3.1). The flavan-3-ols, catechin, epicatechin, flavan-3-ol_1, flavan-3-ol_2, flavan-3-ol_3, flavan-3-ol_4 and polymeric polyphenols, were predominantly localised in the seed. Lower concentrations of catechin and epicatechin were the only flavan-3-ols detected in the skin but these compounds where absent from the pulp (Supplementary Table S3.2). Flavonols, quercetin-glucoside, quercetin-galactoside, flavonol_4 and flavonol_5, were found at the highest concentrations in the skin. Lower

Stellenbosch University https://scholar.sun.ac.za

concentrations of quercetin-glucoside were present in the seed whereas no flavonols were detected in the pulp (Supplementary Table S3.3).

The log_2 fold change of each individual polyphenol was calculated to determine the effect of a HL microclimate on the concentration and distribution of these compounds in the grapes. A positive value

Stellenbosch University https://scholar.sun.ac.za

indicates a higher concentration against the LL microclimate (background/control) and a negative value a decrease. Table 3.2 shows the log2 fold changes for the whole berries, skin, pulp and seed. Caftaric and coutaric acid concentrations were increased in the whole berries, skin and pulp. Caffeic acid only increased in the whole berries and skin and was unaffected in the pulp. Coutaric acid showed the biggest response to the HL microclimate, with significant impacts on the whole berries (0.6), skin (0.7) and pulp (0.6). The hydroxycinnamic acids response was found to be the strongest in the skin, whereas caftaric and caffeic acid were quantified in the seed (Supplementary Table S3.1), but were unresponsive to the HL microclimate.

The response of flavan-3-ols to the HL microclimate was exclusive to the skin and whole berries. A response for catechin was observed in the skin and the polymeric polyphenols in the whole berries. Flavan-3-ol_2 and flavan-3-ol_3 did not respond, but was higher in the LL microclimate whole berry, as indicated by the negative fold change value. In the seeds polymeric polyphenols did not respond to the HL microclimate but a response was observed in the whole berries as content increased by 0.9 log_2 fold.

Similar to the flavan-3-ols, flavonol response to the HL microclimate was only observed in the skin and whole berry. All the quantified flavonols, quercetin-glucoside, quercetin-galactoside, flavonol_4 and flavonol_5 showed strong responses in the skin. The flavonols were the most responsive group of polyphenols generating log_2 fold change values between 3.8 and 7.8.

Different parts of the polyphenol biosynthesis pathway were affected in the different tissues of Sauvignon Blanc grapes with a high phenolic potential (HP) compared to grapes with a low phenolic potential (LP) (figure 3.2 A). The part of the biosynthesis pathway responsible for HCA synthesis was predominantly affected in the skin of high phenolic potential grapes and concurrently reflected in the whole berries. The branches that lead to synthesis of caftaric acid, caffeic acid and coutaric acid were all affected in the skin of high phenolic potential grapes. The ANOVA plots (Figure 3.2 B) indicate that the caftaric acid and caffeic acid branches were significantly affected in the HP skin as these tissues accumulated more of these compounds. In the pulp, the branches responsible for caffeic acid synthesis was unaffected whereas none of the above mention branches were affected in the seeds.

The part of the flavonoid biosynthesis pathway responsible for flavan-3-ols synthesis was mostly unaffected in high phenolic potential grapes. The branch that was affected was the one that lead to catechin synthesis.

The branches responsible for flavonol synthesis was significantly affected in the skin of high phenolic potential grapes. The branches that lead to the synthesis of quercetin-galactoside, flavonol_4 and flavonol_5 synthesis was significantly affected in the HP grapes as none of these compounds were

Stellenbosch University https://scholar.sun.ac.za

Table 3.2: The log_2 fold change of the polyphenol concentrations (µg/gDW) of Sauvignon Blanc whole berries and tissues after harvest from low light (LL) and high light (LL) microclimates.

	LL vs HL (log_2 fold change)			
	WB	Skin	Pulp	Seed
Caftaric acid	0.4*	0.4*	0.3*	0.0
Coutaric acid	0.6*	0.7*	0.6*	0.0
Caffeic acid	0.4*	0.5*	0.0	-0.1
Total hydroxycinnamic acids	0.4*	0.5*	0.3*	0.0
Catechin	0.1	0.4	0.0	0.0
Epicatechin	0.1	0.0	0.0	0.0
Flavan-3-ol_1	0.0	0.0	0.0	-0.1
Flavan-3-ol_2	-0.3*	0.0	0.0	0.0
Flavan-3-ol_3	-0.3*	0.0	0.0	0.1
Flavan-3-ol_4	0.0	0.0	0.0	0.0
Polymeric phenolics	0.9*	0.0	0.0	0.0
Total flavan-3-ols	0.4*	0.3	0.0	0.0
Quercetin-Gal	0.0	7.2*	0.0	0.0
Quercetin-Glu	4.1*	3.8*	0.0	0.0
Favonol_4	0.0	7.8*	0.0	0.0
Flavonol_5	0.0	5.8*	0.0	0.0
Total flavonols	4.4*	4.3*	0.0	0.0
Total Phenolics	0.7*	1.6*	0.3*	0.0

Stellenbosch University https://scholar.sun.ac.za

Log_2 fold change

The log fold change is highlighted by a colour scale from -1 to >4 fold change. Statistical significance is indicated with an ∗ ($p<0.05$) and determined with an ANOVA F-test with a confidence interval of 95%.

Stellenbosch University https://scholar.sun.ac.za

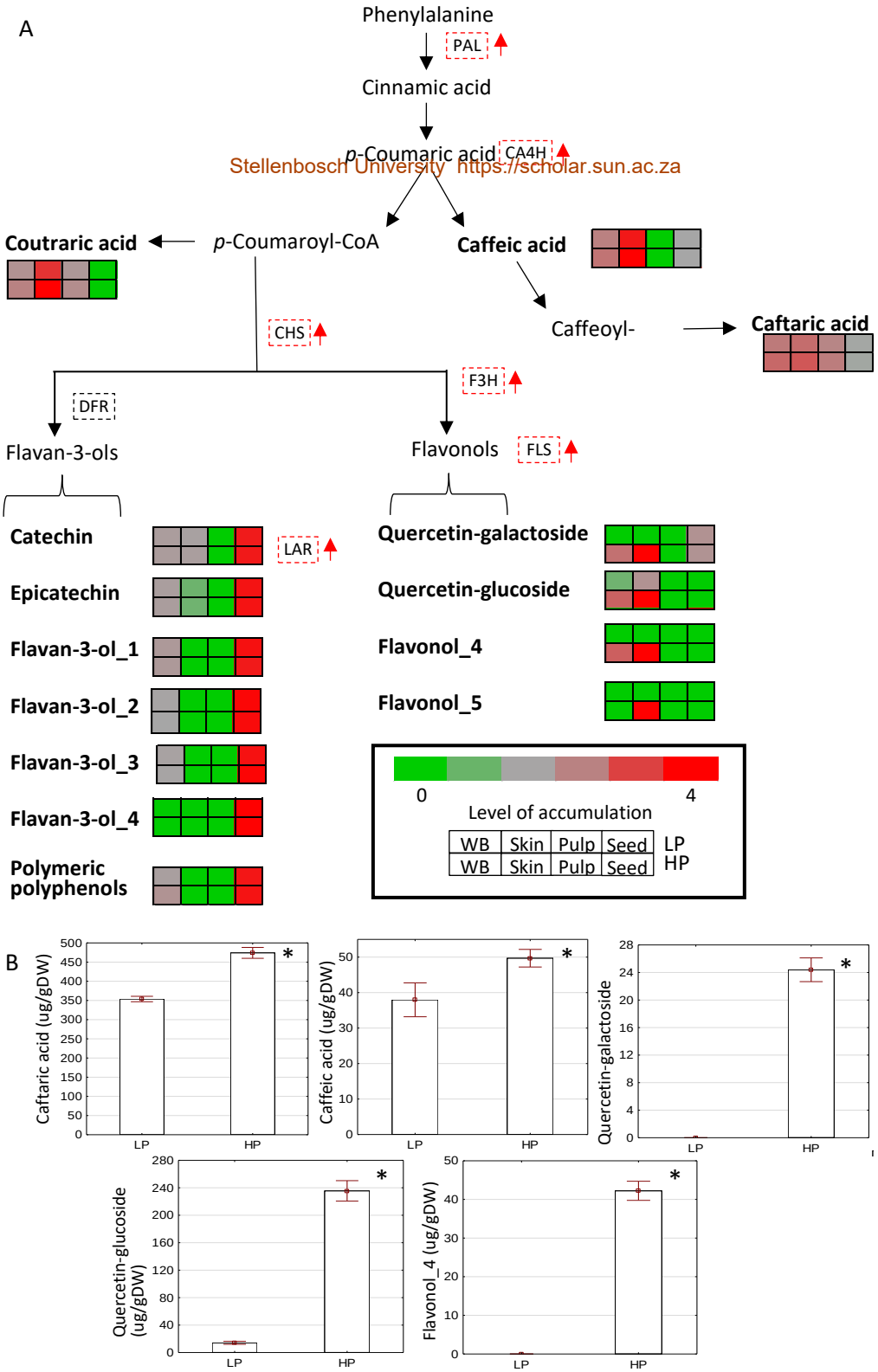


Figure 3.2: A simplified version of the polyphenol biosynthesis pathway of grapes showing (A) the level of accumulation of each compound in Sauvignon Blanc tissues at harvest with a low phenolic potential (LP) and high phenolic potential (HP) microclimate (this study). Dotted red rectangles and red arrows indicate the enzyme encoding genes that were up regulated in the HP grapes compared to LP grapes according to the study of . PAL (phenylalanine -ammonia-lyase), CHS (chalcone synthase), CA4H (trans-cinnamate-4-monooxygenase), FLS (flavonol synthase), DFR (dihydrolflavonol reductase), LAR (leucoanthocyanidin reductase) (Berli et al. 2011; Du Plessis 2017). (B) Mean plots indicate the concentrations of polyphenols most affected by the HL microclimate in the skin. Statistical significance is indicated with an ∗ ($p<0.05$) and determined with an ANOVA F-test with a confidence interval of 95%.

t

Stellenbosch University https://scholar.sun.ac.za

3.4. Discussion

The polyphenol composition and distribution of ripe Sauvignon Blanc grape berry tissues (skin, pulp, seed) was determined with the aim to establish its phenolic potential. The phenolic potential refers to the total and variety of all quantified polyphenols that can potentially be extracted during standard winemaking practices. By establishing the phenolic potential, it provides an estimate on the contribution of each tissue type (skin, pulp seed) towards the potential polyphenol composition of the resulting juice and wine.

Stellenbosch University https://scholar.sun.ac.za

3.4.1. Distribution of phenolic groups in Sauvignon Blanc tissues and the effect of a HL microclimate on the phenolic potential

Grapes from a HL microclimate was confirmed to have a high phenolic potential (HP) compared to the low phenolic potential (LP) of grapes from a LL microclimate due to a significantly higher total polyphenol concentration. This result is in accordance with literature as it has been well established that the total polyphenol concentration is increased in white and red grapes from HL microclimates (Bergqvist et al. 2001; Ristic et al. 2006; Song et al. 2015; Du Plessis 2017; Šebela et al. 2017).

The phenolic potential of the grapes was not increased as a result of the cumulative response of all phenolic groups, in all tissues, to the HL microclimate. The polyphenol composition and content of the skin was most affected by the HL microclimate where less extreme content changes were observed in the pulp. In essence, the higher phenolic potential of the grapes from the HL microclimate was primarily due to the total increase of the phenolic groups present in the skin. The polyphenol composition of the skin of grapes with a high phenolic potential was also more focussed on the accumulation of flavonols compared to the accumulation of hydroxycinnamic acids in low phenolic potential grapes. The results of other studies also revealed that the flavonoid and non-flavonoid content of the berry increased, to an extent, in response to light exposure (Haselgrove et al. 2000; Downey et al. 2004; Berli et al. 2011; Gregan et al. 2011; Koyama et al. 2012).

The skin is the first line of defence of the berry and polyphenols have been reported to exert a protective effect against both environmental stress (Downey et al. 2006) and fungal infection (Guidoni et al. 2008; Mosetti et al. 2016). It can therefore be assumed that skin accumulates a larger variety of compounds as precautionary measure against environmental stress and infection. The increase in flavonol concentrations, and hydroxycinnamic acids to lesser extent, indicated the response of the skin to the altered microclimate (HL) and elucidated its protective role against environmental light stress as these compounds act as a sunscreen by absorbing ultraviolet radiation (Gregan et al. 2011) or scavengers of reactive oxygen species induced by photo damage (Agati et al. 2013).

In a LL microclimate phenolic group distribution was also not uniform in the tissues of the grape berry (figure 3.1). Each of the tissues (skin, pulp and seed) had a characteristic composition, since a specific group

Stellenbosch University https://scholar.sun.ac.za

of polyphenols were primarily accumulated. The skin and pulp primarily accumulated hydroxycinnamic acids whereas the seeds accumulated flavan-3-ols. These results correspond well with what has been reported in literature for Sauvignon Blanc and other white cultivars (Rodríguez Montealegre et al. 2006; Di Lecce et al. 2014; Dragana et al. 2016). Unlike the pulp and seeds, the skin was the most versatile as it accumulated noticeable levels of flavan-3-ols and flavonols.

Stellenbosch University https://scholar.sun.ac.za

3.4.2. Distribution of individual polyphenols

The composition of individual polyphenols of each tissue correlated with what is generally found for Sauvignon Blanc grape tissues and other white cultivars (Rodríguez Montealegre et al. 2006; Castillo-Munoz et al. 2010; Di Lecce et al. 2014; Dragana et al. 2016). However, comparisons between different studies are complicated by different analysis methods. Polyphenols react differently to different extraction solvents, which results in varying compounds being extracted to varying concentrations. Extraction is also dependent on the harshness of the solvent and extraction conditions (Agustin-Salazar et al. 2014; Su et al. 2014; Bosso et al. 2016). The sensitivity of analytical instruments and the conditions under which they are operated also contributes to the variation of compound and their concentrations that are reported in literature (Kennedy and Waterhouse 2000; Di Lecce et al. 2014; Terblanche 2017). In this study, acidified methanol was the extraction solvent, coupled with reverse phase HPLC analysis, as it was previously used by (Du Plessis 2017) for polyphenol extraction from berries from the same model vineyard. Agustin-Salazar et al. (2014) and Su et al.(2014) reported methanol as an effective solvent for polyphenol extraction from grape pomace and litchi pulp respectively. Our analyses did not include certain compound classes, such as the stilbenes, that would also contribute to the (full) phenolic potential of the grapes. Stilbenes, are only detected when HPLC is coupled with mass spectrometry (Kammerer et al. 2004; Di Lecce et al. 2013; Di Lecce et al. 2014).

Polymeric polyphenols were detected in the seeds but was surprisingly not detected in the skin of low phenolic potential or high phenolic potential grapes. The skin of white cultivars have been reported to at least contain procyanidins dimers (polymers of catechin and/or epicatechin) (Rodríguez Montealegre et al. 2006; Skroza et al. 2010). The identity of flavan-3-ol_1, flavan-3-ol_2, flavan-3-ol_3 and flavan-3-ol_4 in the seeds and flavonol_4 and flavonol_5 in the skin (HP grapes only) is unknown due to a lack of reference standards used in this study. Their identity can be speculated to be procyanidins with a low degree of polymerization (dimers or trimers) constituted by catechin and/or epicatechin (Kennedy and Waterhouse 2000). The polymeric polyphenols was eluted as a non-resolved peak and has been reported as a collection of procyanidins (tannins) at various degrees of polymerization. Aleixandre-Tudo et al. (2018) found a strong correlation between the non-resolved polymeric phenol peak and values from obtained from the methyl cellulose precipitable tannin assay (MCP) which estimates the total tannin concentration. The polymeric polyphenols therefore only provide an estimation of the tannin content but not as an absolute value.

Stellenbosch University https://scholar.sun.ac.za

In the study of Du Plessis (2017), the genes that encode the enzymes responsible for the synthesis of individual hydroxycinnamic acids and flavonols (Figure 3.2 and Supplementary Table S3.4) were upregulated in whole Sauvignon Blanc grapes harvested from a HL microclimate. In this study, individual hydroxycinnamic acids and flavonols accumulated to a greater extent in the whole berry, skin and pulp of high phenolic potential grapes which suggest, based on the data of Du Plessis (2017), that the genes responsible for the synthesis of these compounds were also upregulated.

Stellenbosch University https://scholar.sun.ac.za

3.5. Conclusions

In this study leaf removal, to create a HL microclimate (light stress), has been shown to be an effective viticultural treatment to increase the phenolic potential of Sauvignon Blanc grapes. The treatment was effective at increasing all the measured phenolic groups, specifically the flavonols. The phenolic composition and content of the seeds were unaffected whereas a marginal increase in pulp content was induced. The HL microclimate exerted the biggest effect on the skin and its flavonol composition and content. This data provides a quantified baseline for the grapes from the HL and LL microclimates terms of their phenolic potentials, particularly when investigating further the extractability of these additional phenolic compounds in the high phenolic potential grapes, under winemaking conditions, and in comparison to the low phenolic potential grapes.

3.6. References

Adams DO (2006) Phenolics and Ripening in Grape Berries. Am J Enol Vitic 3:249–256.

Agati G, Brunetti C, Di M, Ferrini F, Pollastri S, Tattini M (2013) Plant Physiology and Biochemistry Functional roles of fl avonoids in photoprotection : New evidence , lessons from the past. Plant Physiol Biochem 72:35–45. doi: 10.1016/j.plaphy.2013.03.014

Agustin-Salazar S, Medina-Juárez LA, Soto-Valdez H, Manzanares-López F, Gámez-Meza N (2014) Influence of the solvent system on the composition of phenolic substances and antioxidant capacity of extracts of grape (Vitis vinifera L.) marc. Aust J Grape Wine Res 20:208–213. doi: 10.1111/ajgw.12063

Aleixandre-Tudo JL, Nieuwoudt H, Olivieri A, Luis J (2018) Phenolic profiling of grapes , fermenting samples and wines using UV- Visible spectroscopy with chemometrics.

Arapitsas P, Guella G, Mattivi F (2018) The impact of SO2 on wine flavanols and indoles in relation to wine style and age. Sci Rep 1–13. doi: 10.1038/s41598-018-19185-5

Bergqvist J, Dokoozlian N, Ebisuda N (2001) Sunlight Exposure and Temperature Effects on Berry Growth and Composition of Cabernet Sauvignon and Grenache in the Central San Joaquin Valley of California. 1:3–9.

Berli FJ, Fanzone M, Piccoli P (2011) Solar UV-B and ABA Are Involved in Phenol Metabolism of Vitis vinifera L . Increasing Biosynthesis of Berry Skin Polyphenols. J Agric Food Chem 4874–4884. doi: 10.1021/jf200040z

Bledsoe AM, Kliewer WM, Marois JJ (1988) Effects of Timing and Severity of Leaf Removal on Yield and Fruit Composition of Sauvignon blanc Grapevines. J Enol Vitic 39:49–54.

Stellenbosch University https://scholar.sun.ac.za

Bosso A, Guaita M, Petrozziello M (2016) Influence of solvents on the composition of condensed tannins in grape pomace seed extracts. 207:162–169.

Castillo-Munoz N, Gomez-Alonso S, Garcıa-Romero E, Hermosın-Guitierrez I (2010) Flavonol profiles of Vitis vinifera white grape cultivars. J Food Compos Anal 23:699–705. doi: 10.1016/j.jfca.2010.03.017

Stellenbosch University https://scholar.sun.ac.za

Di Lecce G, Arranz S, Jáuregui O, Tresserra-rimbau A, Quifer-rada P, Lamuela-raventós RM (2014) Phenolic profiling of the skin , pulp and seeds of Albariño grapes using hybrid quadrupole time-of-flight and triple-quadrupole mass spectrometry. Food Chem 145:874–882. doi: 10.1016/j.foodchem.2013.08.115

Di Lecce G, Boselli E, D'Ignazi G, Frega NG (2013) LWT - Food Science and Technology Evolution of phenolics and glutathione in Verdicchio wine obtained with maceration under reductive conditions. LWT - Food Sci Technol 53:54–60. doi: 10.1016/j.lwt.2013.03.006

Downey MO, Dokoozlian NK, Krstic MP (2006) Cultural Practice and Environmental Impacts on the Flavonoid Composition of Grapes and Wine : A Review of Recent Research. Am J Enol Vitic 3:257–268.

Downey MO, Harvey JS, Robinson SP (2003) Analysis of tannins in seeds and skins of Shiraz grapes throughout berry development. Aust J Grape Wine Res 15–27.

Downey MO, Harvey JS, Robinson SP, Industry CP, Unit H, Box PO, Osmond G (2004) The effect of bunch shading on berry development and flavonoid accumulation in Shiraz grapes. Aust J Grape Wine Res 55–73.

Dragana C, Davidovic SM, Todic SR, Bešlic ZS, Tešic L (2016) Identification and quantification of phenolic compounds in berry skin , pulp , and seeds in 13 grapevine varieties grown in Serbia. 211:243–252.

Du Plessis K (2017) The evaluation of the impact of microclimatic factors on grapevine berries in a vineyard setting through molecular profiling. Thesis, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

Fulcrand H, Salas E, Cheynier V (2006) Phenolic Reactions during Winemaking and Aging. 3:289–297.

Garrido J, Borges F (2013) Wine and grape polyphenols - A chemical perspective. Food Res Int 54:1844–1858. doi: 10.1016/j.foodres.2013.08.002

González-Manzano S, Rivas-Gonzalo JC, Santos-Buelga C (2004) Extraction of flavan-3-ols from grape seed and skin into wine using simulated maceration. Anal Chim Acta 513:283–289. doi: 10.1016/j.aca.2003.10.019

Gregan SM, Wargent JJ, Liu L, Shinkle J, Hofmann R, Winfield C, Trough M, Jordan B (2011) Effects of solar ultraviolet radiation and canopy manipulation on the biochemical composition of Sauvignon Blanc grapes. Aust J Grape Wine Res. doi: 10.1111/j.1755-0238.2012.00192.x

Guidoni S, Oggero G, Cravero S, Rabino M, Cravero MC, Balsari P (2008) Manual and mechanical leaf removal in the bunch zone (Vitis vinifera L cv., Barbera): Effects on berry composition , health, yield and wine quality in a warm temperature area. J Sci 49–58.

Haselgrove L, Botting D, van Heeswijck PB, Dry PR, Ford C, Iland PG (2000) Canopy microclimate and berry composition : The effect of bunch exposure on the phenolic composition of Vitis vinifera L cv . Shiraz grape berries. Aust J Grape Wine Res 6:141–149.

Honeth. C (2018) Evaluating the effects of UVB radiation on field grown grape berry metabolites under high and low light microclimates and the consequent impacts on the resulting wines. Thesis, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

Stellenbosch University https://scholar.sun.ac.za

Kammerer D, CLaus A, Carle R, Schieber A (2004) Polyphenol Screening of Pomace from Red and White Grape Varieties (Vitis vinifera L .) by HPLC-DAD-MS / MS. J Agric Food Chem 52:27–30.

Kennedy JA, Matthews MA, Waterhouse AL (2000) Changes in grape seed polyphenols during fruit ripening. 55:77–85.

Stellenbosch University https://scholar.sun.ac.za

Kennedy JA, Saucier C, Glories Y (2006) Grape and wine phenolics: History and perspective. Am J Enol Vitic 57:239–248.

Kennedy JA, Waterhouse AL (2000) Analysis of pigmented high-molecular-mass grape phenolics using ion-pair , normal-phase high-performance liquid chromatography. J Chromatogr A 866:25–34.

Koyama K, Ikeda H, Poudel PR, Goto-yamamoto N (2012) Phytochemistry Light quality affects flavonoid biosynthesis in young berries of Cabernet Sauvignon grape. Phytochemistry 78:54–64. doi: 10.1016/j.phytochem.2012.02.026

Mosetti D, Herrera JC, Sabbatini P, Green A, Alberti G, Peterlunger E, Lisjak K, Castellarin S (2016) Impact of leaf removal after berry set on fruit composition and bunch rot in ' Sauvignon blanc ' Impact of leaf removal after berry set on fruit composition and bunch rot in ' Sauvignon blanc '. Vitis. doi: 10.5073/vitis.2016.55.57-64

Pastrana-Bonilla E, Akoh C, Sellapan S, Krewers G (2003) Phenolic Content and Antioxidant Capacity of Muscadine Grapes.

Ristic R, Downey MO, Iland PG, Bindon K, Francis L, Herderich M, Robinson S (2006) Exclusion of sunlight from Shiraz grapes alters wine colour ,. Aust J Grape Wine Res 53–65.

Rodríguez Montealegre R, Romero Peces R, Chacón Vozmediano JL, Martínez Gascueña J, García Romero E (2006) Phenolic compounds in skins and seeds of ten grape Vitis vinifera varieties grown in a warm climate. J Food Compos Anal 19:687–693. doi: 10.1016/j.jfca.2005.05.003

Šebela D, Turóczy Z, Olejníčková J, Kumšta M, Sotolář R (2017) Effect of Ambient Sunlight Intensity on the Temporal Phenolic Profiles of Vitis Vinifera L . Cv . Chardonnay During the Ripening Season – A Field Study. South African J Enol Vitic 38:94–102.

Skroza D, Generalic I, Moz SS, Abramovic H (2010) Polyphenolic profile , antioxidant properties and antimicrobial activity of grape skin extracts of 14 Vitis vinifera varieties grown in Dalmatia (Croatia). 119:715–723. doi: 10.1016/j.foodchem.2009.07.019

Somers TC, Verette E, Pocock KF (1987) Hydroxycinnamate Esters of Vitis vinifera : Changes during White Vinification , and Effects of Exogenous Enzymic Hydrolysis. 67–78.

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

Su D, Zhang R, Hou F, Zhang M, Guo J, Huang F, Deng Y (2014) Comparison of the free and bound phenolic profiles and cellular antioxidant activities of litchi pulp extracts from different solvents. 1–10.

Suklje K, Antalick G, Coetzee Z, Schmidtke LM, Basa Cesnic H, Brandt J, Toit WJ, Lisjak K, Deloire A (2014) Effect of leaf removal and ultraviolet radiation on the composition and sensory perception of Vitis vinifera L . cv . Sauvignon Blanc wine. Aust J Grape Wine Res 10:223–233. doi: 10.1111/ajgw.12083

Terblanche E (2017) Development of novel methods for tannin quantification in grapes and wine by.

Young PR, Eyeghe-bickong HA, Plessis K, Alexandersson E, Jacobson DA, Coetzee Z, Deloire A, Vivier MA (2016) Grapevine Plasticity in Response to an Altered Microclimate : Sauvignon Blanc Modulates Speci fi c Metabolites in Response to Increased Berry Exposure 1. 170:1235–1254. doi: 10.1104/pp.15.0177

Stellenbosch University https://scholar.sun.ac.za

Supplementary data to Chapter 3.

Supplementary Table S3.1: The hydroxycinnamic acid concentrations of Sauvignon Blanc whole grapes and tissues (µg/gDW) at harvest.

Stellenbosch University https://scholar.sun.ac.za

		Caftaric acid	Coutaric acid	Caffeic acid	**Total Hydroxycinnamic acids**
Whole berry	LL	353.7±9.2 d	10.6±1.3 e	37.9±6.2 d	402.4±16.7 d
	HL	474.1±18.4 b	16.±1.2 c	49.7±3.2 c	540.4±22.9 c
	Population minimum	346.1	8.7	28.8	383.6
	Population maximum	505.0	18.7	54.1	577.9
	Population average	413.9	13.6	43.8	471.4
Skin	LL	442.2±10.5 c	46.9±3.1 b	128.3±11.7 b	617.5±40.8 b
	HL	594.2±22.7 a	76.8±1.4 a	180.3±5.8 a	851.1±76.6 a
	Population minimum	432.2	131.8	42.9	593.8
	Population maximum	619.5	79.1	193.8	892.5
	Population average	430.6	42.8	99.1	734.3
Pulp	LL	267.9±21.2 f	8.0±0.7 f	N.D	275.9±22.0 f
	HL	331.8±19.4 e	12.3±2.1 d	N.D	344.1±21.5 e
	Population minimum	237.7	7.3	N.D	245.0
	Population maximum	351.3	15.2	N.D	366.5
	Population average	299.9	10.1	N.D	310.0
Seed	LL	45.5±1.2 g	N.D	6.0±0.5 e	51.6±1.7 g
	HL	45.2±2.1 g	N.D	5.8±0.3 e	51.0±2.5 g
	Population minimum	42.3	N.D	5.3	47.7
	Population maximum	48.3	N.D	6.9	55.2
	Population average	45.4	N.D	5.9	51.3

Abbreviations: LL, low light; HL, high light

Different letters indicate statistical significance (One-way ANOVA with Fisher's LSD post hoc test, $p<0.05$) and is relevant between the tissues within each phenolic group.

Stellenbosch University https://scholar.sun.ac.za

Supplementary data of Chapter 3

Supplementary Table S3.2: The flavan-3-ol concentrations of Sauvignon Blanc whole grapes and tissues (µg/gDW) at harvest.

		Catechin	Epicatechin	Flavan-3-ol_1	Flavan-3-ol_2	Flavan-3-ol_3	Flavan-3-ol_4	Polymeric polyphenols	**Total Flavan-3-ols**
Whole berry	LL	206.6±16.7 c	120.1±11.1 b	47.7±2.8 c	46.7±8.8 c	65.8±11.2 c	N.D.	305.5±23.7 c	792.6±85.6 d
	HL	214.0±17.1 c	125.0±14.5 b	47.9±3.2 c	38.2±6.5 c	54.8±9.6 c	N.D	578.4±18.4 d	1058.6±84.1 c
	Population minimum	183.7	104.3	42.2	28.3	41.6	N.D	281.7	682.0
	Population maximum	240.0	147.4	52.0	55.8	85.1	N.D	600.5	1181.0
	Population average	210.3	122.6	47.8	42.5	60.3	N.D	441.9	925.6
Skin	LL	145.6±11.2 e	34.7±1.7 c	N.D	N.D	N.D	N.D	N.D	180.3±14.7 f
	HL	193.8±15.7 d	16.8±3.3 c	N.D	N.D	N.D	N.D	N.D	229.3±10.3 e
	Population minimum	118.6	30.5	N.D	N.D	N.D	N.D	N.D	162.4
	Population maximum	197.9	39.7	N.D	N.D	N.D	N.D	N.D	237.7
	Population average	109.1	22.5	N.D	N.D	N.D	N.D	N.D	204.8
Pulp	LL	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	HL	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Population minimum	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Population maximum	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Population average	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Seed	LL	1808.6±19.5 a	1208.1±36.5 a	365.7±26.4 a	875.7±38.6 a	892.0±37.4 b	138.4±7.3	3331.5±1.5 b	8620.1±240.0 a
	HL	1828.4±15.3 a	1212.5±7.6 a	341.9±24.2 b	850.5±20.4 b	957.3±45.3 a	135.8±5.5	3409.4±1.8 a	8736.2±218.9 a
	Population minimum	1786.1	1139.3	303.1	822.7	831.4	127.2	3147.1	8157.0
	Population maximum	1839.0	1235.7	409.8	948.7	962.5	146.1	3543.7	9085.8
	Population average	1818.5	1210.3	353.8	863.1	924.6	137.1	3370.4	8678.1

Abbreviations: LL, low light; HL, high light

Different letters indicate statistical significance (One-way ANOVA with Fisher's LSD post hoc test, $p<0.05$) and is relevant between the tissues within each phenolic group.

Stellenbosch University https://scholar.sun.ac.za

Supplementary Table S3.3: The flavonol concentrations of Sauvignon Blanc whole grapes and tissues (µg/gDW) at harvest.

		Quercetin-galactoside	Quercetin-glucoside	Favonol_4	Flavonol_5	**Total flavonols**
Whole berry	LL	N.D	14.1±2.7 d	N.D	N.D	14.1±2.7 d
	HL	24.2±2.2 b a	235.4±19.3 b	42.2±3.2	N.D	302.1±24.8 b
	Population minimum	N.D	10.4	N.D	N.D	10.4
	Population maximum	26.8	262.7	46.5	N.D	336.0
	Population average	12.2	124.8	21.1	N.D	158.1
Skin	LL	N.D	85.4±12.4 a	N.D	N.D	85.4±14.7 c
	HL	147.2±5.2	572.5±16.3 c	223.7±13.0	54.9±5.1 a	1634.0±10.3 a
	Population minimum	N.D	85.4	N.D	N.D	82.4
	Population maximum	156.6	1240.1	243.3	65.8	1706.0
	Population average	47.3	415.9	71.7	17.6	859.7
Pulp	LL	N.D	N.D	N.D	N.D	0.00
	HL	N.D	N.D	N.D	N.D	0.00
	Population minimum	N.D	N.D	N.D	N.D	0.00
	Population maximum	N.D	N.D	N.D	N.D	0.00
	Population average	N.D	N.D	N.D	N.D	0.00
Seed	LL	11.2±1.5 c	N.D	N.D	N.D	11.22±1.5 de
	HL	10.8±1.8 c	N.D	N.D	N.D	10.85±1.8 de
	Population minimum	8.4	N.D	N.D	N.D	8.4
	Population maximum	14.1	N.D	N.D	N.D	14.1
	Population average	11.0	N.D	N.D	N.D	11.0

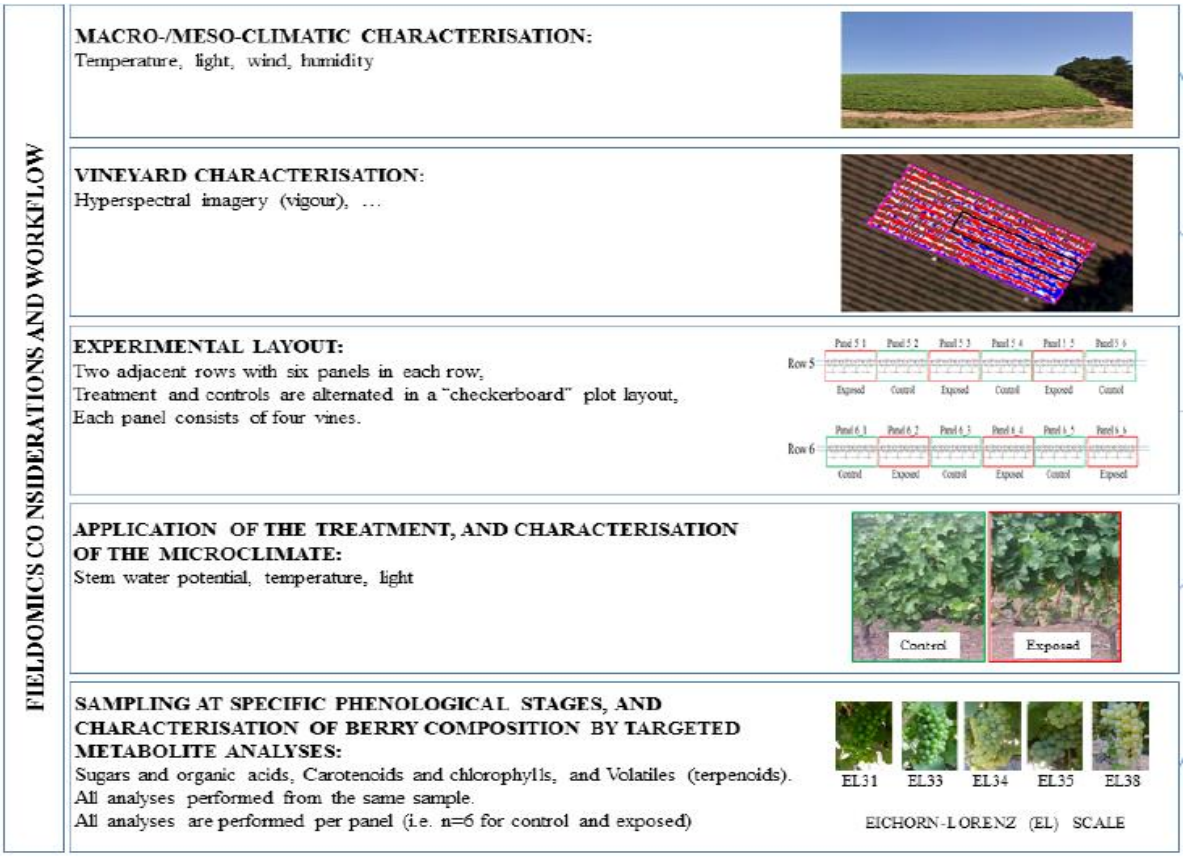
Abbreviations: LL, low light; HL, high light

Different letters indicate statistical significance (One-way ANOVA with Fisher's LSD post hoc test, $p<0.05$) and is relevant between the tissues within each phenolic group.

Stellenbosch University https://scholar.sun.ac.za

Supplementary Table S4.4: Metabolite concentrations and gene expression levels of Sauvignon Blanc whole berries at four developmental stages (Du Plessis 2017). Concetration values are given as log_2 fold change of the grapes from high light microclimates vs the low light microclimate control.

	EL38
phenylalanine ammonia-lyase 2 (PAL2)	***0.84***
Phenylalanine ammonia-lyase	***-1.28***
Phenylalanine ammonia-lyase	***1.27***
trans-cinnamate 4-monooxygenase	***0.68***
trans-cinnamate 4-monooxygenase	***0.84***
P-coumaroyl shikimate 3'-hydroxylase isoform 1	***0.64***
hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyltransferase	0.39
4-coumarate-CoA ligase	0.24
4-coumarate-CoA ligase	***0.68***
4-coumarate-CoA ligase 2	-0.34
4-coumarate-CoA ligase 3	0.26
4-coumarate-CoA ligase	0.10
OPCL1 (OPC-8:0 COA LIGASE1)	-0.17
quercetin 3-O-methyltransferase 1	0.18
caffeic acid 3-O-methyltransferase	-0.21
Caffeic acid O-methyltransferase	-0.23
Caffeoyl-CoA O-methyltransferase	-0.15
caffeoyl-CoA O-methyltransferase 1	***0.61***
chalcone synthase 1	***1.07***
chalcone synthase 2	***1.96***
chalcone synthase 3	***-0.42***
chalcone isomerase [Vitis vinifera]	0.33
Chalcone-flavanone isomerase	0.07
Vitis vinifera dihydroflavonol reductase (DFR) mRNA XM_002281822.1	-0.15
flavanone-3-hydroxylase (F3H)	***0.51***
Flavanone 3-hydroxylase (F3H)	***0.70***
Vitis vinifera dihydroflavonol reductase (DFR) mRNA XM_002281822.1	-0.15
Vitis vinifera dihydroflavonol reductase (DFR) mRNA XM_002281822.1	-0.15
leucoanthocyanidin dioxygenase (LDOX or ANS)	-0.32
Leucoanthocyanidin dioxygenase	-0.19
anthocyanidin reductase (BAN) [Vitis vinifera] GeneID: 100232981	0.41
Flavonol synthase	***0.55***
Flavonol synthase Vitis vinifera	***3.75***



Supplementtary Figure S3.1: The vineyard layout and field-omics workflow of Young et al. (2016).

Stellenbosch University https://scholar.sun.ac.za

Chapter 4

Research Results

The distribution of polyphenols from grapes with a low and high phenolic potential to juice and wine during wine making

Stellenbosch University https://scholar.sun.ac.za

Chapter 4 - The distribution of polyphenols from grapes with a low and high phenolic potential to juice and wine during wine making

4.1. Introduction

Grape berry polyphenols, housed primarily in the vacuoles of the skin and pulp cells (Fontes et al. 2011), require extraction during winemaking otherwise these compounds will inevitably not be transferred to derived juices and wines. The first two sequential steps in standard white winemaking, namely crushing and pressing, extract juice that contains polyphenols from the berry through physical disruption of the grape cells. During these two steps the first contact and extraction between the juice and tissues that contain the polyphenols are made. Polyphenol extraction can be enhanced through manipulation of the conditions during this contact period. Prolonged contact, referred to as pre-fermentative skin contact or cold maceration between the skin and juice has been shown to be effective, specifically at low temperatures, at enhancing polyphenol extraction (Gomez-Miguez et al. 2007; Cejudo-Bastante et al. 2011a; Olejar et al. 2015). With typical white winemaking procedure, hydroxycinnamic acid and flavonoid extraction are both enhanced with skin contact, but extraction of flavonoids are enhanced when contact occurs for brief periods at low temperatures (Gomez-Miguez et al. 2007; Hernanz et al. 2007).

The pressure at which grapes are pressed is also an important parameter which influences the polyphenol content of juice. It has been reported that low pressing pressures (<0.5 bar) favours an increase in hydroxycinnamic acids whereas higher pressures (1-2 bar) extracts both hydroxycinnamic acids along with flavonoids (Maggu et al. 2007; Patel et al. 2010; Ferreira-lima et al. 2016). The internal structure of grapes is constituted by three zones: the central zone (rich in sugars and malic acid), intermediate zone (rich sugars and tartaric acid) and the periphery (rich in polyphenols and mineral salts). During skin contact and pressing, the juice is first released from the intermediate area and lastly released from the periphery with increasing pressing pressure (0.5-2 bar) (Darias-Martín et al. 2004).

A fraction of polyphenols settle out in the sediment that forms after juice clarification (Fang et al. 2006) and would be discarded when the clear juice is racked off (separated from the sediment) for fermentation. It was shown that when wine was made by fermenting turbid juice (i.e. not clarified, or in contact with the sediment), it had the potential to increase the higher alcohols in the wines (van Jaarsveld et al. 2005), but that it could also cause harsh, low quality wine (Singleton et al. 1975; van Jaarsveld et al. 2005) with a possible higher polyphenol content for some cultivars (Singleton et al. 1975; Liu et al. 1987).

An increase in juice or wine polyphenol content are linked to increased susceptibility to oxidation and browning, especially flavonoids (Oliveira et al. 2011). The enzyme, polyphenol oxidase, is released along with other compounds during the early stages of winemaking and is responsible for oxidizing polyphenols to *o*-quinones. These quinones subsequently react with glutathione to form the grape reactive product which is typically linked to browning (Singleton et al. 1985; Recamales et al. 2006; Maggu et al. 2007; Patel et al.

Stellenbosch University https://scholar.sun.ac.za

2010). Enhanced polyphenol extraction is also associated with increased perceived bitterness and astringency of white wines, as reported in various cultivars such as Chardonnay, Chenin Blanc, Gewürzstraminer, Aíren and Riesling (Cejudo-Bastante et al. 2011a; Aleixandre-Tudo et al. 2015; Sokolowsky et al. 2015; Ballester et al. 2018). Catechin and condensed tannins (polymeric flavan-3-ol) are the main compounds responsible for the perceived bitterness and astringency of wine and the intensity of this perception is influenced by pH and ethanol content (Fischer and Noble 1994; Brossaud et al. 2000; Gawel et al. 2017).

Little information is available on how the phenolic potential of grapes is distributed or transferred to the grape juice and how grapes with differential phenolic potential influences the phenolic composition and quantity in the juice. Ristic et al. (2006) reported red wines derived from grapes with a higher phenolic potential to have a higher total polyphenol, anthocyanin and tannin content. In chapter 3 of this thesis, evidence was provided that confirmed that grapes from a high light (HL) microclimate had a higher phenolic potential (HP) with quantified changes in polyphenol profiles, compared to grapes from a low light (LL) and therefore lower phenolic potential (LP). The aim here was to determine the polyphenols distribution from grapes with differential phenolic potentials to the juice and wine under standard white winemaking practices. In addition to this, modulations to the wine-making procedures to potentially increase extractability of polyphenols from the grape tissues before and during fermentation were also studied.

4.2. Materials and methods

4.2.1. Winemaking

The grapes with a low phenolic potential (LP) and high phenolic potential (HP) that were harvested and cooled down as described in chapter 3 were crushed and destemmed, keeping the three biological repeats separate. Figure 4.1 presents a summary of the winemaking process, outlining the different treatments and sampling points for further analyses. The polyphenol analysis of the juice and wine matrices are presented here, whereas the polyphenol analysis of the pomace and juice sediment fractions will be presented in chapter 5.

Standard white winemaking commenced and sulfur dioxide (SO_2, 2.5% w/v) was added to the grapes after crushing and destemming. The crushed grapes were immediately pressed using a basket press (1.5 kPa). The free SO_2 of the pressed juices were adjusted to 30 ppm (2.5% w/v) and treated with Rapidase Clear (1 ml/hL, Oenobrands, Montpelier) and stored overnight at 4^{o}C to clarify the juice. The clear juice was racked and fermented with Cross Evolution (30 g/hL, Lallemand), a commercial wine yeast. Three wine making treatments were applied: skin contact (Sc), sediment contact (Sed) and skin contact combined with sediment contact (ScSed). Skin contact was conducted for 24 h at 4^{o}C and the must pressed (1.5 kPa), enzyme clarified, racked and fermented according to standard white winemaking procedures. Sediment contact (Sed), standard or with skin contact, entailed treating pressed juice with clarifying enzyme but instead of racking the clear juice the sediment was resuspended prior to yeast inoculation. At the end of alcoholic fermentation,

Stellenbosch University https://scholar.sun.ac.za

the free SO_2 of the wine was adjusted to 50 ppm followed by cold stabilization for 2 weeks at -4°C. The cold stabilized wines were racked from the lees and sediment and the free SO_2 adjusted to 40 ppm. The final wine was bottled and aged for 6 months at 15^0 C where after chemical and sensory analysis were performed. Juice fractions were sampled at each of the processing steps (free run, pressed and enzyme clarified juice) and from all the treatments (Figure 4.1). Sediment was collected after racking of the enzyme clarified juice and wine were sampled after cold stabilization and after aging.

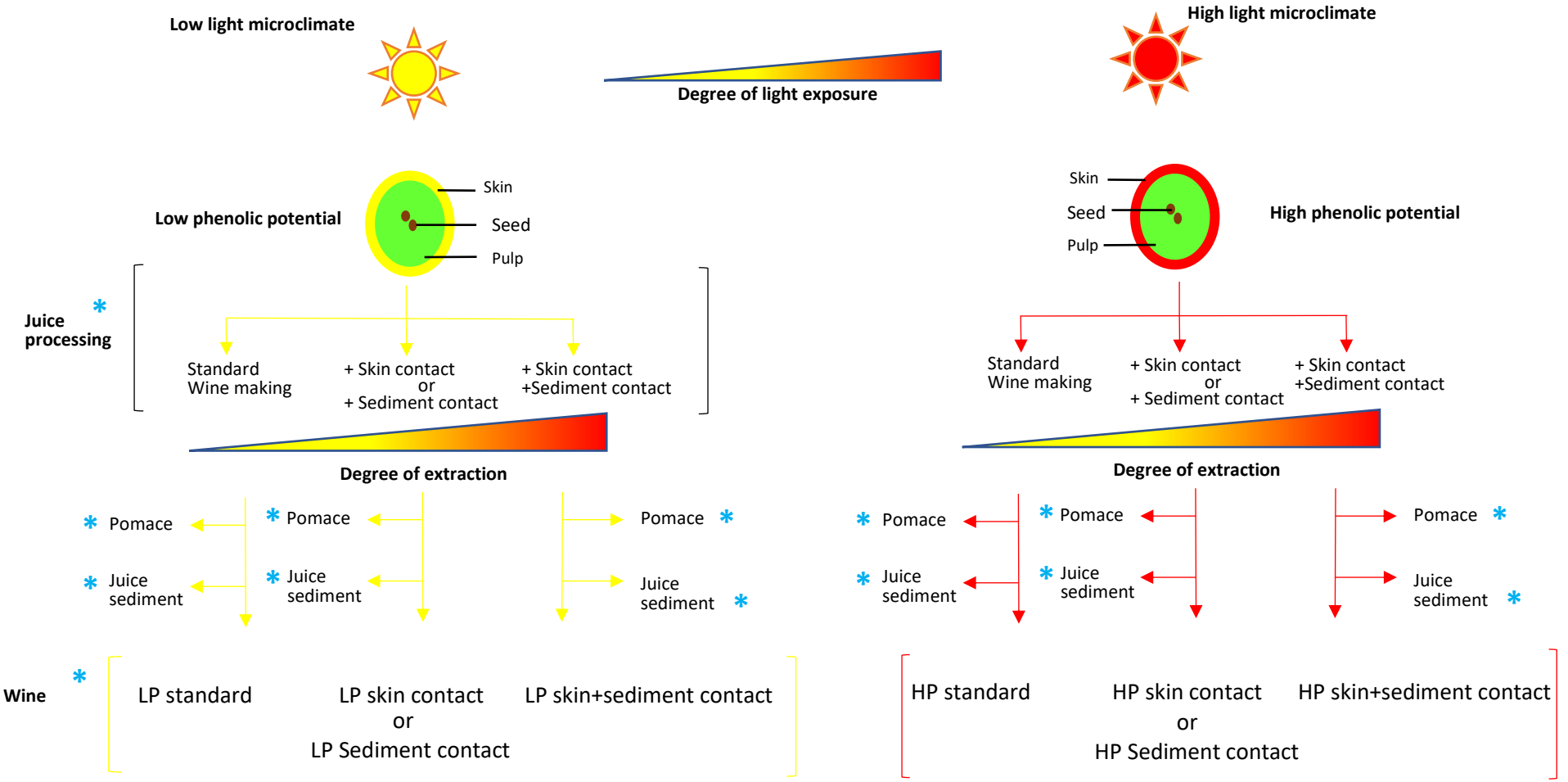
4.2.2. Fermentation metabolites in juice and wine

The organic acids, sugars and alcohols of the clarified juice and the wine at the end of alcoholic fermentation were determined using HPLC as described by Eyéghé-Bickong et al. (2012) The juice and wine samples was mixed with an internal standard to 1:1 ratio. Ribitol (4 g/L) and adipic acid (2 g/L) was used as internal standards. The samples were filtered (0.22 µm, cellulose acetate) into glass vials with metal clamp caps and analysed. An Aminex HPX-87H column (300 nm x 7.8 mm) was used with a Bio-Rad guard column (30 mm x 4.6 mm). The system was equipped with a diode array detector (DAD) and refractive index detector (RID) to detect organic acids and sugars respectively. The HPLC method (SUG_PY5) was run at 55°C using an isocratic gradient of 5 mM H_2SO_4 at a flow rate of 0.5 ml/min for 39 min with an injection volume of 10 µl. The relative concentration of each metabolite was determined by extrapolating from a standard curve generated by a serial dilution of major sugars and organic acids and expressed as mg/L after normalization. Data presented are the means of three biological repeats.

4.2.3. Polyphenol identification and quantification

Polyphenol analysis of the juice and wine was performed using high performance liquid chromatography (HPLC) as previously described in chapter 3. Juice and wine samples (1 mL) were mixed 1:1 with p-coumaric acid (50 mg/L, Merck, Germany) which served as an internal standard, filtered (0.22 µm cellulose acetate membrane) and analysed. Data was expressed as mg/L and are the means of three biological repeats.

Stellenbosch University https://scholar.sun.ac.za



*** = Samples of matrices taken**

Figure 4.1: A flow diagram of the winemaking steps from Sauvignon Blanc grapes with a low phenolic potential (LP) and high phenolic potential (HP). Sampling points are indicated with an asterix (*).

Stellenbosch University https://scholar.sun.ac.za

4.2.4. Wine sensory analysis

Descriptive sensory analysis was conducted as described by Honeth (2018). Briefly a panel consisting of 10 females and 2 males were trained 3 times a week using the wines produced within this study until consensus was reached on the appropriate descriptive terms describing the taste and mouthfeel
of the wine. Panellists were trained to accurately describe and score each descriptor when presented, in a blind test, with a serial dilution of mock samples for each taste descriptor i.e. bitter, astringent, body, sweet and sour as well as the duration of intensity of each descriptor. Upon completion of the training wines were randomised and given a 3-digit code based on the William design Latin-square and presented to the panel in black glasses for evaluation. Each biological repeat of the wine was evaluated in triplicate.

4.2.5. Statistical analysis

Factorial and way One-way analysis of variance (ANOVA) was be performed using Tibco Statistica (version 13, Oklahoma, USA) to determine the statistical significance of light exposure, skin contact and sediment contact within the juice and wine and which phenolic compounds differed significantly within these matrices. ANOVA results are reported as P-values and the highest P-value correlates to the greatest significance. Fischer's least square difference (LSD) post hoc test was conducted to pinpoint which polyphenols responded statistically significant to a particular treatment. All significant differences were interpreted on a 5% significance level ($p<0.05$). Multivariate analysis was performed using SIMCA (Version 14.1 from MKS Umetrics AB). The data was analysed using principle component analysis (PCA) and orthogonal partial least squares – discriminant analysis (OPLS-DA) to investigate trends and outliers within the data and to correlate the variables (X, measured polyphenols) with the qualitative treatments (Y, class e.g. light exposure, skin contact, sediment contact) respectively.

Hierarchical clustering of polyphenols was done as described by Honeth (2018) to confirm treatment responses in the different juice and wine matrices. This was done using Expander (Developed at Ron Shamir's Computational Genomics group, Tel Aviv University, version 7.2).

4.3. Results

4.3.1. Grape, Juice and wine parameters

The harvested grapes from the LL and HL microclimates were analysed for basic ripeness parameters (Table 4.1), showing the measured sugars and organic acids. Similarly, supplementary Tables S4.1 and S4.2 report on the sugars and organic acids measured in all the juice and wine samples taken throughout the juice processing and wine-making steps, as outlined in Figure 4.1. Overall, the organic acids were similar in the different samples of the low and high phenolic potential juices (originating from the LL and HL microclimates respectively), except malic acid which was higher in the low phenolic potential juices. Glucose and fructose

Stellenbosch University https://scholar.sun.ac.za

levels were both higher in the free run high phenolic potential juices, but this difference did not persist for glucose throughout all the juice processing steps. In contrast, fructose levels were still higher in pressed and clarified high phenolic potential juices when skin contact was implemented. The grapes from the HL microclimates were slightly advanced in their sugar accumulation at harvest (Figure 4.1) and the high phenolic potential wines also had the highest residual sugar concentrations, as well as marginally higher ethanol concentrations compared to the corresponding low phenolic potential wines.

Table 4.1: The concentration of sugars and organic acids (mg/gFW) of grapes with a low phenolic potential (LP) and high phenolic potential (HP).

	Whole berry	
	LP	**HP**
Citric acid	N.D.	N.D.
Tartaric acid	6.0±0.3 b	7.1±0.4 a
Malic acid	3.9±0.0.3 a	1.6±0.1 b
Succinic acid	1.8±0.8 a	1.2±0.2 b
Glucose	83.9±2.1 b	95.7±2.2 a
Fructose	88.1±2.2 b	101.2±2.2 a

Abbreviations: N.D. (Not detected)
Different letters indicate statistical significance (One-way ANOVA with Fisher's LSD post hoc test, $p<0.05$)

4.3.2. The comparative impact of juice processing and skin contact on polyphenol levels

The grape juice matrices were analysed for polyphenols at each stage of processing i.e. in the free run, pressed and enzyme clarified juice, to elucidate polyphenol transference from the berry into the juice and how the composition and concentration changed throughout the juice processing steps (Table 4.2), comparing also the impacts between the low and high phenolic potential juices and wines. A PCA analysis (Figure 4.2A) showed that juice samples separated according to the processing steps (from right to left: free run, pressed, clarified) along the first component (PC1, 54.4% explained variance) and separated further along the second component (PC2, 30.5% explained variance) because of the skin contact treatment. A strong separation is observed in all juice samples between low and high phenolic potential, which does not manifest in the standard clarified juice. High phenolic potential samples had lower levels hydroxycinnamic acids in the free run juice but higher levels of flavan-3-ols (Table 4.2). However, high phenolic potential juices had higher levels of hydroxycinnamic acids and flavan-3-ols in the standard pressed samples. Enzyme clarification lowered polyphenol levels in both low and high phenolic potential juices where after these samples were comparable. There was also no discernible difference in hydroxycinnamic acid content in the clarified juices.

Stellenbosch University https://scholar.sun.ac.za

Table 4.2: Polyphenol concentration of the different juices during processing (mg/L) of grapes with a low phenolic potential (LP) and high phenolic potential (HP).

	Free run juice		Pressed juice		Pressed juice with skin contact		Clarified juice		Clarified juice with skin contact	
	LP	HP	LP	HP	LP	HP	LP	HP	LP	HP
Caftaric acid	135.0±11.9 a	124.7±7.2 b	93.6±12.8 d	108.5±14.9 c	107.8±5.6 c	101.7±8.4 cd	4.8±0.5 e	5.5±1.1 e	2.3±1.1 e	6.5±1.5 e
Coutaric acid	N.D.	N.D.	10.2±0.7 d	11.2±2.2 c	20.6±7.4 a	21.4±8.3 a	9.4±4.0 c	9.3±1.2 c	16.1±1.6 a	18.5±1.0 ab
Caffeic acid	5.0±09 de	3.7±0.4 e	3.7±0.4 e	5.7±0.6 cde	7.0±3.3 cd	4.2±0.8 cde	10.0±3.5 b	12.8±3.3 a	14.9±3.4 a	7.8±2.3 bc
Total phenolic acids	**140.0±12.3 a**	**128.5±12.6 b**	**107.4±13.5 c**	**125.4±15.8 b**	**134.4±16.9 ab**	**127.4±9.7 b**	**24.3±8.0 d**	**27.7±5.3 d**	**32.4±5.9 d**	**32.9±4.7 d**
Catechin	84.2±8.2 c	139.7±8.9 a	65.4±7.2 d	78.7±7.5 c	77.7±6.8 c	110.1±9.4 b	24.1±2.3 g	31.5±5.6 fg	36.5±3.4 f	55.6±8.9 e
Epicatechin	4.8±0.2 cde	4.4±0.1 e	4.5±0.3 de	4.9±0.2 cde	6.0±1.5 a	5.7±1.4 ab	4.7±0.8 cde	5.2±0.2 bc	5.3±0.3 abc	5.1±0.5 cd
Flavan-3-ol_1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Flavan-3-ol_2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Flavan-3-ol_3	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Flavan-3-ol_4	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Total Flavan-3-ols	**89.0±8.4 c**	**144.1±8.6 a**	**69.9±7.5 d**	**84.7±7.7 c**	**83.7±16.9 c**	**115.9±20.5 b**	**28.9±8.0 g**	**36.8±5.3 fg**	**41.9±12.5 f**	**60.7±14.3 e**
Polymeric phenolics	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Quercetin-galactoside	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Quercetin-glucoside	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Favonol_4	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Flavonol_5	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Total phenolics	**229.1±21.3 bc**	**272.7±16.3 a**	**177.3±21.3 e**	**209.1±23.2 d**	**218.2±24.7 cd**	**243.3±25.3 b**	**53.3±11.2 h**	**64.5±11.8 gh**	**74.4±9.7 g**	**93.6±14.0 f**

Abbreviations: N.D. (not detected).

Different letters indicate statistical significance (One-way ANOVA with Fisher's LSD post hoc test, $p<0.05$)

Stellenbosch University https://scholar.sun.ac.za

The skin contact treatment increased flavan-3-ols in all the pressed juices but only increased hydroxycinnamic acids levels in the low phenolic potential juice. Hydroxycinnamic acid levels were higher in low phenolic potential skin contact pressed juice but flavan-3-ol levels were higher in high phenolic potential samples. The skin contact clarified juice samples also had lower levels of polyphenols but were higher in both low and high phenolic potential juices compared to the standard samples. Hydroxycinnamic acid concentrations were similar in all the clarified skin contact juices but flavan-3-ol levels were higher in high phenolic potential samples.

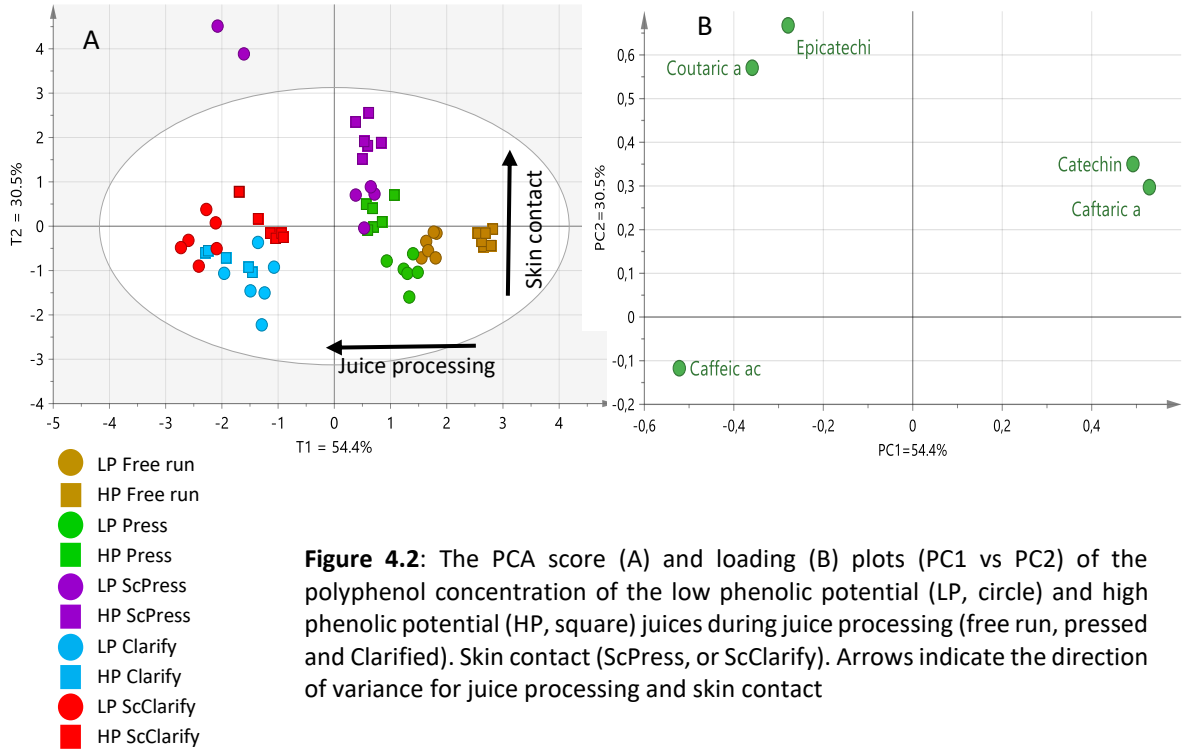


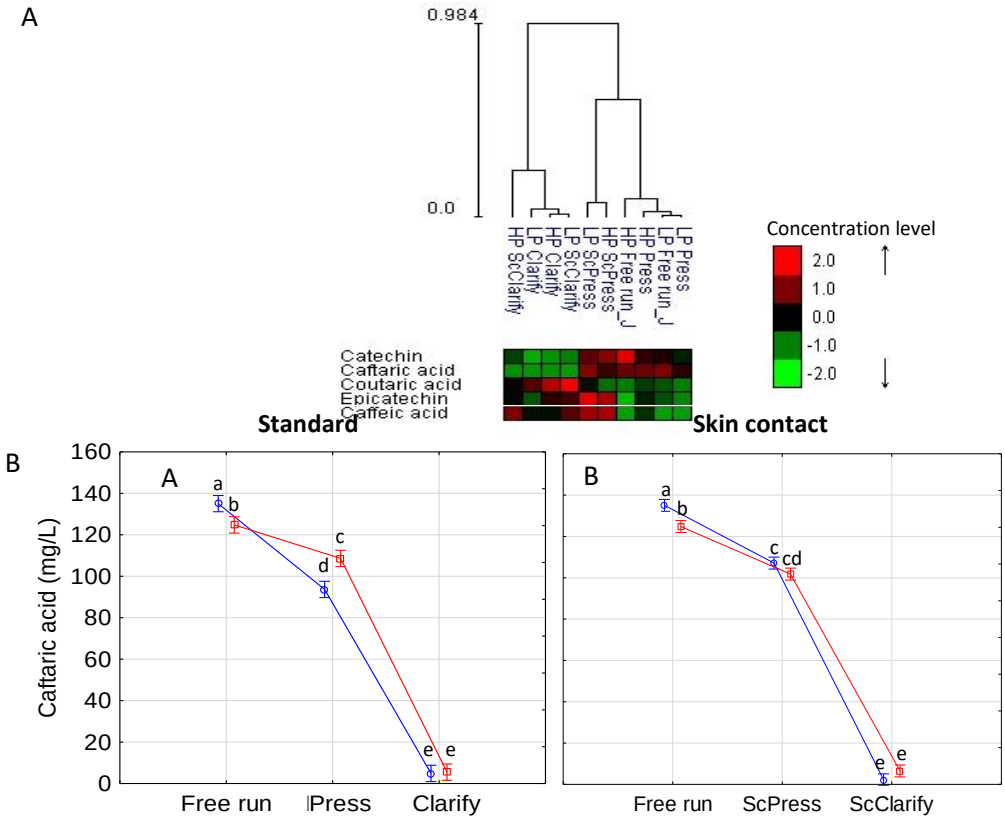
Figure 4.2: The PCA score (A) and loading (B) plots (PC1 vs PC2) of the polyphenol concentration of the low phenolic potential (LP, circle) and high phenolic potential (HP, square) juices during juice processing (free run, pressed and Clarified). Skin contact (ScPress, or ScClarify). Arrows indicate the direction of variance for juice processing and skin contact

Given the interesting patterns observed with the hydroxycinnamic acids, hierarchical cluster analysis was also performed to visualize the effect of vine treatment or juice processing steps on the polyphenol concentration (Figure 4.3A) of the juice. The samples first grouped according to juice processing step, followed by skin contact and then phenolic potential which indicates the significance of each treatment on polyphenol concentration in that order. The concentration of the different phenolic compounds are shown in Table 4.2 and ANOVA plots were used to illustrate (Figure 4.3B) how the levels of individual polyphenolic compounds were affected during the juice processing steps. Juice processing decreased caftaric acid and catechin levels (Figure 4.3B: A, B, G and H) in both low and high phenolic potential juices. On the contrary, coutaric acid and caffeic acid increased from crushing to pressing whereas the concentration of caffeic acid was the highest after juice clarification (Figure 4.3B: C, D, E and F). Epicatechin was the only polyphenol whose concentration was comparable throughout juice processing (Figure 4.3B: I and J). The effect of skin contact, which took place between crush and press, can be seen in the values for pressed juice (Figure 4.3B: B, D, F, H and J). All the polyphenols, except caftaric acid, increased in the HP juice. For example, coutaric acid concentration in pressed juice was approximately 10 mg/L when no skin contact was applied, but with skin

Stellenbosch University https://scholar.sun.ac.za

contact, the concentration was 20 mg/L. However, these increases were not maintained for all the phenolic compounds throughout juice clarification. Phenolic potential appeared to be more dominant in the free run and standard pressed juice samples as groupings were first formed according to phenolic potential followed by juice processing (Figure 4.3A). Caftaric acid was not increased in clarified juices as concentration levels were similar to juice derived from LP grapes. Catechin was the only polyphenol where the high phenolic potential of the grapes reflected in all processing steps except in the standard clarified juice. Coutaric acid and epicatechin were comparable between low phenolic potential and high phenolic potential standard and skin contact juices. Caffeic acid appeared to be unaffected by phenolic potential within the free run juice but was found to respond after clarification and with skin contact

Figure 4.3: Hierarchical cluster analysis (A) and the ANOVA plots (B) of all measured polyphenols of low phenolic

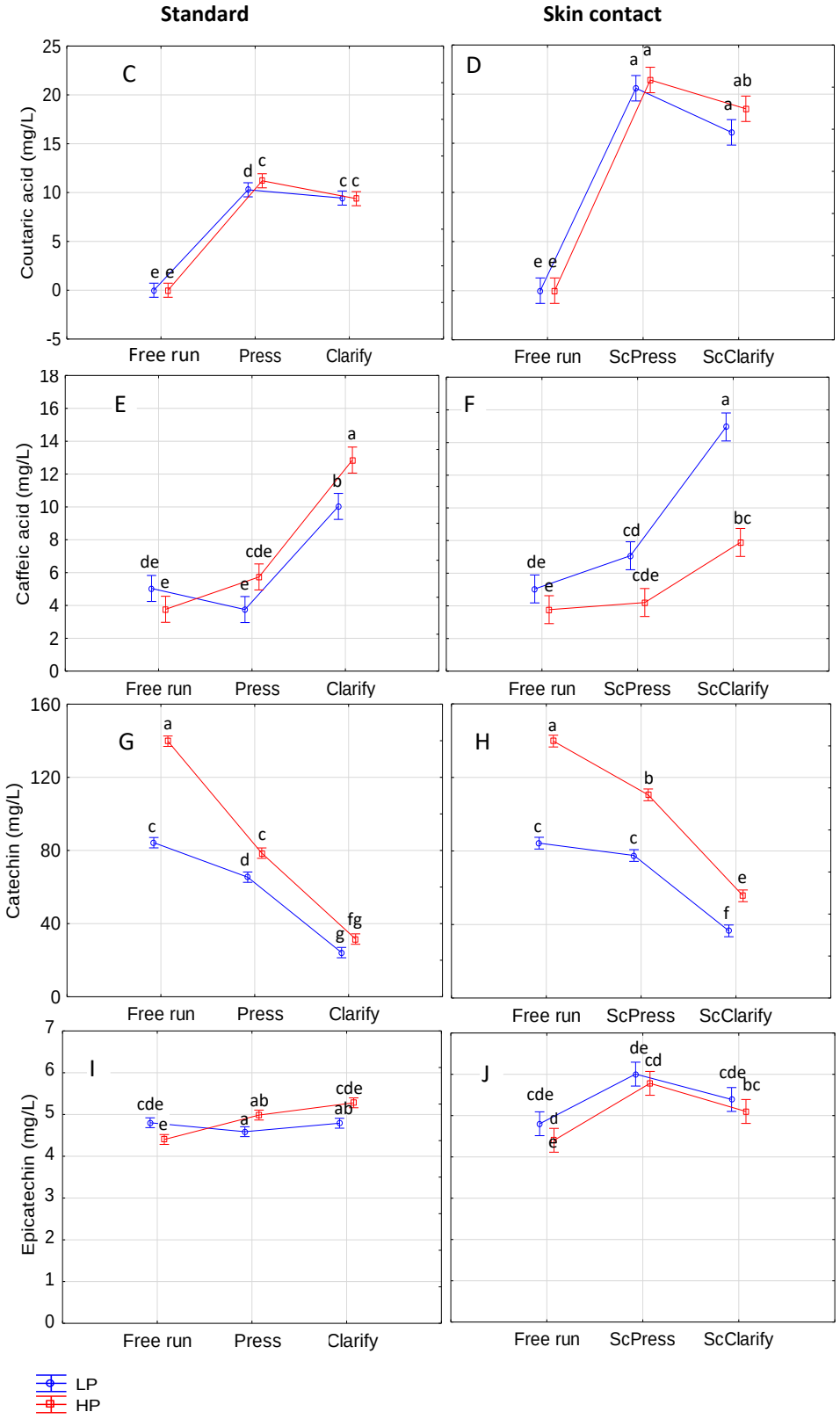


potential (LP) and high phenolic potential (HP) juices during processing. Juices were subjected to skin contact (Sc, treatment) or standard (as the standard control).

(Continued)

Stellenbosch University https://scholar.sun.ac.za

Figure 4.3: Continued.
The following polyphenols were measured: caftaric acid (control [A], treatment [B]), coutaric acid (control [C], treatment [D]), caffeic acid (control [E], treatment [F]), catechin (control [G], treatment [H]) and epicatechin (control [I], treatment [J]). The different letters indicate significance according to Fisher's LSD post hoc tests with a 95% confidence interval.



Stellenbosch University https://scholar.sun.ac.za

4.3.3. Polyphenol analysis of wine

A total of eight different wines were produced as a result of phenolic potential, standard wine making practice and three treatments (skin contact [Sc], sediment contact [Sed]), and a combination of skin and sediment contact [ScSed]. Only four polyphenols could be quantified within all wine samples (Table 4.3). None of the treatments or combinations thereof altered the phenolic composition/profiles of the wines (i.e. the same compounds were present in all samples), but definitive changes in the concentration of compounds were observed.

A two component PCA model, based on the polyphenol data (Table 4.3), showed that the samples separated along PC1 (sediment contact) which explained 45.4% of the variance as well as PC2 (Skin contact), explaining 32.3% of the variance (Figure 4.6 A). The score plot shows each wine type separating from the rest, with a distinct grouping of wines that were racked (R) and those which had sediment contact during fermentation (Sed). Sediment contact wines had higher total polyphenol concentrations compared to their respective controls, standard and skin contact racked wines (Table 4.3).

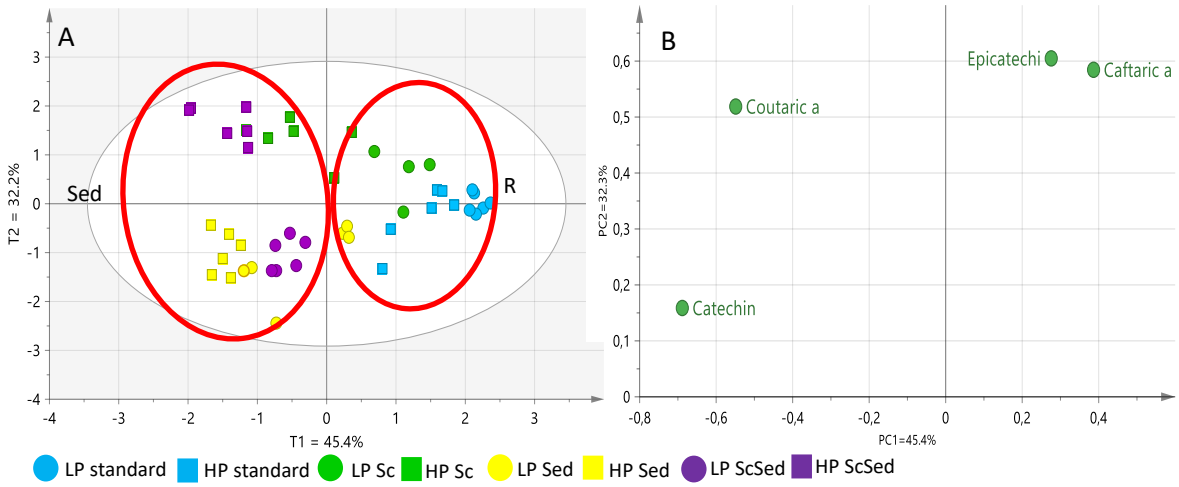


Figure 4.4: The PCA score (A) and loading (B) plots (PC1 vs PC2) of the polyphenol concentration of the wines after cold stabilization. Abbreviations: low phenolic potential (LP, circle), high phenolic potential (HP, square), skin contact (Sc), sediment contact (Sed), Skin contact and sediment contact (ScSed), racked (R). Red ovals indicate wines which grouped according to treatment and control: sediment contact or racking respectively.

Stellenbosch University https://scholar.sun.ac.za

Table 4.3: The polyphenol concentration of wines (mg/L) produced from grapes with a low phenolic potential (LP) and high phenolic potential (HP).

	Standard (no skin or sediment contact)		Skin contact		Sediment contact		Skin and sediment contact	
	LP	**HP**	**LP**	**HP**	**LP**	**HP**	**LP**	**HP**
Caftaric acid	37.5±1.8 a	36.6±2.5 a	38.1±4.1 a	36.6±3.6 a	26.1±3.3 a	25.9±3.6 a	31.5±1.5 b	36.8±1.3 a
Coutaric acid	6.6±1.2 e	9.4±2.3 de	14.8±2.5 c	20.2±5.0 b	12.2±3.1 cd	13.9±1.4 c	15.6±3.4 c	25.4±3.9 a
Caffeic acid	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Total hydroxycinnamic acids	**44.1±3.1 d**	**45.9±4.9 d**	**53.1±6.6 c**	**57.1±8.6 c**	**38.3±6.4 b**	**39.5±4.9 e**	**47.0±7.9 de**	**62.2±5.1 a**
Catechin	47.1±3.7 e	55.6±7.9 d	56.7±6.4 d	89.9±13.7 b	76.7±6.5 c	106.2±12.0 a	76.5±13.4 c	105.3±15.1 a
Epicatechin	2.5±0.0 c	2.4±0.1 bc	2.5±0. 6c	2.5±0.1 c	2.4±0.1 bc	2.4±0.1 b	2.3±0.0 a	2.5±0.1 bc
Flavan-3-ol_1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Flavan-3-ol_2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Flavan-3-ol_3	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Flavan-3-ol_4	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Polymeric phenolics	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Total Flavan-3-ols	**49.6±3.8 e**	**58.1±8.9 d**	**59.30±6.6 d**	**92.6±13.8 b**	**79.2±6.5 c**	**108.4±12.1 a**	**78.8±13.4 c**	**107.7±15.2 a**
Quercetin-galactoside	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Quercetin-glucoside	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Flavonol_4	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Flavonol_5	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Total phenolics	**93.8±7.5 f**	**104.0±16.9 e**	**112.6±7.6 d**	**149.1±27.4 b**	**117.3±12.9 c**	**148.5±16.9 a**	**125.9±21.4 d**	**169.9±20.3 b**

Abbreviations: N.D. (not detected)

Different letters indicate statistical significance (One-way ANOVA with Fisher's LSD post hoc test, $p<0.05$)

Stellenbosch University https://scholar.sun.ac.za

Hierarchical cluster analysis (Figure 4.5A) of the wines supported the PCA model where Sediment contact was the main driver behind polyphenol concentration of the wine since the samples first grouped according to sediment contact, followed by skin contact and lastly by phenolic potential. ANOVA plots (Figure 4.5B) confirmed that skin and/or sediment contact influenced the levels of the four individual phenolic compounds that was measured, in different ways in the different wines. For example, caftaric acid concentrations were not influenced by skin contact in either the LP or HP wines (Figure 4.5B: A), while sediment contact reduced the levels of caftaric acid to the same degree in both LP and HP wines (Sed, Fig 4.5B: B). A combination of skin and sediment contact "protected" the caftaric acid levels from this reduction (more prominent in high phenolic potential wine) (Figure 4.6B: B). Coutaric acid concentrations on the other hand increased in both LP and HP wines that had skin contact (Figure 4.5B: C), sediment contact and a combination of the two (ScSed) (Figure 4.5B: D). Catechin was the only polyphenol where the high phenolic potential of the grapes had a prominent and consistent effect in enhancing the concentration for this compound for all the wines types compared to their equivalents made from low phenolic potential grapes (Figures 4.5B: E and F). Moreover, skin and sediment contact, but not the combination of skin and sediment contact increased the concentrations in both the LP and HP wines (Figure 4.5B: F). Epicatechin, the polyphenol with the lowest concentration, was not increased by the HP nor skin contact (Figure 4.5B: G). However, when sediment contact was applied in combination with skin contact, an increase in concentration was observed in wines produced from HP grapes (Figure 4.5B: F). A surprising decrease in epicatechin concentration was observed for LP wines when sediment and skin contact was applied in combination (Figure 4.5 F).

Stellenbosch University https://scholar.sun.ac.za

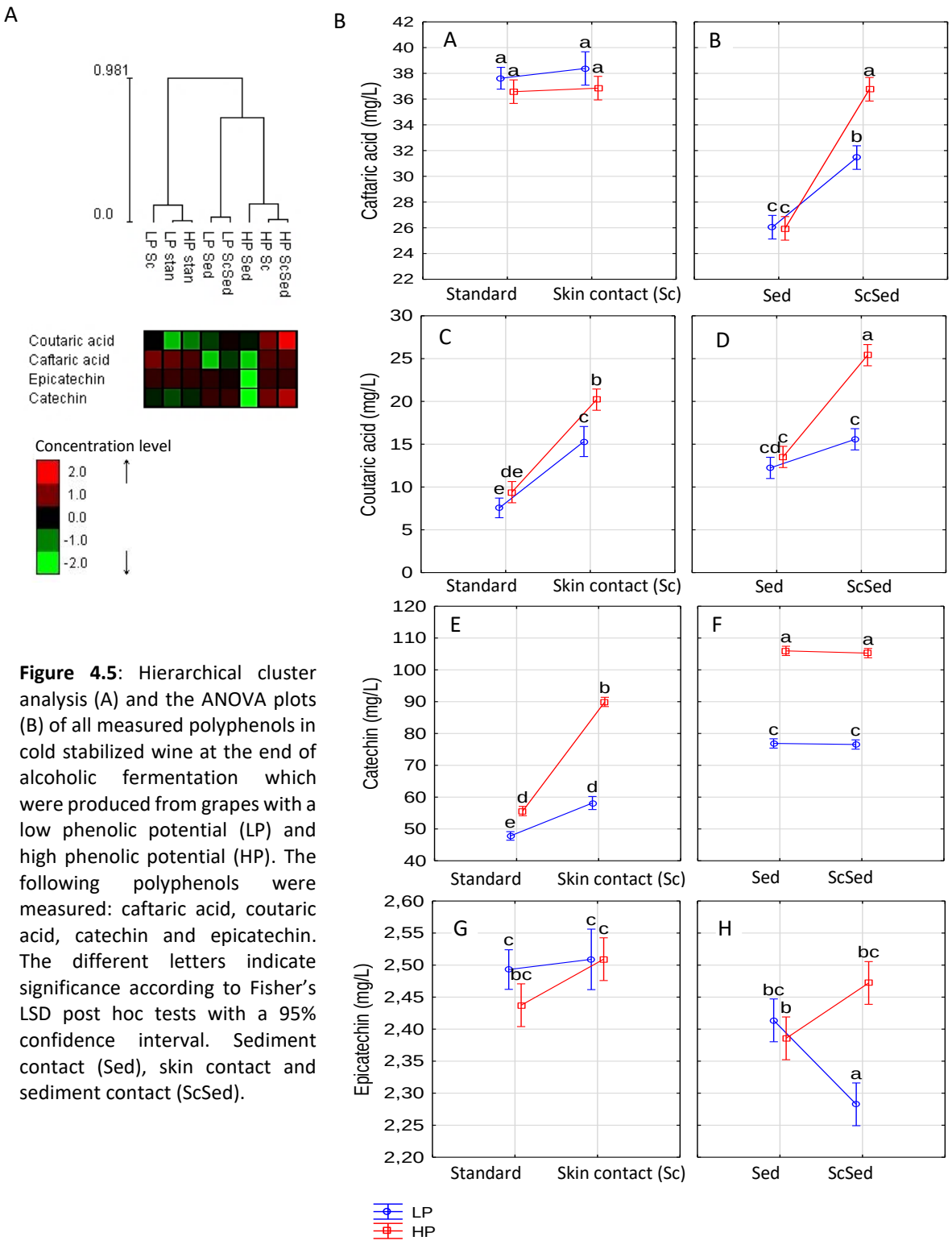


Figure 4.5: Hierarchical cluster analysis (A) and the ANOVA plots (B) of all measured polyphenols in cold stabilized wine at the end of alcoholic fermentation which were produced from grapes with a low phenolic potential (LP) and high phenolic potential (HP). The following polyphenols were measured: caftaric acid, coutaric acid, catechin and epicatechin. The different letters indicate significance according to Fisher's LSD post hoc tests with a 95% confidence interval. Sediment contact (Sed), skin contact and sediment contact (ScSed).

Stellenbosch University https://scholar.sun.ac.za

4.3.4. Wine descriptive sensory analysis

Descriptive sensory analysis was performed on all wines bottled wines. Although wines were analysed for both aroma and taste/mouth feel properties, the focus was placed on the latter for the purpose of this study (the aroma descriptors are provided in supplementary Table S4.3). The descriptive data for taste/mouthfeel is presented in Table 4.4 and radar charts (Figure 4.6) and contains all wine types. The chart showed that the panellists scored the high phenolic potential standard and skin contact wines high for the descriptors "body" and "sweetness" whereas the corresponding low phenolic potential wines were described as "sour" and "predominantly sour with a degree of sweetness". Sediment contact during fermentation (Sed) generally lead to wines being described as "bitter" and "sour". All of the wines received a low score for astringency.

Table 4.4: The descriptive sensory analysis of the tastes scores of the wines.

Taste descriptors	Standard		Sediment contact		Skin contact		Skin and sediment contact	
	LP	HP	LP	HP	LP	HP	LP	HP
Sweet	1.6±0.11 d	4.6±1.6 ab	1.9±0.2 cd	2.3±0.2 ab	2.8±0.8 ab	5.0±1.2 a	2.3±1.7 ab	3.3±1.1 ab
Sour	4.8±0.2 a	3.0±0.7 bc	4.7±0.2 a	4.3±0.1 ab	3.9±0.7 ab	2.4±0.1 c	4.0±0.9 ab	3.6±0.5 bc
Bitter	1.7±0.1 a	1.3±0.4 a	1.5±0.1 a	1.8±0.3 a	1.5±0.1 a	1.2±0.5 a	1.8±0.4 a	1.7±0.5 a
Astringent	1.2±0.1 a	0.8±0.2 a	1.3±0.2 a	1.2±0.1 a	1.2±0.1 a	0.8±0.3 a	1.1±0.2 a	1.2±0.2 a
Body	2.3±0.1 cd	3.2±0.5 ab	2.2±0.1 d	2.6±0.2 bc	2.6±0.3 d	3.4±0.2 bc	2.4±0.6 cd	2.6±0.2 c
Sourness	1.0±0.1 c	0.2±0.3 ab	0.8±0.1 c	0.8±0.1 c	0.7±0.2ac	0.2±0.1 b	0.7±0.3 ac	0.5±0.3 ad
Sweetness	<LOD	0.6±0.4 ab	0.01±0.0 b	0.1±0.0 ab	0.1±0.1 ab	0.6±0.3 a	0.1±0.3 ab	0.3±0.3 ab
Bitterness	0.2±0.1 a	0.1±0.2 a	0.3±0.2 a	0.4±0.1 a	0.1±0.1 a	0.1±0.1 a	0.3±0.2 a	0.2±0.2 a

Abbreviations: LP (Low phenolic potential), HP (high phenolic potential), LOD (Limit of detection).

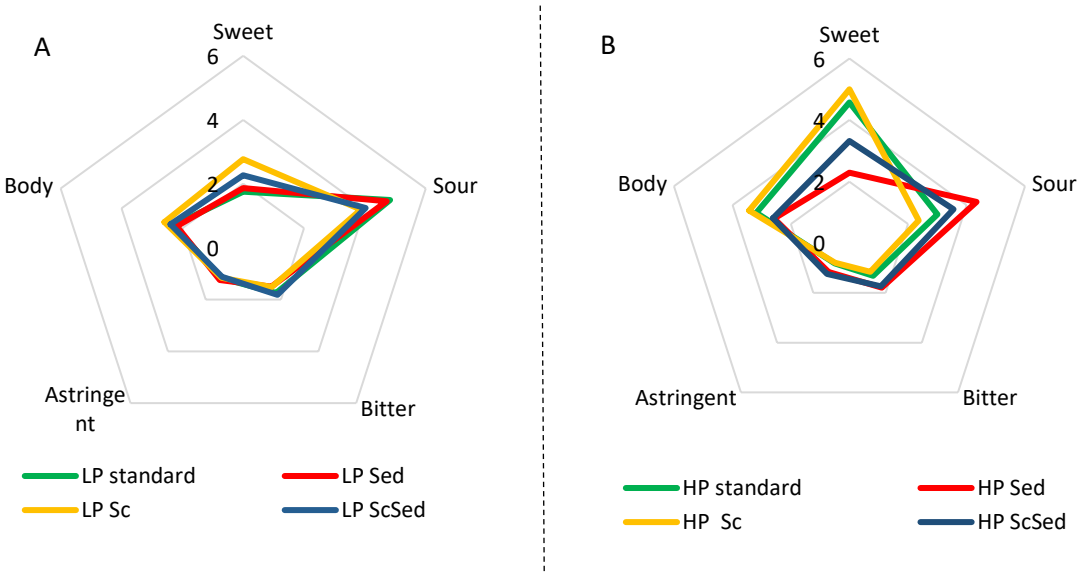


Figure 4.6: The descriptive sensory data for the taste and mouthfeel properties of the different wines produced from grapes with a low (LP, A) and high phenolic potential (HP, B). Abbreviations: skin contact (Sc), sediment contact (Sed), skin contact and sediment contact (ScSed).

Stellenbosch University https://scholar.sun.ac.za

4.4. Discussion

The polyphenol composition and concentration was compared between the ripe Sauvignon Blanc grapes and its derived juice to determine the extent to which the polyphenol potential of the grape was transferred. The phenolic potential of the grapes refers to the total content and profile of phenolic compounds. Furthermore, polyphenols were monitored throughout juice processing to establish the effect of each processing step (crushing, pressing, enzyme clarification) on the polyphenol composition and concentration of the juice. Skin contact was introduced as an attempt to evaluate the extent to which the transference of the phenolic potential can be enhanced from grapes, with a low and high phenolic potential, to juice. Additionally, the polyphenol composition and concentration of the different wine types were determined to evaluate the efficacy of skin contact and sediment contact treatments on utilizing the phenolic potential of the grapes, the juice and grape residues of the sediment. The polyphenol composition of the clarified juice was linked to that of the wine to establish whether polyphenols were gained or lost during winemaking.

4.4.1. Polyphenol analysis of juice during juice processing

A total of eleven polyphenols were identified within the low phenolic potential whole berries and fourteen within high phenolic potential berries (Table 3.1). Of these, only five were transferred to the free run, pressed and clarified juices: caftaric acid, coutaric acid, caffeic acid, catechin and epicatechin (Table 4.2). The remaining polyphenols (flavan-3-ols, quercetin-glucoside, quercetin-galactoside, flavonol_4 and flavonol_5) were either not extracted into the juice or were below the instrument's detection limits. The juice contained no polymeric phenolics but some catechin and epicatechin, the monomeric precursors of proanthocyanidins, were present. The origin of these juice flavan-3-ols can be the skin or the seed of the grapes (Table 3.1) but it has been reported in literature (González-Manzano et al. 2004) that more flavonoids are extracted from the skin. Even under fermentative conditions, flavanols were extracted earlier and at lower alcohol concentrations from the skins than from the seeds. Direct comparison of the polyphenol concentrations between the juice and solid grape tissue is not possible as juice yield was not measured in this study and the sample preparation differed (methanol extraction for grape tissue and simple filtration for juice).

In Chapter 3 we saw that a high light (HL) microclimate increased the concentration of all the phenolic compound groups as well as all the individual phenolic compounds in the skin and pulp of Sauvignon Blanc grapes, except for epicatechin. Interestingly these differences in phenolic concentration were maintained/transferred to the corresponding free run and standard pressed juices (when comparing the total values for the different phenolic groups) but once skin contact was administered there were no longer a difference between the total hydroxycinnamic acid concentrations of low and high phenolic potential juices. Furthermore, for standard clarified juices there were no differences observable for any of the phenolic groups. Similarly, Honeth (2018) found that higher phenolic compound concentrations of HL juices were only visible within free run juice and becomes comparable with low light concentrations after pressing and

Stellenbosch University https://scholar.sun.ac.za

enzyme clarification. In our study we see that the skin contact treatment (which was not part of the study by Honeth, 2018) did yield higher phenolics in general (Table 3.1) and this was caused by the higher concentration of catechin in HL juices. The results in this study correlated with the findings of Honeth (2018) as flavanol concentrations were comparable in the clarified juice. Based on the findings of this project, it appears that a high phenolic potential only meaningfully led to an increase in catechin concentration as it reflects after juice clarification. The clarified juice sets the benchmark for the phenolic composition of the sequential wine and the concentration differences prior to clarification will not have an influence on the fermentation and the phenolic composition of the final wine.

As we see from Figure 4.1, the different juice processing steps were the most important determining factor for the variation in the polyphenol concentration of the juices, followed by the skin contact treatment. Total polyphenol concentration decreased during juice processing, most significantly due to clarification, regardless of skin contact (Table 4.2). This decline was mainly due to a decrease in catechin (± 57% for low and high phenolic potential) and caftaric acid (± 96% for low and high phenolic potential) which were present at the highest quantities within all of the juices (Figure 4.3). Caftaric acid and catechin have been reported as being the major hydroxycinnamic acid and flavonoid respectively within juice implying that a decline in these two polyphenols would mean a decline in total phenolic concentration (Hernanz et al. 2007; Cejudo-Bastante et al. 2011a; Ferreira-lima et al. 2016). A decrease in hydroxycinnamic acids, flavonols and total polyphenols after juice pressing and enzyme clarification have previously been described by Honeth (2018). However, studies which focussed on the effect of pressing conditions on polyphenol concentration reported increases in caftaric acid, coutaric acid, caffeic acid and two flavonoids, of which the identity was unknown, after pressing (Maggu et al. 2007; Patel et al. 2010; Ferreira-lima et al. 2016). These increases after pressing correlated with increases observed for caffeic acid and coutaric acid within this study. Not all polyphenols increase after pressing, for example decreases in caftaric acid have been reported (as seen in this study) and this usually corresponded with an increase in S-glutathional caftaric acid (Maggu et al. 2007; Patel et al. 2010).

Oxidized phenols reacts with reduced glutathione to form grape reactive product (GRP) and the presence of GRP (e.g. S-glutathional caftaric acid) is an indication of polyphenol oxidation in the juice (Singleton et al. 1985; Coetzee et al. 2013). Furthermore, the high caftaric and catechin concentration of the juices could make them more susceptible to oxidation (Ferreira-lima et al. 2016). In our study dry ice and SO_2 were added to the juices as precautionary measures against oxidation. However, the HPLC method that was used to analyse the juices was not set up to detect GRP and it cannot be confirmed whether any polyphenols were oxidized. The browning index of the juices were not measured but in the case that oxidation occurred it would be expected that the high phenolic potential juices would be more brown due to a higher total polyphenol content (Cejudo-Bastante et al. 2011b; Smith et al. 2011; Ferreira-lima et al. 2016).

The statistically significant higher total polyphenol concentration within juices that had extended skin contact (Table 4.2) was expected since the grape skins are rich in hydroxycinnamic acids and flavonols

Stellenbosch University https://scholar.sun.ac.za

(Rodríguez Montealegre et al. 2006; Dragana et al. 2016). It is well documented that skin contact, which is time and temperature dependent, can significantly increase phenolic acid and flavonoid concentration of juices, although some polyphenols were unaffected by the treatment. The extracting effect from the skins also favour flavonoids (Darias-Martín et al. 2000; Gomez-Miguez et al. 2007; Hernanz et al. 2007; Maggu et al. 2007; Cejudo-Bastante et al. 2011a). In this study, given the efficacy of skin contact to enhance polyphenol extraction from the skin it was expected that juice derived from high phenolic potential grapes would have significantly higher levels of all compounds based on the findings in chapter 3.

The increase (coutaric and caffeic acid) and decrease (caftaric acid and catechin) in polyphenol concentration after enzyme clarification (Clarify and ScClarify) compared to the free run juice may be explained by their reactive nature and ability to bind to other polyphenols and structures (Mojzer et al. 2000; Bindon and Smith 2013; Renard et al. 2017). Bindon et al. (2010a) had previously shown that proanthocyanidins have a high binding affinity to cell wall material of grape cells, specifically pectin. It is plausible that the polyphenols within the juice may also have bound to insoluble cell wall material during the 24 h clarification period and settled out in the sediment during application of the pectinase enzyme. Within this project the opposite may be true to account for the increase in coutaric and caffeic acid as enzymatic depolymerization of pectin polymers within the grape cell walls could have led to these compounds being liberated (Bindon et al. 2010; Bautista-Ortín et al. 2016; Bindon et al. 2016).

4.4.2. Polyphenol analysis of the wine

The total polyphenol concentrations of the standard wine, and the skin contact wines (Table 4.3) were lower (Olejar et al. 2015) and higher (Patel et al. 2010) respectively than what is reported in literature for Sauvignon Blanc wine. Within these studies different HPLC columns and mobile phase solvents were used which could affect both the ability to identify and quantify phenolic compounds (Waterhouse 2002). Furthermore, it should also be acknowledged that the analytical method used in this study was limitated in its ability to detect certain phenolic compounds such as the stilbenes, which are only detected when HPLC is coupled with mass spectrometry (Kennedy and Waterhouse 2000; Gomez-Alonso et al. 2007; Di Lecce et al. 2014). The total polyphenol concentrations of these wine were also higher compared to their respective clarified juices (Table 4.2). This contradicts the results of Patel et al. (2010) where the total polyphenol concentrations were lower in Sauvignon Blanc wine compared to the juice. Similar results were seen in Chardonnay, Manzoni and Garganeg (Ferreira-lima et al. 2016). On the other hand, the results of Hernanz et al. (2007) and Vicario et al. (2007) on the Zalema cultivar, correlates with this study as their wines had a higher total polyphenol concentration than the juice. However, differences in viticultural and winemaking practices or sampling and analysis methods may contribute to varying results.

All the polyphenols that were present within the clarified juice were also present within the resulting wine except for caffeic acid (Table 4.2). This is contradictory to previous studies that reported between 1

Stellenbosch University https://scholar.sun.ac.za

mg/L – 5 mg/L caffeic acid in white wine which is sometimes higher or lower than the levels in the corresponding juice but not to the point where it is not detectable (Gomez-Miguez et al. 2007; Hernanz et al. 2007; Patel et al. 2010; Ferreira-lima et al. 2016). A wine fermentation is a complex biochemical process where polyphenols could react with a plethora of compounds which could influence their bioavailability. White cultivars contain a higher degree of hydroxycinnamoyltartaric acids compared to red cultivars which can be partially hydrolysed during fermentation to generate free HCA. One possible explanation could be that the higher caftaric acid in the standard and skin contact wine (for low and high phenolic potential) was liberated during alcoholic fermentation from the hydrolysis of hydroxycinnamoyltartaric acid (Kallithraka et al. 2001; Di Lecce et al. 2013; Garrido and Borges 2013).

Similarly, catechin concentrations increased during alcoholic fermentation and correlated to the results of other studies on white wine (Gomez-Miguez et al. 2007; Garrido and Borges 2013; Ferreira-lima et al. 2016). The proposed mechanism for catechin increase also correlates to hydrolysis where proanthocyanidins are hydrolysed to catechin or epicatechin under the acidic wine conditions (Cejudo-Bastante et al. 2011b). The decrease in epicatechin was subtle, from ±5 mg/L in the juice (Clarify and ScClarify, low and high phenolic potential) to ±2.5 mg/L in the wine (low and high phenolic potential, standard and skin contact). This correlated with literature as epicatechin concentration was reported to be between 2 mg/L to 8 mg/L in juice and white wine where small changes (increases or decreases) in concentration were observed during alcoholic fermentation (Gomez-Miguez et al. 2007; Hernanz et al. 2007; Di Lecce et al. 2013; Ferreira-lima et al. 2016). Epicatechin and catechin are monomers of procyanidins where the former polymerizes to form the latter (Garrido and Borges 2013). An indirectly proportional relationship has been observed between epicatechin and the formation of procyanidin A (dimeric flavan-3-ol) in white wine (Di Lecce et al. 2013) which could explain the lower epicatechin concentration observed in the wine from our study. The presence of these compounds could however not be confirmed because the analysis method that was used was unable to identify these oligomers. Furthermore, although we saw an unresolved peak that proposedly represent polymeric polyphenols with varying degrees of polymerisation (Waterhouse 2002; Aleixandre-Tudo et al. 2018) in the fresh grape tissues, this was absent in the juice as well as the wine samples. This is similar to the finding by multiple studies that also reported the absence of polymeric polyphenols within white wine (Gomez-Miguez et al. 2007; Hernanz et al. 2007; Patel et al. 2010; Cejudo-Bastante et al. 2011b; Ferreira-lima et al. 2016). It can be speculated that procyanidins, with a low degree of polymerization (2-3 subunits), were present but below the detection limit of the analysis method. These potentially undetectable polymers may also relate to their potential hydrolysis in wine and the liberation of catechin as previously mentioned. Another potential explanation might be that epicatechin was oxidized in the wine, leading to lower concentrations, since an inverse relationship has previously been reported for epicatechin and wine browning (Kallithraka et al. 2001). The conversion of glucose and fructose to ethanol during fermentation may also contribute towards flavonoid increases as ethanol has been shown to be effective at liberating

Stellenbosch University https://scholar.sun.ac.za

flavan-3-ols in wine-like conditions (González-Manzano et al. 2004) and as extraction solvent for tissue (Su et al. 2014).

All eight different wines had the same polyphenol composition/profile, but the concentration of the individual phenolic compounds varied. The winemaking treatments, skin contact and sediment contact, had the biggest impact on wine polyphenol concentration when solely applied and this is shown in Figure 4.5B. Quantitative analysis, in combination with PCA analysis revealed that hydroxycinnamic acids reacted to skin contact whereas flavan-3-ols responded predominantly to sediment contact (Figure 4.5B). The increases observed for skin contact wines were as expected and had been reported throughout literature. However, not all concentration increases have been reported to be significant (Gomez-Miguez et al. 2007; Hernanz et al. 2007; Maggu et al. 2007; Aleixandre-Tudo et al. 2018). These increases were expected for catechin and coutaric acid as their corresponding clarified juices (low and high phenolic potential skin contact) had higher concentrations of these polyphenols (Table 4.3). Similarly, caftaric acid concentration was comparable between skin contact and standard clarified juices therefore skin contact and standard wines were comparable. On the contrary, skin contact appeared to increase caftaric acid in sediment contact wine. The lower caftaric acid concentration within sediment contact wine, compared to standard wine, may arise from interaction with protein or cell wall material that was reintroduced during fermentation which precipitated during cold stabilization (Fang et al. 2006; Van Sluyter et al. 2015; Bindon et al. 2016). Sediment contact most notably increased catechin concentration, and the higher concentration of catechin previously seen in skin contact clarified juice was no longer visible in the corresponding wines. Singleton et al. (1975) and Liu et al. (1987) found that white wines produced from turbid juices has similar total phenolic concentration to clear juice wines. Although their results contradict the findings of this study, they only used calorimetric assays which gives no indication of the individual or phenolic groups that were affected. The effect of sediment contact on individual polyphenol concentration is yet to be reported in literature and it is therefore unknown which concentration or compositional changes can be expected.

The effect of phenolic potential on polyphenol concentration did not reflect within the wine as it did within the grape berry (chapter 3) and the juice (specifically caffeic acid and catechin). Standard and sediment contact low and high phenolic potential wines had similar concentrations for all quantified polyphenols except for catechin where the higher phenolic potential was evident. Interestingly, in the presence of skin contact, coutaric acid and catechin concentration increased within high phenolic potential skin contact wines, as well as caftaric acid and coutaric acid in skin and sediment contact (ScSed) wines. On the contrary, caftaric acid and catechin within standard and sediment contact high phenolic potential wine did not increase in the presence of skin contact respectively. Caftaric acid may have potentially not responded to the high phenolic potential of the grapes or skin contact treatment whereas the different biochemical conditions within the sediment contact fermentations may have led to a light response (Garrido and Borges 2013). In the standard wine, the phenolic potential of the grapes and juice did not affect polyphenol concentrations, with the

Stellenbosch University https://scholar.sun.ac.za

exception being catechin. However, when skin contact, sediment contact or a combination of the two was applied, the initial increase in polyphenol content was more prevalent. These results indicate that winemaking treatments are effective in increasing the polyphenol concentration of wine, thus utilizing the phenolic potential of the grapes. Furthermore, the high phenolic potential of the grapes only reflected in the wine through augmentation with winemaking treatments. Changes in epicatechin with a higher phenolic potential, skin contact or sediment contact were negligible.

4.4.3. The impact of polyphenol concentration on wine taste

The eight different wines formed two main groupings based on their sensory profiles (Figure 4.6). In this study the perception of bitterness and astringency correlated with the polyphenol content of the wines. Standard and skin contact high phenolic potential wines received similar scores for bitter, astringent, sweet, body as well as the duration of bitter (bitterness) (Table 4.4). However, the skin contact wine contained a significantly higher catechin concentration, but comparable epicatechin and hydroxycinnamic acid concentrations. Catechin is known for inducing the perception of bitterness in model solutions and wine (Robichaud and Noble 1990; Brossaud et al. 2000; Aleixandre-Tudo et al. 2015; Sokolowsky et al. 2015). These wines had high residual sugar concentrations which could potentially have masked the perception of bitterness and explains the sweetness descriptor. Standard and skin contact low phenolic potential wines received similar scores for bitter and astringent as sediment contact wines (Sed and ScSed) (Table 4.4). However, the duration of bitter (bitterness) was longer in sediment contact wines which correlated to the increased intensity of this descriptor with an increase in catechin content (Arnold et al. 1980; Robichaud and Noble 1990). Sediment contact as well as a combination of skin and sediment contact (ScSed) increased wine bitterness and astringency regardless of phenolic potential.

The wines received low scores for “astringent” which is not surprising as polymeric polyphenols (tannins), which induces the astringent taste (Brossaud et al. 2000; Teissedre and Glories 2008), were not detected in any of the wines. It may also be possible that the wines contained low amounts of polymeric polyphenols, which could not be detected by the analytical instrument, hence the perception of “astringent”.

The aroma descriptors of the standard wines (Supplementary Table S4.3) correlates to what is reported in literature for wines produced from grapes that originates from a LL and HL microclimate. Standard Sauvignon Blanc wines produced from grapes from a LL microclimate are typically described as having green herbaceous aromas whereas those from a HL microclimate are described as floral and tropical (Suklje et al. 2014; Honeth 2018). Interestingly, low phenolic potential wines received higher scores for certain tropical attributes (pineapple, banana, passionfruit) when skin contact was applied but also received higher scores for the green attributes (gherkin, cooked veg, asparagus). This may indicate that the green attributes mask the perception of the floral and tropical aromas. Sediment contact wines received high and low scores for

Stellenbosch University https://scholar.sun.ac.za

the green and tropical aromas respectively. It has been reported that polyphenols may suppress the perception of 3MH, a thiol linked with passionfruit aroma, in Sauvignon Blanc (Lund et al. 2009).

4.5. Conclusion

The phenolic potential of grapes was shown to increase the quantity of polyphenols that were distributed into the juice but did not affect the profiles. These increases were subtle on individual phenolic level but proved to be cumulatively significant. Juice processing had the biggest effect on the polyphenol content of the juice, which could not be overshadowed by the effect of a higher phenolic potential and/or pre-fermentative skin contact. The phenolic potential of the grapes only truly reflected on individual level in the wine when skin contact was applied alone, or in combination of sediment contact. Producing white wine from grapes with a higher phenolic potential, or employing winemaking steps with the goal to enhance phenolic content, contributes towards the perception of “bitter” and “sour” sensory attributes. However, the results of this study provides novel insights into polyphenol distribution from white grapes to the juice, how the phenolic potential of the grapes affects distribution, and the impact of juice processing and winemaking treatments on distribution to the wine.

4.6. References

Aleixandre-Tudo J, Weightman C, Panzeri V, Nieuwoudt HH, Toit WJ (2015) Effect of Skin Contact Before and During Alcoholic Fermentation on the Chemical and Sensory Profile of South African Chenin. South African J Enol Vitic 36:366–377.

Aleixandre-Tudo JL, Nieuwoudt H, Olivieri A, Luis J (2018) Phenolic profiling of grapes , fermenting samples and wines using UV- Visible spectroscopy with chemometrics.

Arnold R, Noble A, Singleton V (1980) Bitterness and Astringency of Phenolic Fractions in Wine. J Agric Food Chem 675–678. doi: 10.1021/jf60229a026

Ballester J, Magne M, Perrine J, Noret L, Nikolantonaki M, Coelho C, Id DG (2018) Sensory Impact of Polyphenolic Composition on the Oxidative Notes of Chardonnay Wines. Beverages 1–17. doi: 10.3390/beverages4010019

Bautista-Ortín AB, Abdallah R Ben, Castro-López L del R, Jiménez-Martínez MD, Gómez-Plaza E (2016) Technological implications of modifying the extent of cell wall-proanthocyanidin interactions using enzymes. Int J Mol Sci. doi: 10.3390/ijms17010123

Bindon KA, Li S, Kassara S, Smith PA (2016) Retention of Proanthocyanidin in Wine-like Solution Is Conferred by a Dynamic Interaction between Soluble and Insoluble Grape Cell Wall Components. J Agric Food Chem 64:8406–8419. doi: 10.1021/acs.jafc.6b02900

Bindon KA, Smith PA (2013) Comparison of the affinity and selectivity of insoluble fibres and commercial proteins for wine proanthocyanidins. Food Chem 136:917–928. doi: 10.1016/j.foodchem.2012.08.016

Bindon KA, Smith PA, Holt H, Kennedy JA (2010) Interaction between Grape-Derived Proanthocyanidins and Cell Wall Material . 2 . Implications for Vinification. J Agric Food Chem 10736–10746. doi: 10.1021/jf1022274

Stellenbosch University https://scholar.sun.ac.za

Brossaud F, Cheynier V, Noble ANNC (2000) Bitterness and astringency of grape and wine polyphenols. 33–39.

Cejudo-Bastante M, Castro-Vazquez L, Hermosín-Gutierrez MP-C (2011a) Combined Effects of Prefermentative Skin Maceration and Oxygen Addition of Must on Color-Related Phenolics , Volatile Composition , n White Wine and Sensory Characteristics of Air e. J Agric Food Chem 12171–12182. doi: 10.1021/jf202679y

Cejudo-Bastante MJ, Hermosín-Gutierrez I, Castro-Vasquez LI, Perez-Coello M (2011b) Hyperoxygenation and Bottle Storage of Chardonnay White Wines : Effects on Color-Related Phenolics , Volatile Composition , and Sensory Characteristics. J Agric Food Chem 4171–4182. doi: 10.1021/jf104744q

Coetzee C, Lisjak K, Nicolau L (2013) Oxygen and sulfur dioxide additions to Sauvignon blanc must : effect on must and wine composition. 155–167. doi: 10.1002/ffj.3147

Darias-Martín JJ, Diaz-Gonzalez D, Diaz-romero C (2004) Influence of two pressing processes on the quality of must in white wine production. J Food Eng 63:335–340. doi: 10.1016/j.jfoodeng.2003.08.005

Darias-Martín JJ, Rodríguez O, Díaz E, Lamuela-Raventós RM (2000) Effect of skin contact on the antioxidant phenolics in white wine. Food Chem 71:483–487. doi: 10.1016/S0308-8146(00)00177-1

Di Lecce G, Arranz S, Jáuregui O, Tresserra-rimbau A, Quifer-rada P, Lamuela-raventós RM (2014) Phenolic profiling of the skin , pulp and seeds of Albariño grapes using hybrid quadrupole time-of-flight and triple-quadrupole mass spectrometry. Food Chem 145:874–882. doi: 10.1016/j.foodchem.2013.08.115

Di Lecce G, Boselli E, D'Ignazi G, Frega NG (2013) LWT - Food Science and Technology Evolution of phenolics and glutathione in Verdicchio wine obtained with maceration under reductive conditions. LWT - Food Sci Technol 53:54–60. doi: 10.1016/j.lwt.2013.03.006

Dragana C, Davidovic SM, Todic SR, Bešlic ZS, Tešic L (2016) Identification and quantification of phenolic compounds in berry skin , pulp , and seeds in 13 grapevine varieties grown in Serbia. 211:243–252.

Eyéghé-Bickong HA, Alexandersson EO, Gouws LM, Young PR, Vivier MA (2012) Optimisation of an HPLC method for the simultaneous quantification of the major sugars and organic acids in grapevine berries. J Chromatogr B Anal Technol Biomed Life Sci 885–886:43–49. doi: 10.1016/j.jchromb.2011.12.011

Fang Z, Zhang M, Tao G, Sun Y, Sun J (2006) Chemical composition of clarified bayberry (Myrica rubra Sieb, et Zucc.) juice sediment. J Agric Food Chem 54:7710–7716. doi: 10.1021/jf0618980

Ferreira-lima NE, Burin VM, Caliari V (2016) Impact of Pressing Conditions on the Phenolic Composition , Radical Scavenging Activity and Glutathione Content of Brazilian Vitis vinifera White Wines and Evolution During Bottle Ageing. Food Bioprocess Technol 944–957. doi: 10.1007/s11947-016-1680-7

Fischer U, Noble a (1994) The Effect of Ethanol, Catechin Concentration, and pH on Sourness and Bitterness of Wine. Am J Enol Vitic 45:6–10.

Fontes N, Delrot S, Gualtar C De, Functional G (2011) Grape Berry Vacuole: A Complex and Heterogeneous Membrane System Specialized in the Accumulation of Solutes. Am J Enol Vitic. doi: 10.5344/ajev.2011.10125

Garrido J, Borges F (2013) Wine and grape polyphenols - A chemical perspective. Food Res Int 54:1844–1858. doi: 10.1016/j.foodres.2013.08.002

Gawel R, Day M, van Sluyter S, Holt H, Waters E, Smith P (2017) Compositional Factors Affecting the Mouth-feel of White Wine. J Agric Food Chem 62:10008–10014.

Stellenbosch University https://scholar.sun.ac.za

Gomez-Alonso S, Garcia-Romero E, Hermosın-Gutierrez I (2007) Analysis of diverse grape and wine phenolics using direct injection and multidetection by DAD and fluorescence. J Food Compos Anal 20:618–626. doi: 10.1016/j.jfca.2007.03.002

Gomez-Miguez JM, Gonzalez-Miret LM, Hernanz D, Fernandez AM, Vicario IM, Heredia FJ (2007) Effects of prefermentative skin contact conditions on colour and phenolic content of white wines. J Food Eng 78:238–245. doi: 10.1016/j.jfoodeng.2005.09.021

González-Manzano S, Rivas-Gonzalo JC, Santos-Buelga C (2004) Extraction of flavan-3-ols from grape seed and skin into wine using simulated maceration. Anal Chim Acta 513:283–289. doi: 10.1016/j.aca.2003.10.019

Hernanz D, Recamales ÁF, González-Miret ML, Gómez-Míguez MJ, Vicario IM, Heredia FJ (2007) Phenolic composition of white wines with a prefermentative maceration at experimental and industrial scale. J Food Eng 80:327–335. doi: 10.1016/j.jfoodeng.2006.06.006

Honeth. C (2018) Evaluating the effects of UVB radiation on field grown grape berry metabolites under high and low light microclimates and the consequent impacts on the resulting wines. Thesis, University of Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa. South Africa.

Kallithraka S, Arvanitoyannis IS, Kefalas P, El-zajouli A (2001) Instrumental and sensory analysis of Greek wines ; implementation of principal component analysis (PCA) for classication according to geographical origin.

Kennedy JA, Waterhouse AL (2000) Analysis of pigmented high-molecular-mass grape phenolics using ion-pair , normal-phase high-performance liquid chromatography. J Chromatogr A 866:25–34.

Liu J., Gallander JF, Wilker KL (1987) Effect of Juice Clarification on the Composition and Quality of Eastern US Table Wines. Am J Enol Vitic 38:147–150.

Lund CM, Nicolau L, Gardner RC, Kilmartin PA (2009) Effect of polyphenols on the perception of key aroma compounds from Sauvignon Blanc wine. Aust J Grape Wine Res 15:18–26. doi: 10.1111/j.1755-0238.2008.00028.x

Maggu M, Winz R, Kilmartin PA, Trought MCT, Trought M, Nicolau L (2007) Effect of Skin Contact and Pressure on the Composition of Sauvignon Blanc Must. J Agric Food Chem 55:10281–10288.

Mojzer E, Hrncic M, Skerget M, Knez Z, Bren U (2000) Polyphenols: Extraction Methods, Antioxidative Action, Bioavailability and Anticarcinogenic Effects. doi: 10.3390/molecules21070901

Olejar KJ, Fedrizzi B, Kilmartin PA (2015) Influence of harvesting technique and maceration process on aroma and phenolic attributes of Sauvignon blanc wine. Food Chem 183:181–189. doi: 10.1016/j.foodchem.2015.03.040

Oliveira C, Silva Ferreira A, De Freitas V, Silva A (2011) Oxidation mechanisms occurring in wines. Food Res Int 44:1115–1126. doi: 10.1016/j.foodres.2011.03.050

Patel P, Herbst-Johnstone M, Lee SA, Gardner RC, Weaver R, Nicolau L, Kilmartin PA (2010) Influence of Juice Pressing Conditions on Polyphenols , Antioxidants , and Varietal Aroma of Sauvignon blanc Microferments. Agric Food Chem 58:7280–7288. doi: 10.1021/jf100200e

Recamales F, Sayago A, Gonza ML, Hernanz D (2006) The effect of time and storage conditions on the phenolic composition and colour of white wine. 39:220–229. doi: 10.1016/j.foodres.2005.07.009

Renard CMGC, Watrelot AA, Bourvellec C Le (2017) Trends in Food Science & Technology Interactions between polyphenols and polysaccharides : Mechanisms and consequences in food processing and digestion. 60:43–51.

Stellenbosch University https://scholar.sun.ac.za

Ristic R, Downey MO, Iland PG, Bindon K, Francis L, Herderich M, Robinson S (2006) Exclusion of sunlight from Shiraz grapes alters wine colour ,. Aust J Grape Wine Res 53–65.

Robichaud JL, Noble AC (1990) Astringency and Bitterness of Selected Phenolics in Wine. 343–353.

Rodríguez Montealegre R, Romero Peces R, Chacón Vozmediano JL, Martínez Gascueña J, García Romero E (2006) Phenolic compounds in skins and seeds of ten grape Vitis vinifera varieties grown in a warm climate. J Food Compos Anal 19:687–693. doi: 10.1016/j.jfca.2005.05.003

Singleton VL, Salgues M, Zaya J, Trousdale E (1985) Caftaric Acid Disappearance and Conversion to Products of Enzymic Oxidation in Grape Must and Wine. Am J Enol Vitic 36:50–56.

Singleton VL, Sieberhagen HA, Wet P De, Wyk CJ Van (1975) Composition and sensory qualities of wines prepared from the white grapes by fermentation with and without grape solids.

Smith MR, Penner MH, Bennett SE, Bakalinsky AT (2011) Quantitative Colorimetric Assay for Total Protein Applied to the Red Wine Pinot Noir. 6871–6876. doi: 10.1021/jf200547u

Sokolowsky M, Rosenberger A, Fischer U (2015) Sensory impact of skin contact on white wines characterized by descriptive analysis , time – intensity analysis and temporal dominance of sensations analysis. Food Qual Prefer 39:285–297. doi: 10.1016/j.foodqual.2014.07.002

Su D, Zhang R, Hou F, Zhang M, Guo J, Huang F, Deng Y (2014) Comparison of the free and bound phenolic profiles and cellular antioxidant activities of litchi pulp extracts from different solvents. 1–10.

Suklje K, Antalick G, Coetzee Z, Schmidtke LM, Basa Cesnic H, Brandt J, Toit WJ, Lisjak K, Deloire A (2014) Effect of leaf removal and ultraviolet radiation on the composition and sensory perception of Vitis vinifera L . cv . Sauvignon Blanc wine. Aust J Grape Wine Res 10:223–233. doi: 10.1111/ajgw.12083

Teissedre P, Glories Y (2008) Effect of pH , ethanol and acidity on astringency and bitterness of grape seed tannin oligomers in model wine solution. 19:286–291. doi: 10.1016/j.foodqual.2007.08.004

van Jaarsveld FP Van, Blom M, Hattingh S, Marais J (2005) Effect of Juice Turbidity and Yeast Lees Content on Brandy Base Wine and Unmatured Pot-still Brandy Quality. South African J Enol Vitic 26:116–130.

Van Sluyter SC, McRae JM, Falconer RJ, Smith PA, Bacic A, Waters EJ, Marangon M (2015) Wine protein haze: Mechanisms of formation and advances in prevention. J Agric Food Chem 63:4020–4030. doi: 10.1021/acs.jafc.5b00047

Waterhouse AL (2002) Wine Phenolics. Ann New York Acad Sci 36:21–36.

Stellenbosch University https://scholar.sun.ac.za

Supplementary data to Chapter 4

Supplementary Table S4.1: The concentration of sugars and organic acids (g/L) of juice from different processing stages of grapes with a low phenolic potential (LP) and high phenolic potential (HP).

	Free run juice		Pressed juice		Pressed juice after skin contact		Clarified		Clarified juice after skin contact	
	LP	HP	LP	HL	LP	HP	LP	HP	LP	HP
Citric acid	0.2±0.0 cd	0.2±0.01 c	0.2±0.0 acd	0.2±0.0 ab	0.2±0.0 cd	0.2±0.0abd	0.2±0.0 abcd	0.2±0.0 ab	0.2±0.0 c	0.2±0.0 b
Tartaric acid	7.8±0.4 ab	8.4±0.2 a	7.0±0.3 bc	7.2±0.4 b	5.6±0.3 d	5.1±0.9 d	5.9±2.1 cd	5.4±1.5 d	3.5±0.3 e	3.7±0.4 e
Malic acid	3.5±0.4 a	2.6±0.2 f	3.2±0.2 b	2.3±0.2 d	2.9±0.1 ce	1.8±0.2 g	3.0±0.2 bc	2.2±0.1 d	2.7±0.1 ef	1.8±0.1 g
Succinic acid	1.6±0.2 ade	1.28±0.14 bc	1.7±0.1 adf	1.4±0.1 bce	1.9±0.1 f	1.5±0.1 ade	1.6±0.1 ade	1.4±0.0 bce	1.9±0.1 df	1.2±0.5 c
Glucose	98.71±5.5 bcd	112.5±0.6.9 a	101.0±5.3 bcd	105.2±5.8 b	96.3±3.7 cd	101.9±6.7 bc	95.2±6.9 de	95.6±6.9 cde	89.7±2.2 e	95.4±5.7 de
Fructose	96.6±4.5 cd	112.9±7.1 a	99.8±5.2 bc	105.2±5.7 b	96.6±4.2 cd	103.8±6.0 b	94.3±6.5 cd	96.0±5.9 cd	91.0±2.7 d	97.7±5.3 c

Supplementary Table S4.2: The concentration of sugars and organic acids (g/L) of wine produced from grapes with a low phenolic potential (LP) and high phenolic potential (HP).

	Standard		Skin contact		Sediment contact		Skin and sediment contact	
	LP	HP	LP	HP	LP	HP	LP	HP
Citric acid	0.1±0.0 a	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Tartaric acid	3.2±0.7 a	3.0±0.2 ab	2.4±0.2 bc	1.9±0.9 c	3.0±0.51 ab	3.0±0.1 ab	2.3±0.1 c	2.1±0.0 c
Malic acid	2.2±0.4 a	1.8±0.1 bcd	2.1±0.0 ab	0.5±0.8 e	2.1±0.15 abc	1.7±.1 cd	2.2±0.1 abc	1.5±0.0 d
Succinic acid	1.6±0.3 d	1.6±0.1 d	2.0±1.0 bc	1.9±0.1 c	1.9±0.0 c	2.0±0.0 c	2.5±0.1 a	2.2±0.1 b
Glucose	<LOD	0.4±0.4 b	<LOD	1.0±0.9 a	<LOD	<LOD	<LOD	0.3±0.2 bc
Fructose	1.1±1.7 cde	9.7±3.7 ab	6.0±0.7 bcd	14.0±8.3 a	3.4±2.9 e	2.5±1.3 e	0.9±1.5 de	7.3±2.7 bc
Ethanol	99.6±22.3 b	115.6±7.4 a	109.7±7.4 ab	115.0±4.1 a	105.9±6.4 ab	116.2±3.0 a	111.0±4.4 a	113.3±4.1 a

Abbreviations: Low phenolic potential (LP), high phenolic potential (HP), limit of detection (LOD)

Stellenbosch University https://scholar.sun.ac.za

Supplementary Table S4.3: The descriptive sensory analysis of the taste scores of the wines.

Abbreviations: LP (Low phenolic potential), HP (high phenolic potential)

Aroma descriptors	Standard		Sediment contact		Skin contact		Skin and sediment contact	
	LP	HP	LP	HP	LP	HP	LP	HP
Pineapple	3.1±0.2 ab	3.3±0.2 ab	2.8±0.2 ab	2.8±0.1 ab	3.4±0.2 a	3.3±0.3 a	2.8±0.3 ab	2.4±0.2
Peach	2.6±02 ab	2.8±0.2 ab	1.4±0.4 c	1.5±0.3 ab	2.8±0.1 a	2.9±0.0 a	1.5±0.8 bc	0.8±0.0 c
Banana	0.8±0.1 ab	1.0±0.1 ab	0.6±0.1 ab	0.4±0.1 bc	1.0±0.1 a	0.8±0.3 bc	0.5±0.3 c	0.4±0.1 ab
Passionfruit	2.0±0.6 ab	2.0±0.2 ab	1.4±0.3 a	1.5±0.2 ab	2.4±0.0 b	2.3±0.3 ab	1.4±0.7 b	0.9±0.1 b
Grapefruit	2.3±0.1 ab	2.3±0.0 ab	2.1±0.1 b	2.2±0.3 ab	2.6±0.2 a	2.4±0.4 ab	2.4±0.3 ab	1.9±0.1 b
Floral	1.1±0.2 ab	1.8±0.0 a	0.4±0.3 ab	0.4±0.2 a	1.2±0.2 ab	1.3±0.5 ab	0.3±0.5 c	0.2±0.0 bc
Baked apple	2.3±0.1 a	2.7±0.2 ab	1.5±0.1 bc	1.7±0.1 cd	2.0±0.0 bc	2.2±0.3 ab	1.2±0.4 d	1.3±0.1 cd
Gherkin/dill	0.2±0.2 b	0.1±0.1 b	0.8±0.3 sb	0.7±0.2 b	0.4±0.1 b	0.3±0.1 b	0.8±0.3 ab	1.5±0.0 a
Asparagus	0.1±0.0 c	0.1±0.0 c	0.5±0.1 ab	0.3±0.1 bc	0.1±0.0 c	0.0±0.0 c	0.4±0.2 ab	0.9±0.0 a
Cooked veg	0.2±0.1 b	0.1±0.0 b	1.5±0.6 a	1.2±0.4 ab	0.2±0.0 b	0.1±0.1 b	1.4±0.9 a	1.5±0.1 a

Stellenbosch University https://scholar.sun.ac.za

Chapter 5

Research Results

Linking polyphenol distribution during wine making with the fractions lost in the waste matrices and the berry cell wall composition.

Stellenbosch University https://scholar.sun.ac.za

Chapter 5: Linking polyphenol distribution during wine making with the fractions lost in the waste matrices and the berry cell wall composition.

5.1. Introduction

During winemaking, waste products such as pomace and sediments occur that can also be considered "traps" for compounds that accumulate in them. The pomace constitute of primarily grape skins and is generated after pressing the grapes to collect the juice, whereas the juice sediment is removed during juice clarification. Studies on pressed white grape pomace revealed that these tissues contain residual hydroxycinnamic acids (caftaric acid, caffeic acid, coutaric acid, fertaric acid), flavonols (quercetin-glucoside, kaempferol-glucoside, isorhamnetin-glucoside) and flavan-3-ols (catechin, epicatechin, procyanidin dimers) up to a total of 170 µg/g, 1300 µg/g, 660 µg/g respectively (Kammerer et al. 2004; Rossello and Teissedre 2013). Residual polyphenols within the pomace is a clear indication that winemaking techniques only extract a fraction of polyphenols from the skins.

During white wine making, the grape skins are usually separated from the juice (when no pre-fermentative skin contact is employed) right after the pressing step and thus the extraction of polyphenols from the skin into the juice is limited and the cell wall composition of the skin cells are left, presumably, relatively unchanged. Thus typical white winemaking procedures result in more residual polyphenols found in white grape pomace compared to red grape pomace (Kammerer et al. 2004) where the must is usually fermented while in contact with the skins and is also often treated with cell wall degrading enzymes (Romero-Cascales et al. 2012).

The aim of this chapter was to quantify how much of the phenolic potential of the grape berry at harvest was unused and ended up in the waste matrices during winemaking. The phenolic potential of the grapes refers to the sum and variety of extractable polyphenols under standard white winemaking practices. The cell wall polymers of grape skin are not only a barrier to polyphenol extraction (Ortega-Regules et al. 2006; Pinelo et al. 2006) but extracted/released polyphenols can bind to some of these cell wall polymers when the grape juice is in contact with the pomace or with other grape tissue residues. A fraction of phenolic compounds is also bound to the grape cell wall polymers (Downey et al. 2003; Fournand et al. 2006). The cell walls of grapes consist of pectin (±60%), cellulose (±15%), hemicellulose polymers (±10%) and structural proteins (±5%) (Lecas and Brillouet 1994; Vidal et al. 2001; Arnous and Meyer 2009). A positive correlation exists between the degree of procyanidin polymerization and their adsorption to exposed cell wall material (Bindon et al. 2010b; Bindon et al. 2010a). The use of cell wall degrading enzymes (pectinase, cellulase, hemicellulase) prior to or during fermentation have been shown to deconstruct the cell wall, resulting in enhanced polyphenol extraction (Kammerer et al. 2005; Chamorro et al. 2012; Zietsman et al. 2015b).

Stellenbosch University https://scholar.sun.ac.za

In terms of the juice sediment as a waste component, to our knowledge literature does not contain information on the composition of grape juice sediment. The composition of bayberry juice sediment was previously reported to contain amino acids, fermentable monosaccharides, polyphenols and various minerals (Fang et al. 2006). The sediment is formed after enzymatic treatment (pectinase) of the juice as it increases juice yield, decreases viscosity for filtration purposes and reduces juice turbidity (Tapre and Jain 2014). Fermentation in the presence of the sediment (i.e. turbid juices) are typically associated with imparting negative sensory characteristics on the resulting wine (Liu et al. 1987; van Jaarsveld et al. 2005; Coelho et al. 2018) and is not common practice.

Polyphenol levels in wines are also influenced by the protein contents of the juices and wines, particularly since proteins and polyphenols can form complexes. For example, the protein content of white grape juice and wine play a vital role in the formation of haze which influences consumer perception. These proteins unfold in response to a stimuli (e.g. elevated temperature) and subsequently aggregates and flocculate to form a visible haze (Van Sluyter et al. 2015). Polyphenols contribute to haze formation by binding to proteins or via hydrophobic interaction leading to aggregation and precipitation (Esteruelas et al. 2011; Bindon and Smith 2013; Gazzola et al. 2015). Protein content of juice increase with pre-fermentative skin contact (Vincenzi et al. 2011), but since polyphenol extraction is also known to increase, it would lead to individual levels of both decreasing due to their interaction and complex formation (Tian 2014).

In this study, the polyphenol composition and content of all pomace fractions (crushed, skin contact, pressed) were determined to quantify the residual polyphenols that were not distributed to the juice or wine. Pomace cell wall analysis was used to provide information on the possible impact of juice processing methods on the cell wall structure and the potential to encapsulate/bind polyphenols. Similarly, the composition and content of the juice sediments were determined to quantify the polyphenols that were lost (settled out in the sediment) during enzyme clarification of the juice. By comparing the phenolic analysis of the wines that were fermented with and without sediment contact, one can estimate/speculate how much polyphenols were reintroduced when fermentation occurred in the presence of the sediment (chapter 4). Furthermore, by looking for correlations between phenolic and protein content of the juice and wine we tried to account for phenolic losses due to interaction between these two compound groups.

5.2. Materials and methods

5.2.1. Preparation of cell walls from grapes and pomace

Grape cell walls, referred to as the alcohol insoluble residue (AIR), were extracted (four AIR extractions were prepared from each biological repeat) from the partitioned pulp and skins and deseeded pomace (crushed, after skin contact, after press) as described by Gao et al (2015). The above-mentioned grape tissues were frozen with liquid nitrogen and milled (Retch Mixer Mill MM 400, Germany) at 30 Hz for 1 min until a fine

Stellenbosch University https://scholar.sun.ac.za

powder was obtained. Approximately 6 g of tissue was mixed with twice the volume of absolute ethanol (Merck, Germany) and boiled 95°C for 20 min. The solid residues were collected by centrifugation (3000 rpm, 3 min) and washed with absolute ethanol on a rotating wheel for 2 h. This extraction step was followed by a methanol: chloroform (1:1) (Merck, Darmstadt, Germany) wash and then a methanol: acetone (1:1) (Merck, Germany) wash. The final pellet was washed in acetone and allowed to dry until most of the acetone was evaporated. The pellet was then re-suspended in MilliQ water and stored at -80°C. A fine dry powder of AIR was obtained after lyophilising (Martin-Christ, Germany) the frozen sample for 96h.

5.2.2. Comprehensive microarray polymer profiling (CoMMP) analysis of cell walls.

CoMMP analysis was performed by Dr. Julia Schückel (University of Copenhagen) as previously described in Moller et al (2007). Briefly, pectin and hemicellulose rich extracts were obtained by sequential extraction of the isolated cell wall material (AIR) with 0.05 M Cyclohexane diamine tetra acetic acid (CDTA) and 4 M Sodium hydroxide (NaOH), both at room temperature. These extracts were spotted five times onto nitrocellulose membranes and probed with primary monoclonal antibodies and carbohydrate-binding modules that recognise specific cell wall antigen domains. A mean fluorescence signal from a secondary monoclonal antibody was calculated per spot and the relative abundance of the specific cell wall polymer or protein was normalized to the highest signal in the dataset and a cut-off value of five was employed.

5.2.3. Mid infrared spectroscopy analyses of cell walls

Cell walls isolated from the skin, pulp and pomace (crushed, after skin contact and pressed) were also analysed with infrared spectroscopy. Cell wall material was scanned on an Alpha-P ATR Fourier transform mid-infrared (FT-MIR) spectrometer (Bruker Optics, Ettlingen, Germany), fitted with a single bounce diamond crystal (area 2 mm^2) maintained at 30°C. After each scan the sample area was cleaned using distilled water and lens paper. Each sample was scanned five times and the following scanning parameters were used: 4000-400 cm^{-1} wavenumber ranges, 4 cm^{-1} resolution, 7.5 kHz scanner velocity and 64 background and sample scans. Sample scans were collected against a background of air which was taken after every 8 sample scans. The spectra were analysed using SIMCA (Version 14.1 from MKS Umetrics AB) where an MSC (multiplicative scatter correction) filter was applied to compensate for scattering and differences in the effective path length. The fingerprint region of the spectra (1400-800 cm^{-1}) conveys unique polysaccharide patterns and information about the functional groups are portrayed by peaks in the 1400 to 1800 cm^{-1} wavelength region (Capek et al. 2000; Szymanska-Chargot and Zdunek 2013).

5.2.4. Polyphenol extraction, identification and quantification

Sediment and pomace (crushed, skin contact and pressed) samples were collected during winemaking (refer to figure 4.1 in chapter 4). The solid residues of the juice sediment was collected with centrifugation (5 min, 5000 rpm) after the enzyme clarified, clear juice was removed to be fermented. Sediment and pomace

Stellenbosch University https://scholar.sun.ac.za

samples were deseeded and kept frozen with liquid nitrogen and milled (Retch Mixer Mill MM 400, Germany) at 30 Hz for 1 min until a fine powder was obtained. The dry weights of the pomace and the sediment samples were determined by allowing moisture to evaporate at 80^{o}C and recording the weights when the readings stabilised**.**

The individual phenolic acids and flavonoids were extracted according to the method of (Du Plessis 2017) and is described in the materials and methods section of Chapter 3.

5.2.5. Juice and wine protein quantification

The juice (free run, pressed and clarified) and wine proteins were quantified using the Pierce^{TM} BCA Protein Assay Kit as described by the manufacturer (Thermo Fischer Scientific, USA). The proteins in the juice and wine samples were precipitated by adding ice cold acetone to a 300 µl sample in a 1:4 ratio and incubated overnight at -20^{o}C. The mixture was centrifuged at 20 000 rpm for 10 min, the supernatant discarded and the pellet re-suspended in 50 µl MilliQ water. In a 96-well microplate, 25 µl of the re-suspended protein sample was mixed with 200 µl of a mixture of reagent A and B (50:1), supplied by the manufacturer, and incubated for 30 min at 37^{o}C. Samples were allowed to cool to room temperature for 10 min and optical density measured at 562 nm using a Bio-Tek PowerWave X Microplate Reader. The data was analysed using KC4 software provided with the instrument. Protein standard curves of albumin stock solutions were prepared in sterile MilliQ water and used to quantify the protein concentrations of the juice and wine. All reactions were performed in triplicate. Juice samples were diluted 20 times prior to the addition of the reagent A and B mixture to ensure that samples were within the detection limits of the standards.

5.2.6. Statistical analysis

Multivariate analysis was performed using SIMCA (Version 14.1 from MKS Umetrics AB). The data was analysed using principle component analysis (PCA) and orthogonal partial least squares – discriminant analysis (OPLS-DA) to investigate trends and outliers within the data and to correlate the variables (X, measured polyphenols) with the qualitative treatments (Y, class e.g. light exposure, skin contact, sediment contact) respectively.

Factorial and One-way analysis of variance (ANOVA) was performed using Tibco Statistica (version 13, Oklahoma, USA) to determine the statistical significance of phenolic potential, skin contact and sediment contact treatments within the pomace and sediment to elucidate which phenolic compounds differed significantly within these matrices. ANOVA results are reported as P-values and the highest P-value correlated to the greatest significance. Fischer's least square difference (LSD) post hoc test was conducted to pinpoint which polyphenols responded statistically significant to a particular treatment. All significance differences were interpreted on a 5% significance level ($p<0.05$).

Stellenbosch University https://scholar.sun.ac.za

5.3. Results

5.3.1. Polyphenol analysis of the pomace during juice processing

The polyphenol composition of the pomace samples, generated during juice processing (from crushing to pressing), was analysed (Table 5.1) to determine polyphenol distribution and to quantify the fractions of the grape berry phenolic potential that accumulated in the waste products of the winemaking process.

Principle component analysis (PCA) was performed which revealed that pomace samples separated along the first principle component (PC1, 53% explained variance) according to juice processing and phenolic potential along the second principle component (PC2, 26% explained variance) (Figure 5.1 A). The total hydroxycinnamic acids decreased in both low and high phenolic potential pomace during juice processing in the standard and skin contact samples (Table 5.1). On the contrary, total flavan-3-ol levels increased during juice processing which increased the total polyphenols in all pomace samples.

ANOVA plots showed that the hydroxycinnamic acid concentrations of low and high phenolic potential pomace decreased during juice processing (Figure 5.2) with all pressed pomace samples having lower concentrations of caftaric (Figure 5.2: A, B), coutaric (Figure 5.2: C, D) and caffeic acid (Figure 5.2: E, F), compared to the pomace at the crushed stage. The effect of skin contact caused increased extraction for certain hydroxycinnamic acids (e.g. coutaric acid in the high phenolic potential pomace was lower in the skin contact pressed samples compared to the standard pressed samples) or no effect (caftaric acid in both low and high phenolic potential pomace). The residual flavonoid concentrations increased from crushing to pressing in most of the pomaces analysed (Table 5.1 and Figure 5.3). The only exceptions were catechin (Figure 5.3: A and B) in high phenolic potential pomace that decreased irrespective of skin contact treatment; and flavonol_4, that were not detected in low

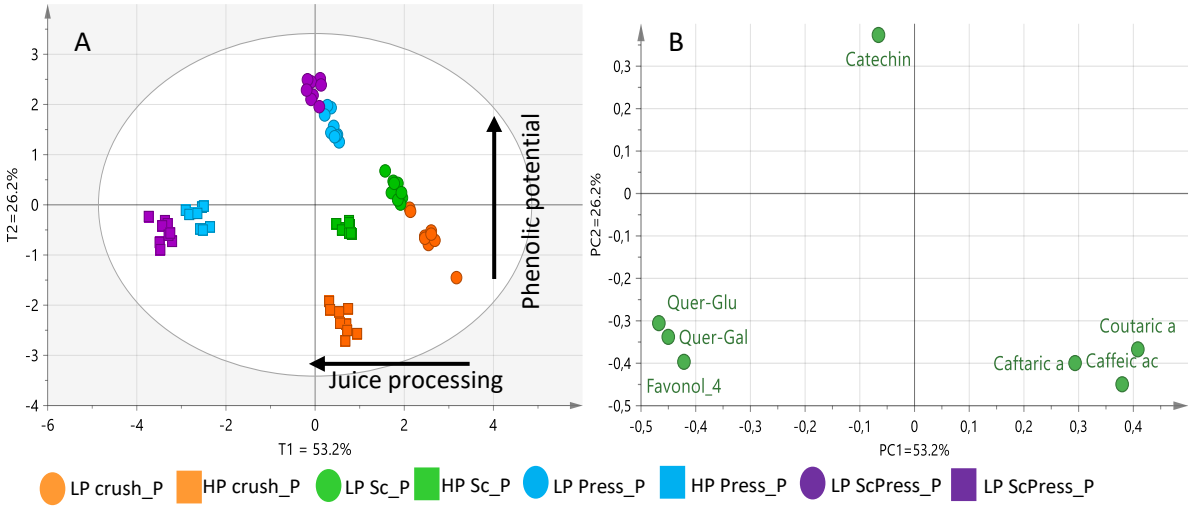


Figure 5.1: The PCA score (A) and loading (B) plots (PC1 vs PC2) of the polyphenols extracted from pomace with a low phenolic potential (LP, circle) and high phenolic potential (HP, square) generated at each stage of processing (crush and press) during winemaking, standard or with skin contact (Sc) applied.

Stellenbosch University https://scholar.sun.ac.za

Table 5.1: The concentration of the residual polyphenols extracted from the pomace, sampled during juice processing (ug/gDW).

	Crushed pomace		Pomace after skin contact		Standard pomace after pressing		Pomace after Skin contact and pressing	
	LP	HP	LP	HP	LP	HP	LP	HP
Caftaric acid	2.9±0.6 a	3.0±0.3 a	2.00±0.23 b	1.7±0.1 bcd	1.8±0.1 bd	1.5±0.1 c	1.8±0.1 bcd	1.6±0.3 cd
Coutaric acid	7.5±0.1 b	9.1±0.8 a	5.93±0.42 c	6.1±0.6 c	3.5±0.2 d	3.0±0.1 e	3.1±0.1 e	N.D.
Caffeic acid	8.0±0.6 b	9.9±0.6 a	7.88±0.45 b	8.0±0.2 b	2.8±1.1 c	0.7±0.8 e	N.D.	0.8±0.8 e
Total hydroxycinnamic acids	**18.4±1.2 b**	**22.1±2.3 a**	**15.94±1.02 c**	**15.9±0.9 c**	**8.3±2.6 d**	**5.5±2.4 e**	**4.9±0.2 e**	**2.6±1.5 f**
Catechin	11.9±1.0 e	16.1±1.3 c	12.74±1.36 e	12.0±0.8 e	17.5±5.3 b	15.1±4.3 d	18.9±5.1 a	12.7±3.3 e
Epicatechin	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Flavan-3-ol_1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Flavan-3-ol_2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Flavan-3-ol_3	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Flavan-3-ol_4	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Polymeric phenolics	N.D.	N.D.	10.02±0.19 e	23.8±2.8 b	20.8±5.3 c	27.9±7.1 a	25.8±3.5 f	54.0±15.3 d
Total Flavan-3-ols	**11.9±1.0 f**	**16.1±1.37 b**	**22.62±1.57 ef**	**25.8±0.8 e**	**38.3±10.7 d**	**43.0±7.4 a**	**44.8±8.7 c**	**66.7±18.6 c**
Quercetin-galactoside	N.D.	3.6±0.1c	N.D.	2.1±0.1 d	1.1±0.5 e	4.4±0.8 b	0.8±0.5 f	5.3±0.3 a
Quercetin-glucoside	7.7±0.54g	37.6±9.7 c	8.31±0.25 g	23.2±4.1 d	15.1±3.2 f	47.8±7.3 b	16.8±2.7 e	60.9±15.3 a
Favonol_4	N.D.	5.3±0.8 c	N.D.	1.3±0.0 d	N.D.	7.8±0.3 b	N.D.	7.0±0.5 a
Total flavonols	**7.7±0.54g**	**46.3±13.0 c**	**8.31±0.25 g**	**26.9±4.1 d**	**16.3±3.3 f**	**58.6±8.8 b**	**17.6±3.2 e**	**73.5±16.2 a**
Total polyphenols	**37.9±2.8 f**	**84.5±16.7 b**	**46.87±2.84 e**	**68.6±5.9 c**	**63.0±18.5 e**	**107.3±18.7 a**	**67.3±12.1 f**	**142.9±38.3 d**

Abbreviations: Low phenolic potential (LP), high phenolic potential (HP)
Different letters indicate statistical significance (One-way ANOVA with Fisher's LSD post hoc test, $p<0.05$)

Stellenbosch University https://scholar.sun.ac.za

phenolic potential pomace. In general, high phenolic potential pomace had a higher residual concentration of flavonoids compared to low phenolic potential and this difference was seen in crushed and pressed samples (except for catechin in pressed pomace). A very distinct pattern was seen for the concentration of quercetin-galactoside (Figure 5.3: D), quercetin-glucoside (Figure: 5.3 F) and flavonol_4 (Figure 5.3: H) (and to a lesser degree for catechin) in high phenolic potential pomaces subjected to skin contact. All these compounds showed a sharp decreased in concentration after skin contact, followed by a sharp increase after pressing (except for catechin, Figure 5.3: B). This decrease was not observed in low phenolic potential pomace.

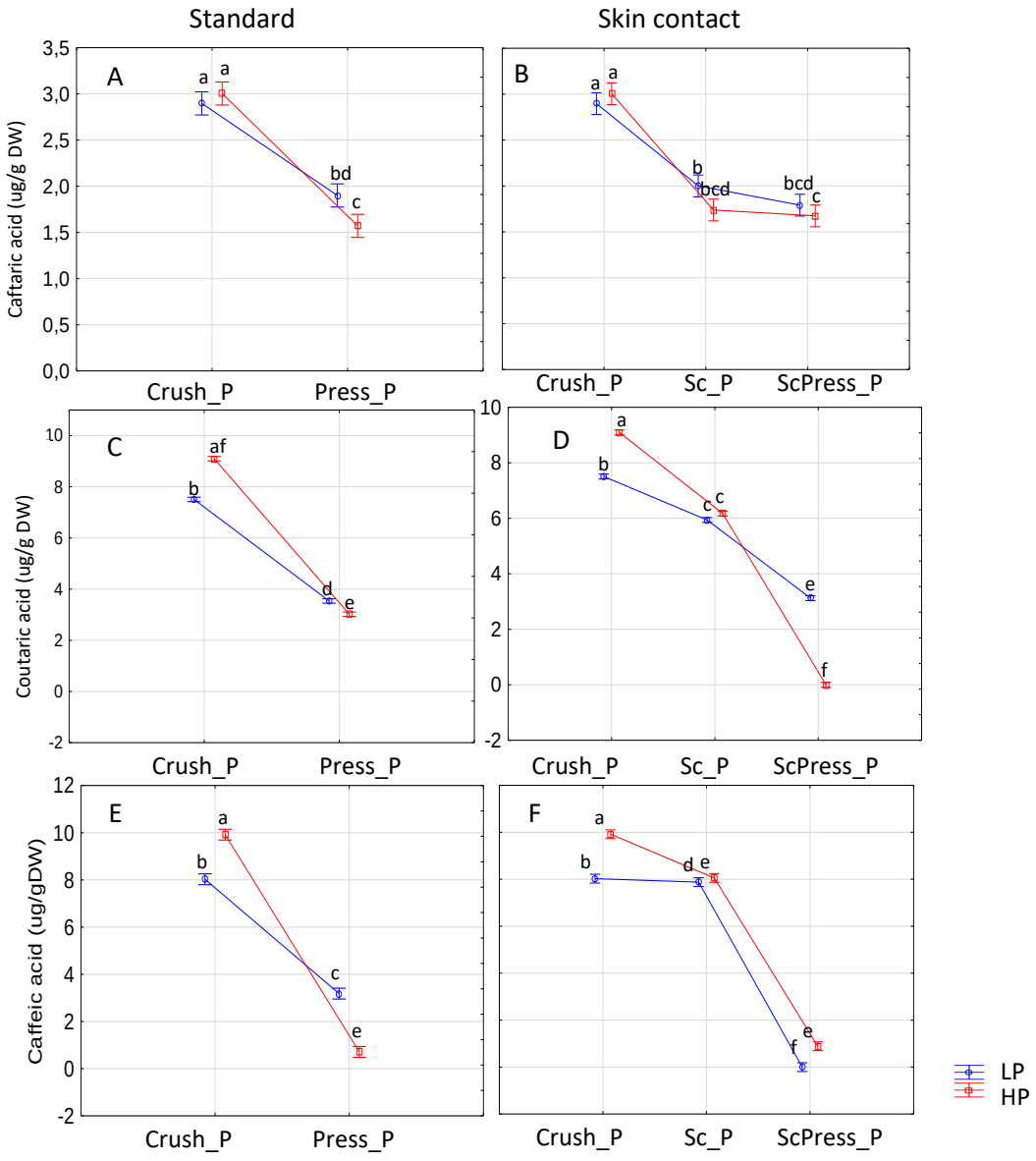


Figure 5.2: The ANOVA plots of residual hydroxycinnamic acids within the pomace with a low phenolic potential (LP) and high phenolic potential (HP) at different processing stages (crush and press) during standard winemaking or with skin contact (Sc, treatment). The following polyphenols were measured: caftaric acid (standard [A], Sc [B]), coutaric acid (standard [C], Sc [D]) and caffeic acid (standard [E], Sc [F]). The different letters indicate significance according to Fisher LSD post hoc tests with a 95% confidence interval.

Stellenbosch University https://scholar.sun.ac.za

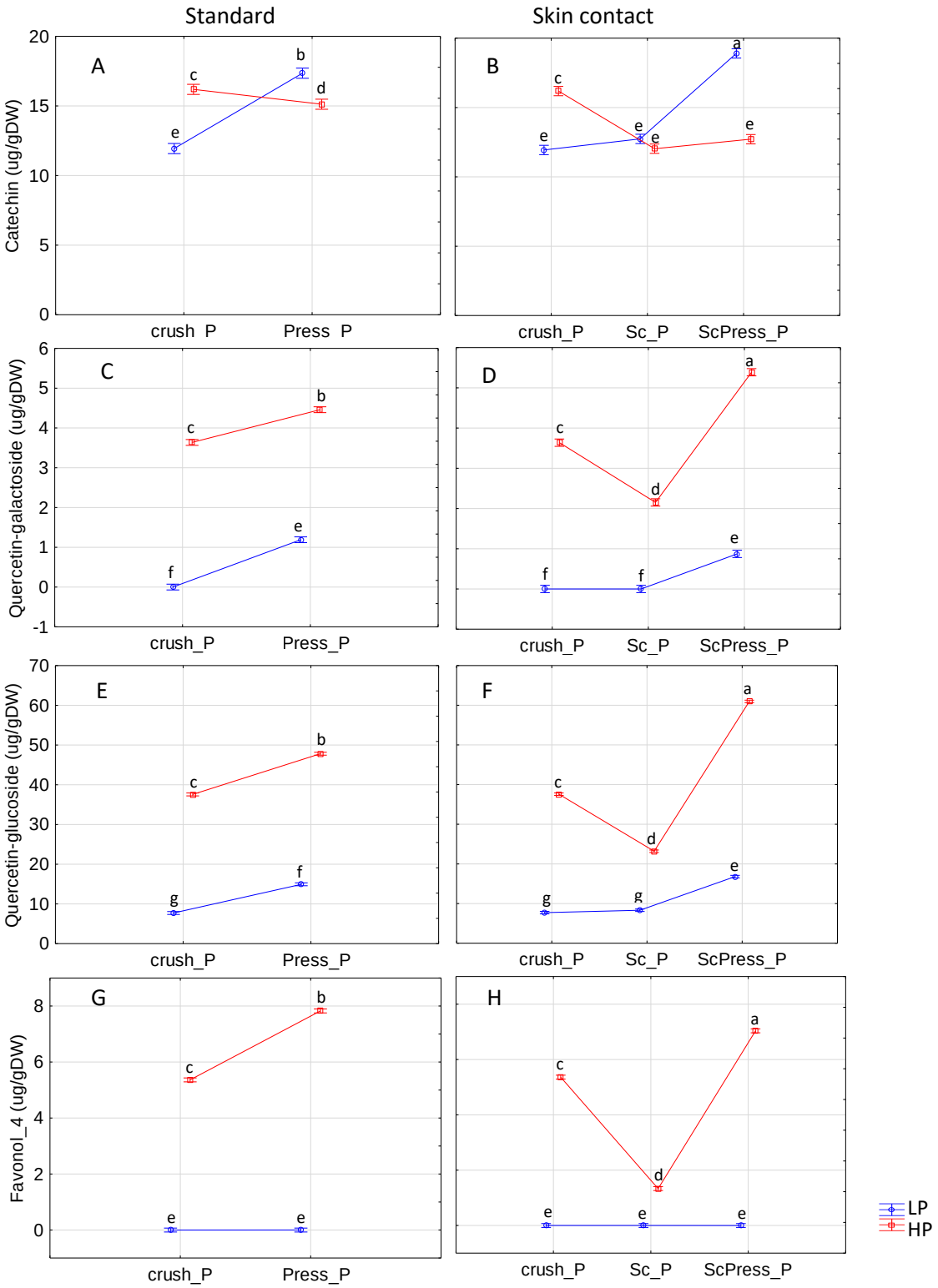


Figure 5.3: The ANOVA plots of residual flavonoids within pomace originating from grapes with a low phenolic potential (LP) and high phenolic potential (HP) at different processing stages during winemaking, standard or including skin contact (Sc, with skin contact). The following polyphenols were measured: catechin (standard [A], skin contact[B]), quercetin-galactoside (standard [C], skin contact [D]), quercetin glucoside (standard [E], skin contact [F]) and flavonol_4 (standard [G], skin contact [H]). The different letters indicate significance according to Fisher LSD post hoc tests with a 95% confidence interval.

Stellenbosch University https://scholar.sun.ac.za

5.3.2. Polyphenol analysis of juice sediment

The polyphenol composition of the juice sediment, collected after enzyme clarification, was determined to quantity how much polyphenol settled out, or precipitated with the sediment particles (Table 5.2). These fractions thus represent a potential loss of polyphenols in terms of what could end up in the final wine. PCA analysis of the data revealed that the strongest driver of variance in the sediment samples (PC1, 52.7 % explained variance) was the skin contact treatment (Figure 5.4 A). Skin contact increased total hydroxycinnamic acid levels in low and high phenolic potential sediment samples but decreased total flavan-3-ol levels (Table 5.2). Phenolic potential seemed to drive the separation along PC2 (25.4% explained variance) (Figure 5.4 A). Total hydroxycinnamic acid levels were higher in the standard and skin contact low phenolic potential sediment samples. In turn, total flavan-3-ols were significantly higher in the high phenolic potential samples.

Table 5.2 Polyphenol concentration extracted from juice sediment after juice clarification (ug/gDW).

	Standard sediment		Skin contact sediment	
	LP	HP	LP	HP
Caftaric acid	407.3±19.3 a	372.9±9.3 bc	393.2±11.9 ab	365.9±17.2 c
Coutaric acid	23.8±2.7 d	34.1±1.8 c	51.3±12.3 b	62.0±9.2 a
Caffeic acid	18.8±2.6 c	25.2±1.0 bc	28.6±7.9 b	41.3±4.0 a
Total hydroxycinnamic acids	**450.0±25.3 ab**	**432.2±11.5 b**	**473.2±32.8 a**	**469.3±33.1 a**
Catechin	658.4±23.0 c	786.0±41.3 a	478.0±19.5 d	702.6±29.3 b
Epicatechin	14.0±0.6 a	14.0±1.3 a	15.9±2.2 a	15.8±4.1 a
Flavan-3-ol_1	N.D.	N.D.	N.D.	N.D.
Flavan-3-ol_2	N.D.	N.D.	N.D.	N.D.
Flavan-3-ol_3	N.D.	N.D.	N.D.	N.D.
Flavan-3-ol_4	N.D.	N.D.	N.D.	N.D.
Polymeric phenolics	203.7±21.4 d	247.7±22.3 b	220.8±25.8 c	271.0±34.3 a
Total Flavan-3-ols	**672.4±23.6 b**	**800.0±43.0 a**	**493.9±23.5 c**	**718.5±34.1 a**
Quercetin-galactoside	N.D.	N.D.	N.D.	N.D.
Quercetin-glucoside	7.1±0.9 d	13.1±1.6 c	19.0±8.0 a	70.4±11.3 b
Favonol_4	N.D.	N.D.	N.D.	N.D.
Flavovol_5	N.D.	N.D.	N.D.	N.D.
Total flavonols	**7.1±0.9 d**	**13.1±1.6 c**	**19.0±8.0 a**	**70.4±11.3 b**
Total phenolics	**1333.3±57.1 a**	**1493.2±55.8 d**	**1207.2±62.3 b**	**1529.3±83.7 c**

Abbreviations: N.D. (not detected.)
Different letters indicate statistical significance (One-way ANOVA with Fisher's LSD post hoc test, $p<0.05$)

Stellenbosch University https://scholar.sun.ac.za

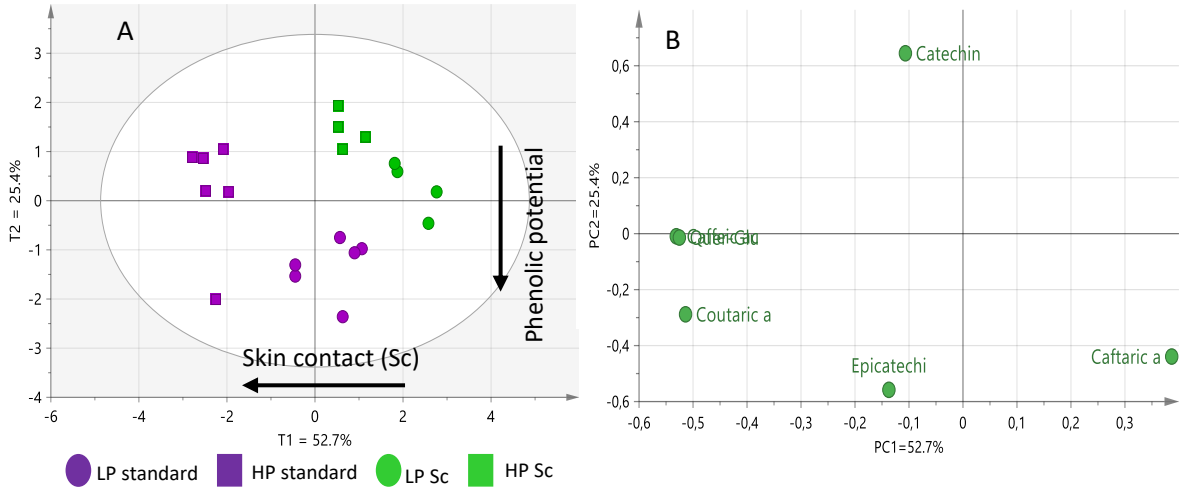


Figure 5.4: The PCA score (A) and loading (B) plots (PC1 vs PC2) of the polyphenols extracted from the sediment collected after juice clarification of grapes with a low phenolic potential (LP, circle) and high phenolic potential (HP, square).

The sediment samples reflected the phenolic potential of the juices from which it derived (e.g. high phenolic potential juice delivered high phenolic potential sediment), except for caffeic acid and epicatechin, where there were no differences between low and high phenolic potential derived sediments (Figure 5.5: C and E). Skin contact led to an increase in the coutaric acid (Figure 5.5: B), caffeic acid (Figure 5.5: C), epicatechin (Figure 5.5: E) and quercetin-glucoside (Figure 5.5: F) concentrations of the sediment for both the low and high phenolic potential samples. These increases were statistically significant for all polyphenols except epicatechin. Conversely, although both caftaric acid (Figure 5.5: A) and catechin (Figure 5.5: D) concentrations decreased within low and high phenolic potential sediments, statistical significance was only observed for catechin.

Stellenbosch University https://scholar.sun.ac.za

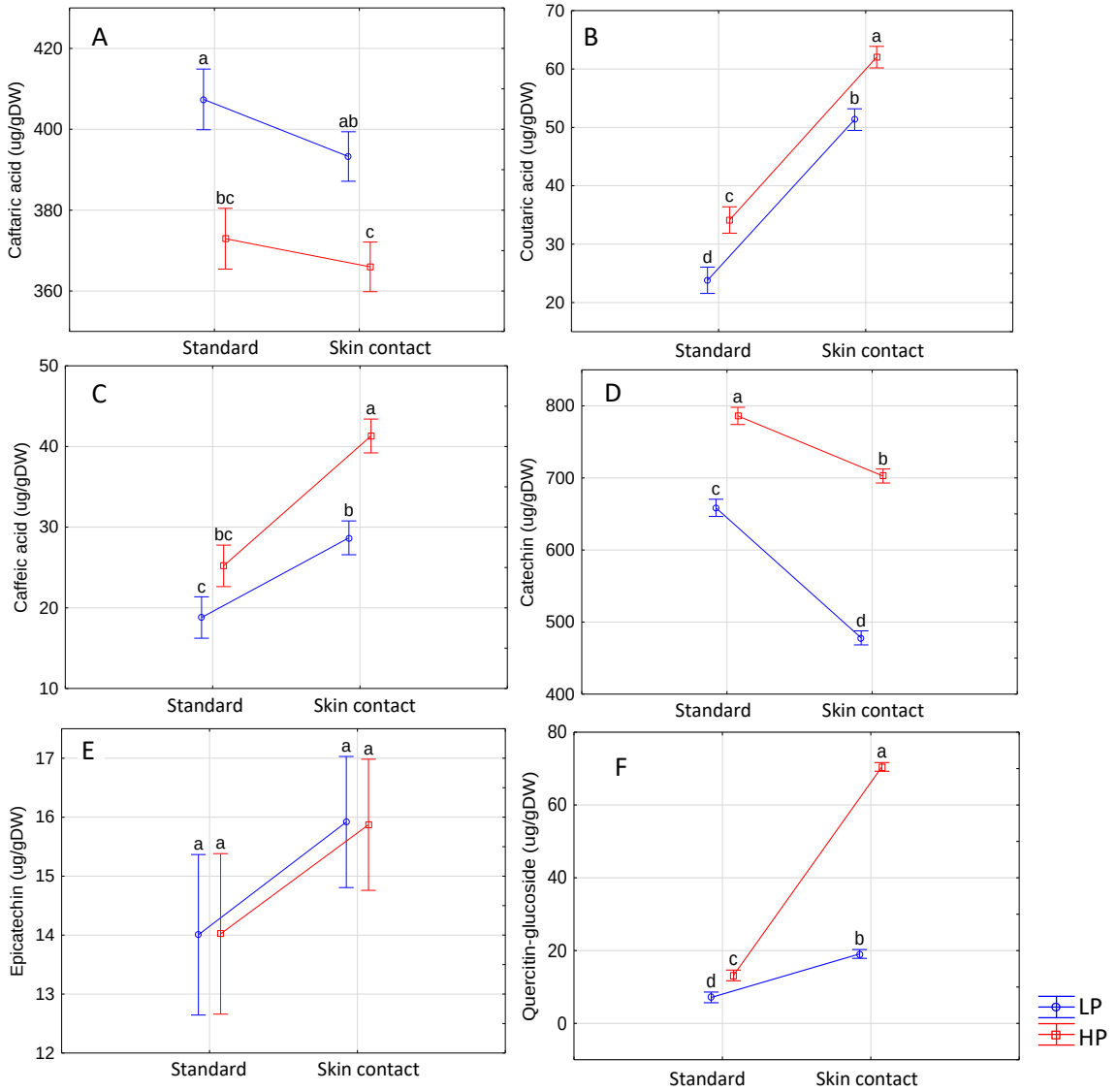


Figure 5.5: The ANOVA plots of the polyphenols extracted from the juice sediment that settled after enzyme clarification. Sediment settled from low phenolic potential (LP) and high phenolic potential (HP) standard juices or with skin contact (Sc). The following polyphenols were measured: caftaric acid (A), coutaric acid (B), caffeic acid (C), catechin (D), epicatechin (E) and quercetin-glucoside (F). The different letters indicate significance according to Fisher's LSD post hoc tests with a 95% confidence interval.

5.3.3. Cell wall compositional analysis of ripe berry skin, pulp and pomace collected during juice processing

A heatmap of the COMPP analysis performed on the isolated cell walls, from the skin, pulp and pomace residue collected at harvest and during juice processing, showed the relative abundance of each polymer extracted with either CDTA or NaOH (Tables 5.2 and 5.3) (the relative abundance of each polymer was expressed as a percentage of the entire dataset in these heatmaps). CDTA and NaOH were used for sequential extraction of cell wall glycans as these solvents are known to solubilize pectins and hemicellulose respectively (Moller et al. 2007). Differences in epitope signals are considered significant when values differ by a margin of 10 units. Based on the heatmap (Table 5.2), the CDTA fractions extracted from low phenolic potential skin and pulp were predominantly comprised of homogalacturonan and rhamnogalacturonan I (RGI) (backbone and side chains). The corresponding NaOH fractions of each tissue were primarily rich in xyloglucan. Compositional differences were observed between the skin and pulp pomace cell walls (Table 5.2) as the skin

Stellenbosch University https://scholar.sun.ac.za

extracts had a higher signal for xyloglucan (mAb LM15 and LM25, NaOH fraction) and homogalacturonan with a low degree of esterification (mAb JIM5, CDTA fraction), whereas the pulp extract had higher RGI, arabinan (mAbs INRA-R1 and –R2, LM13, CDTA and NaOH fractions), extensin and arabinogalactan protein (AGPs) (both CDTA and NaOH fractions) signals.

When comparing the cell walls derived from the tissues of the high and low phenolic fresh grapes, higher levels of extensins (mAb LM1) were seen in the high phenolic potential pulp, while the low phenolic potential skin and pulp had higher signals for xyloglucan (mAb LM15, NaOH) and RGI (mAb INRA-R2) respectively. This was partly in contrast to the results observed for the pomace cell wall analysis (Table 5.3) since all high phenolic potential pomace cell walls had a lower RGI signal than the low phenolic potential cell walls in the CDTA fraction. Fourier transform mid-infrared (FT-MIR) analysis of the AIR (alcohol insoluble residue) from the fresh grapes revealed that pectin was more abundant within low and high phenolic potential pulp cell walls as they had slightly higher peak areas within the spectrum (1095 cm^{-1},1157 cm^{-1},1230-1350 cm^{-1} and 1610 cm^{-1}) compared to their corresponding skins (Supplementary Figure S5.1 A). These pulp cell walls also had higher peaks within the cellulose, xyloglucan and cell wall protein regions of the spectrum (1230-1350 cm^{-1} and 1610 cm^{-1}).

It seems that the skin contact treatment might have caused a change in the cell wall polymer composition because there are differences in many of the epitope signals (mAb INRA-R1 and –R2, LM13, LM15, LM25, LM1 and JIM13) between the crushed or pressed pomace and their counterparts that were subjected to skin contact (NaOH fraction, Table 5.3). The FT-MIR spectrum generated from all the pomace cell walls showed little to no change in peak intensity at any of the characteristic wavenumbers (Supplementary Figure S5.1 B). High phenolic potential crushed pomace (crush_P) was the only fraction that showed higher peak intensities within the pectin, cellulose and xyloglucan region (1157-1230 cm^{-1}) compared to the control (low phenolic potential crush_P) and the other fractions.

Stellenbosch University https://scholar.sun.ac.za

Table 5.2: CoMPP analysis of alcohol insoluble residue extracted from low phenolic potential (LP) and high phenolic potential (HP) grape skin and pulp at harvest. The heatmap shows the relative abundance of each cell wall associated glycan epitope within the different grape tissues.

Extraction	Treatment	Tissue	Homogalacturonan							RG1 and side chains					Mannans		Glucans/Xyloglucans			Xylans		Cellulose	Extensins		AGPs			
			Homogalacturonan with a low DE (mAb JIM 5)	Homogalacturonan with a high DE (mAb JIM 7)	Partly methylesterified homogalacturonan (mAb LM18)	Partly methylesterified homogalacturonan (mAb LM19)	Partly methylesterified homogalacturonan (mAb LM20)	Ca cross-linked homogalacturonan (mAb 2F4)	Xylogalacturonan (mAb LM8)	Backbone of rhamnogalacturonan I (mAb INRA-R1)	Backbone of rhamnogalacturonan I (mAb INRA-R2)	(1→4)-β-D-galactan (mAb LM5)	Feruloylated (1→4)-β-D-galactan (mAb LM9)	Linearised (1→5)-α-L-arabinan (mAb LM13)	(1→4)-β-D-mannan (mAb LM21)	(1→4)-β-D-mannan/galactomannan (mAb LM22)	(1→3)-β-D-glucan (mAb BS-400-2)	Xyloglucan (XXXG motif) (mAb LM15)	Xyloglucan (mAb LM25)	(1→4)-β-D-xylan (mAb LM10)	(1→4)-β-D-xylan/arabinoxylan (mAb LM11)	Cellulose (mAb CBM3A)	Extensin (mAb LM1)	Extensin (mAb JIM11)	Extensin (mAb JIM20)	AGP (mAb LM8)	AGP (mAb JIM13)	AGP (mAb JIM14)	AGP, β-linked GlcA (mAb LM2)
CDTA	LP	Skin	74	29	19	21	30	6	0	12	36	0	0	0	0	0	0	0	0	0	0	0	0	0	9	14	0	0
CDTA	LP	Pulp	60	26	17	18	30	6	0	29	78	5	5	11	0	0	0	0	0	0	6	0	10	7	16	6	12	0	0
CDTA	HP	Skin	73	25	21	21	32	0	0	12	31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	8	0	5
CDTA	HP	Pulp	60	23	21	21	32	0	0	31	65	9	7	16	0	0	0	0	0	0	9	0	8	0	10	6	9	0	0
NaOH	LP	Skin	0	0	0	0	0	0	0	0	6	8	0	0	0	0	0	82	71	0	0	0	0	5	0	7	8	0	0
NaOH	LP	Pulp	0	0	0	0	0	0	0	13	23	6	0	10	0	0	0	42	36	6	0	0	17	8	0	16	22	0	0
NaOH	HP	Skin	0	0	0	0	0	0	0	0	8	13	0	0	0	0	5	69	78	0	0	0	6	5	0	0	7	0	0
NaOH	HP	Pulp	0	0	0	0	0	0	0	13	36	12	0	17	0	0	9	35	41	7	0	0	33	11	8	13	24	0	0

Red blocks indicate significant differences for the epitopes signals between samples.

Stellenbosch University https://scholar.sun.ac.za

Table 5.3: CoMPP analysis of alcohol insoluble residue (AIR) extracted from pomace (P) with a low phenolic potential (LP) and high phenolic potential (HP) during standard juice processing steps (crush or press) or with skin contact (Sc). The heatmap shows the relative abundance of each cell wall associated glycan epitope as present within the grape pomace tissues.

Extraction	Treatment	Tissue	Homogalacturonan							RG1 and side chains				Mannans		Glucans/Xyloglucans			Xylans		Cellulose	Extensins			AGPs			
			Homogalacturonan with a low DE (mAb JIM 5)	Homogalacturonan with a high DE (mAb JIM 7)	Partly methylesterified homogalacturonan (mAb LM18)	Partly methylesterified homogalacturonan (mAb LM19)	Partly methylesterified homogalacturonan (mAb LM20)	Ca cross-linked homogalacturonan (mAb 2F4)	Xylogalacturonan (mAb LM8)	Backbone of rhamnogalacturonan I (mAb INRA-R1)	Backbone of rhamnogalacturonan I (mAb INRA-R2)	(1→4)-β-D-galactan (mAb LM5)	Feruloylated (1→4)-β-D-galactan (mAb LM9)	Linearised (1→5)-α-L-arabinan (mAb LM13)	(1→4)-β-D-mannan (mAb LM21)	(1→4)-β-D-mannan/galactomannan (mAb LM22)	(1→3)-β-D-glucan (mAb BS-400-2)	Xyloglucan (XXXG motif) (mAb LM15)	Xyloglucan (mAb LM25)	(1→4)-β-D-xylan (mAb LM10)	(1→4)-β-D-xylan/arabinoxylan (mAb LM11)	Cellulose (mAb CBM3A)	Extensin (mAb LM1)	Extensin (mAb JIM11)	Extensin (mAb JIM20)	AGP (mAb LM8)	AGP (mAb JIM13)	AGP (mAb JIM14)	AGP, β-linked GlcA (mAb LM2)
CDTA	LP	Crush_P	73	29	22	25	31	8	0	15	50	0	0	0	0	0	0	0	0	0	5	0	0	0	6	9	0	0
CDTA	LP	Sc_P	67	31	26	29	35	9	0	19	59	0	0	0	0	0	0	0	0	0	5	0	0	0	5	8	0	0
CDTA	LP	Press_P	77	30	24	28	31	8	0	17	54	0	0	0	0	0	0	0	0	0	0	0	0	0	6	12	0	0
CDTA	LP	ScPress_P	68	29	25	30	33	9	0	19	58	0	0	0	0	0	0	0	0	0	5	0	0	0	0	8	0	0
CDTA	HP	Crush_P	73	28	25	27	34	0	0	17	42	0	0	0	0	0	0	0	0	0	5	0	0	0	6	10	0	0
CDTA	HP	Sc_P	67	27	26	28	34	0	0	17	43	0	0	0	0	0	0	0	0	0	8	0	0	0	0	7	0	0
CDTA	HP	Press_P	75	29	25	29	34	0	0	17	44	0	0	0	0	0	0	0	0	0	5	0	0	0	5	8	0	0
CDTA	HP	ScPress_P	70	27	27	29	34	0	0	17	46	0	0	0	0	0	0	0	0	0	8	0	0	0	0	7	0	0
NaOH	LP	Crush_P	0	0	0	0	0	0	0	0	10	7	0	0	0	0	0	74	66	0	0	0	0	0	8	10	0	0
NaOH	LP	Sc_P	0	0	0	0	0	0	0	0	7	6	0	0	0	0	0	78	68	0	0	0	0	0	9	10	0	0
NaOH	LP	Press_P	0	0	0	0	0	0	0	0	7	7	0	0	0	0	0	75	67	0	0	0	0	0	8	10	0	0
NaOH	LP	ScPress_P	0	0	0	0	0	0	0	0	7	7	0	0	0	0	0	78	70	0	0	0	0	0	8	9	0	0
NaOH	HP	Crush_P	0	0	0	0	0	0	0	0	11	14	0	0	0	0	6	79	88	0	0	0	7	6	0	5	9	0	0
NaOH	HP	Sc_P	0	0	0	0	0	0	0	10	25	13	0	11	0	0	10	58	64	8	0	0	26	11	6	10	22	0	0
NaOH	HP	Press_P	0	0	0	0	0	0	0	0	9	14	0	0	0	0	6	81	90	0	0	0	8	6	0	5	10	0	0
NaOH	HP	ScPress_P	0	0	0	0	0	0	0	10	27	13	0	11	0	0	9	59	68	7	0	0	25	10	6	13	22	0	0

Red blocks indicate significant differences for the epitopes signals between samples.

5.3.4. Juice and wine total protein quantification

Statistically significant decreases in protein concentrations were observed throughout the juice processing steps (comparing free run with clarified juice) for all the juices (Figure 5.6). Skin contact led to a slight increase in the total protein concentration of pressed high phenolic potential juice (comparing standard pressed and skin contact pressed), but this difference did not persist after clarification occurred. Similarly, there was a slight, but not consistent difference between the protein concentrations of the low and high phenolic potential juices after pressing (no skin contact).

Stellenbosch University https://scholar.sun.ac.za

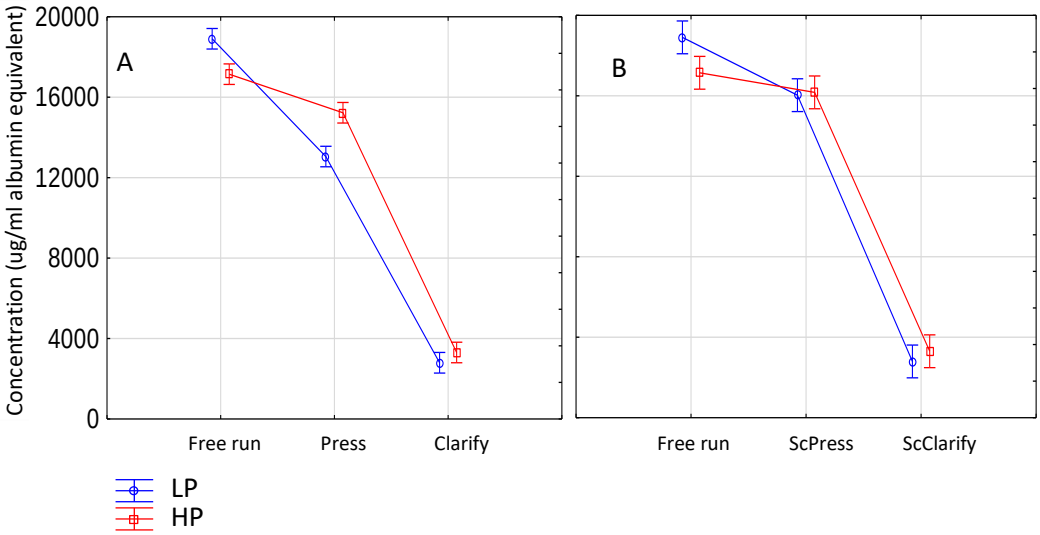


Figure 5.6: The total protein concentration extracted from low phenolic potential (LP) and high phenolic potential (LP) juices during standard juice processing or with skin contact (Sc). (A) standard and (B) skin contact (Sc).

When considering the protein contents of the wines (Supplementary Table S5.2 and Figure 5.7), the data showed that implementation of skin contact and sediment contact led to a decrease in total protein concentration, compared to the levels in the respective standard wines (Figure 5.7). Furthermore, the combination of skin and sediment contact (ScSed) synergistically decreased total wine protein concentration compared to the individual effects of these treatments. The phenolic potentials of the grapes that were used to produce the wines (LP versus HP) did not appear to have a statistically significant effect on total wine protein concentration.

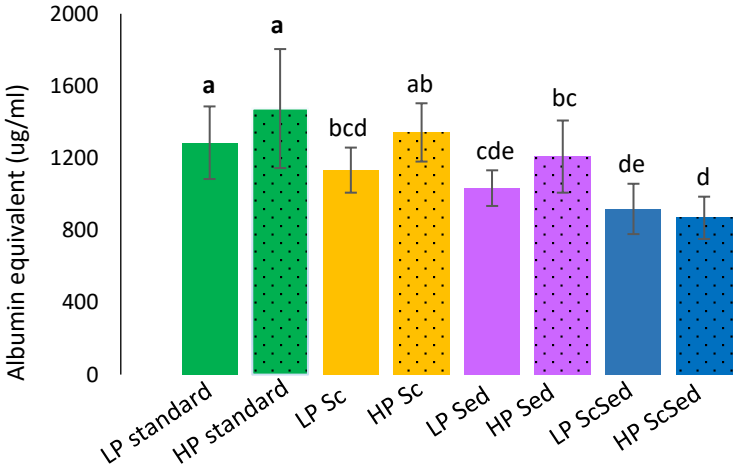


Figure 5.7: The total protein concentration of low phenolic potential (LP) and high phenolic potential (HP) wines after cold stabilization. Skin contact (Sc), sediment contact (Sed) and skin contact and sediment contact (ScSed).

Stellenbosch University https://scholar.sun.ac.za

5.4. Discussion

During winemaking waste is generated after pressing (pomace) and enzyme clarification (sediment) of the juice. These matrices have been shown to have phenolic value (Kammerer et al. 2004; Fang et al. 2006; Rossello and Teissedre 2013) and can therefore be considered as a loss of the phenolic potential of the grapes. Proteins and cell wall material have also been shown to decrease the phenolic content of wine through interaction (Bindon et al. 2010b; Vincenzi et al. 2011; Tian 2014; Bindon et al. 2016).

5.4.1. Residual polyphenols found in pomace and juice sediment

The residual polyphenols within the pomace, sampled during processing, were quantified to determine the fraction that was lost/unused within the pomace when skin contact is not applied. Similarly, the phenolic composition of the sediment was determined to quantify the amount that is discarded when the juice is racked (separated from the sediment that settles at the bottom of the container) before fermentation. PCA analysis (Figure 5.1) revealed that the main driver behind changes in polyphenol concentration of the pomace was processing: crushing, skin contact (Sc) and pressing. A similar pattern was observed in both low and high phenolic potential juice (chapter 4, Figure 4.1). It would be expected that the pomace and its corresponding juice fractions would have opposite concentrations of polyphenols, since the extraction of these compounds typically increase with juice processing, e.g. skin contact (Gomez-Miguez et al. 2007; Hernanz et al. 2007; Maggu et al. 2007) and pressing (Darias-Martín et al. 2004; Patel et al. 2010; Ferreira-lima et al. 2016). However, our juice data revealed a decrease in total polyphenols (chapter 4, Table 4.1) of the juice during juice processing which indicated that polyphenols were lost from the juice matrix after being extracted from the grapes. Furthermore, certain polyphenols (e.g. flavan-3-ol_1_, 2 and _3 and flavonols) did not “move” from the whole berry into the juice or the wine. Initially it was presumed that they were poorly extracted from the berry tissues and were either below the limits of detection, or completely absent in the subsequent matrices (chapter 4). Some of those polyphenols, like the flavan-3-ols, originate from the seeds and it is possible that during crushing, skin contact and pressing, the conditions (no alcohol and low temperature) were not conducive for polyphenol extraction from the seeds (González-Manzano et al. 2004; Romero-Cascales et al. 2005). However, since we did not find any flavan-3-ols in the pomace or the sediment samples, we propose that the more likely explanation could be that they were indeed extracted, but probably polymerized (accumulating either in the wine or sediment as polymeric polyphenols).

The flavonols (glycosylated quercetin) on the other hand, were detected in the pomace (lower concentrations than what was found in fresh skin, Table 3.2) and sediment (only quercetin-glucoside) and could also have been incorporated in polymeric forms. In contrast to our results, juice and white wines have been reported to contain flavonols in its aglycone (free) and glycosylated (bound) forms. Flavonol aglycone concentration increased during winemaking and storing due to the low pH of wine initiating hydrolysis of the

Stellenbosch University https://scholar.sun.ac.za

glycosylated forms (Recamales et al. 2006; Hernanz et al. 2007; Cejudo-Bastante et al. 2011; Ferreira-lima et al. 2016).

The hydroxycinnamic acids were all present in the juice fractions and thus extraction can partially account for their decrease from fresh grapes to crushed pomace, but we did not see a corresponding increase in these compounds when comparing free run with pressed juice (with and without skin contact, Table 4.1). Caftaric acid, caffeic acid and coutaric acid were all found at lower concentrations within the pomace after crushing compared to the berry skins and pulp at harvest (chapter 3, supplementary Table 3.1) and decreased further going from crushed pomace to pressed pomace (standard and with skin contact) (Figure 5.2). Similar observations were made for the flavonoids as catechin, epicatechin, quercetin-glucoside, quercetin-galactoside and flavonol_4 concentrations were lower in the pomace samples after crushing and skin contact, compared to berry skins and pulp at harvest. The increase in residual flavonoid concentrations in the pomace from crushing to pressing, together with their corresponding concentration decrease in juice may potentially indicate that these compounds were extracted into the juice, but then bound to the cell wall material through the formation of hydrogen bonds. The "bonding effect" predominantly affects flavonoids (specifically the polymeric flavonoids) and not phenolic acids (Bindon et al. 2010b; Bindon et al. 2010a; Bautista-Ortín et al. 2015) and has been previously implicated in the decrease of the polyphenol concentration of juice during processing (Bindon et al. 2010b; Hanlin et al. 2010). Literature appears to lack information on the participation of HCA in polyphenol polymerization, or interaction with cell wall material. Hydroxybenzoic acids, which are found at low levels in *Vitis vinifera* berries are the only phenolic acids which have been reported to participate in polyphenol polymerization, where the latter has the ability to bind to cell wall material (Waterhouse 2002; Garrido and Borges 2013). Epicatechin was not detected within the pomace but an increase in polymeric polyphenols (Table 5.1) was observed after skin contact and pressing (standard and with skin contact). It is tempting to speculate that epicatechin may have polymerized during juice processing and bound to pomace cell wall polymers (Garrido and Borges 2013).

The small differences between the crushed pomace coming from high and low phenolic potential grapes in terms of caffeic acid and coutaric acid was again seen in the pomace after pressing, irrespective of skin contact (Figure 5.2). There was a 45% difference in the total polyphenols between low and high phenolic potential fresh grapes (chapter 3, Table 3.1), but this difference decreased to 1% (standard) and 2% (skin contact) in the pressed pomace which suggests that similar quantities of polyphenols were extracted from the respective skins and pulps. The noticeable differences observed between low and high phenolic potential residual quercetin-glucoside, quercetin-galactoside and flavonol_4 concentration (Figure 5.3) were expected as the HL microclimate caused a 4.4 $\log_2$ fold increase in flavonol concentration (chapter 3, Table 3.1).

The juice sediment provides additional information on changes in polyphenol concentration during juice processing and alcoholic fermentation. As with all the grape, juice and wine matrices characterized within this and the other chapters, no compositional changes were observed between the sediments coming from

Stellenbosch University https://scholar.sun.ac.za

differentially treated grapes (low and high phenolic potential) and juices (low and high phenolic potential, skin contact, sediment contact and skin and sediment contact). However, sediments differed in terms of the concentrations of the individual phenolic compounds (Table 5.2).

The polyphenol concentration of the sediment appeared to be influenced by the phenolic potential of the grapes it was derived from and by the skin contact treatment as both these factors led to increased concentrations (Figure 5.5). The lower catechin concentration in the sediment derived from skin contact treated samples can potentially be explained by multiple factors including binding to insoluble cell wall polymers, polymerization or even oxidation to an extent (Singleton et al. 1985; Cheynier et al. 1989; Maggu et al. 2007; Ferreira-lima et al. 2016). Oxidation may also explain the decrease in caftaric acid concentration with skin contact, but is somewhat questionable as none of the other hydroxycinnamic acids decreased. The sediment of bayberry and Muscat juice has previously been described to contain some flavonols and hydroxybenzoic acids which to a certain extent correlates with the results reported in this study (Lee and Talcott 2002; Fang et al. 2006).

A large quantity of the fresh berry tissue polyphenols appeared to have settled to form the sediment, given its total polyphenol concentration (supplementary Table 5.5) being comparable to that of the whole berries (chapter 3, Table 3.1). As the alcohol increases during fermentation, an extraction of polyphenols from the sediment might be possible (González-Manzano et al. 2004; Su et al. 2014). Unfortunately, we did not measure the polyphenols of the sediment after alcoholic fermentation, but when comparing the difference in total polyphenols between clarified juice and wine we see that the largest increase can be seen (Supplementary Table S5.1) for wine that was fermented in contact with the sediment.

5.4.2. Potential interaction between polyphenols, cell wall polymers and proteins

Grape cell wall deconstruction has been shown to enhance polyphenol extraction (Chamorro et al. 2012). Therefore, the cell wall composition of the skin, pulp and pomace (all stages of juice processing) were determined to investigate cell wall compositional changes that occur within the pomace structure during juice processing in an effort to link polyphenol extraction with cell wall deconstruction. The effect of a HL microclimate on cell wall composition was evaluated to determine whether compositional changes would occur and whether these changes would facilitate polyphenol extraction. Interestingly, a HL microclimate did not lead to many compositional changes in the cell wall of the skin and pulp of Sauvignon Blanc grapes (Table 5.3). However, the lower extractability of xyloglucan and RGI that were observed for high phenolic potential grapes compared to low phenolic potential grapes might correspond with the firmness and low extractability of RGI previously reported in pears exposed to sunlight (Raffo et al. 2011). The effect of a HL microclimate on the cell wall composition of ripe grapes is not known yet. It is

Stellenbosch University https://scholar.sun.ac.za

therefore also unknown whether a HL microclimate induces other compositional changes which cannot be detected with the methods used in this study (CoMPP and FT-MIR). A cell wall model for grape pomace was proposed by Gao (2016) which correlated with the skin and pulp compositions reported in this study. CoMPP results revealed that low and high phenolic potential skins had the highest xyloglucan signal, whereas the pulp had the highest rhamnogalacturonan signals. The increased extraction of RGI from pulp cell walls were also observed previously (personal communication with Dr Anscha Zietsman, Stellenbosch University) and might confirm the report by Nunan and co-workers (1998) that described pulp cell walls as thinner and more fragile than skin cell walls. Although not directly comparable, FT-IR analysis somewhat contradicts the CoMPP results as low and high phenolic potential pulp appeared to be richer in pectin, xyloglucan and cellulose. However, the penetration of FT-MIR (Supplementary figure S5.1) might have been less optimal with the more condensed composition of the skin cell walls and may have led to lower signals within these regions (Zietsman et al. 2015a; Gao 2016). Skin contact caused changes in many epitope signals for both the low and high phenolic potential pomace cell walls (compared to crushed pomace cell walls). These changes might indicate a weakened or more porous cell wall structure, which might cause the release or the encapsulation of polyphenols respectively. With the available data at present, we are unable to link these changes to the polyphenol extraction or retention.

The protein concentrations of the juice and wine were determined to correlate how protein-polyphenol interaction could potentially affect polyphenol availability. Juice proteins decreased in concentration (Figure 5.6) as the processing progresses and this decrease was similar to the decrease of some polyphenols, (chapter 4) such as catechin and caftaric acid during juice processing. The largest decrease in protein concentration, which occurred between pressing and clarification, also coincided with the largest decrease in polyphenols. Vincenzi et al. (2011) reported a ±15 % decrease in total protein concentration after pectinase treatment which was much lower than the 93% (standard) and 79-81% (skin contact) decrease for low and high phenolic potential juice seen within this study. The hydroxyl groups on the phenyl rings of polyphenols (Fulcrand et al. 2006) allow binding to grape proteins which can result in the formation of haze aggregates and sediments (Hanlin et al. 2010; Van Sluyter et al. 2015). This interaction, along with polyphenol adsorption to cell wall material, may contribute towards the decrease in polyphenol concentration during juice processing (Bindon et al. 2010b; Vincenzi et al. 2011). It should also be acknowledged that other compounds, to which polyphenols can interact with, are extracted during juice processing which may also influence their levels. Direct interaction between proteins and polyphenols was not investigated in this study and their interaction can therefore only be proposed based on trends in the data and what has been reported in literature. Esteruelas et al. (2011) found hydroxycinnamic acids, flavan-3-ols and flavonols bound to proteins in haze collected from commercial Sauvignon Blanc wines, which confirmed the interaction between these compounds in wine. Tian et al. (2017) reported total protein and total polyphenol content to be indirectly

Stellenbosch University https://scholar.sun.ac.za

proportional in Sauvignon Blanc juice and wine which suggested a complexing relationship and could provide an explanation for the decrease of both compounds within the juice in this study. Neither high phenolic potential of juice or skin contact appeared to affect juice protein concentration since all clarified juices had comparable protein content. Skin contact did however increase the protein content of the pressed juice which correlated to the results of Ough (1969).

Total protein concentration was higher in the standard wines and lower in the skin contact wine compared to their corresponding clarified juices (Supplementary Table S5.2). This result was not surprising for either of the wines and their respective juices given the fact that protein increases (Fukui and Yokotsuka 2003) and decreases (Dizy and Bisson 2000; Vincenzi et al. 2011) have been reported during alcoholic fermentation. Wines made with sediment contact during fermentation had the lowest protein concentration compared to the standard wine (Figure 5.7) and concentrations were further decreased when applied in combination with skin contact (ScSed). Analysis of the sediment and wine polyphenol composition revealed that the sediment was rich in catechin and polymeric polyphenols whereas catechin was the most abundant in wine (chapter 4). Similar to what has previously been reported, the high flavan-3-ol concentration of these wines, coupled with their low protein concentration suggested that binding of the two occurred during alcoholic fermentation which may have extended into cold stabilization (Esteruelas et al. 2011; Vincenzi et al. 2011; Bindon and Smith 2013; Bindon et al. 2016). Flavonoid and protein interaction has been well studied and it is known that polyphenols actively contribute towards haze formation in white wine (Van Sluyter et al. 2015). After cold stabilization, wines which had sediment contact were significantly clearer compared to standard wines suggesting that the sediment, to an extent, exerted a fining effect (data not shown). Insoluble grape cell wall material and polymeric flavan-3-ols, in the juice sediment, may have bound to haze forming proteins during fermentation and settled in the lees-sediment mixture during cold stabilization of the wine.

5.5. Conclusions

Polyphenols were lost/trapped in grape pomace, regardless whether skin contact was used to maximize extraction from the skin. However, skin contact was confirmed effective to utilise more of the phenolic potential from the grapes compared to the standard wine making practice, as expected. This was supported by compositional analysis of the pomace cell walls which indicated that deconstruction occurred to a certain extent. The majority of the phenolic potential of the juices were lost in the sediments after enzyme clarification which was a novel result as this has, to our knowledge, not been reported previously. The enhanced polyphenol content of the sediment after skin contact further confirmed the effectiveness of this winemaking practice at enhancing polyphenol extraction (also seen in the higher polyphenol levels in pressed and clarified juice that were subjected to skin contact). The protein content of the juice and wine did not appear to have an effect on polyphenol availability. Rather, an inverse relationship was observed between protein and polyphenol content. The results generated in this study provide novel insight into the fraction of

Stellenbosch University https://scholar.sun.ac.za

polyphenols that were not distributed from the grapes to the juice and the extracted fraction that was lost during juice processing.

5.6. References

Arnous A, Meyer AS (2009) Quantitative prediction of cell wall polysaccharide composition in grape (vitis vinifera l.) and apple (malus domestica) skins from acid hydrolysis monosaccharide profiles. J Agric Food Chem 57:3611–3619. doi: 10.1021/jf900780r

Bautista-Ortín AB, Ruiz-García Y, Marín F, Molero N, Apolinar-Valiente R, Gómez-Plaza E (2015) Remarkable proanthocyanidin adsorption properties of monastrell pomace cell wall material highlight its potential use as an alternative fining agent in red wine production. J Agric Food Chem 63:620–633. doi: 10.1021/jf503659y

Bindon KA, Li S, Kassara S, Smith PA (2016) Retention of Proanthocyanidin in Wine-like Solution Is Conferred by a Dynamic Interaction between Soluble and Insoluble Grape Cell Wall Components. J Agric Food Chem 64:8406–8419. doi: 10.1021/acs.jafc.6b02900

Bindon KA, Smith PA (2013) Comparison of the affinity and selectivity of insoluble fibres and commercial proteins for wine proanthocyanidins. Food Chem 136:917–928. doi: 10.1016/j.foodchem.2012.08.016

Bindon KA, Smith PA, Holt H, Kennedy JA (2010a) Interaction between Grape-Derived Proanthocyanidins and Cell Wall Material . 2 . Implications for Vinification. J Agric Food Chem 10736–10746. doi: 10.1021/jf1022274

Bindon KA, Smith PA, Kennedy JA (2010b) Interaction between Grape-Derived Proanthocyanidins and Cell Wall Material . 1 . Effect on Proanthocyanidin Composition and Molecular Mass. J Agric Food Chem 2520–2528. doi: 10.1021/jf9037453

Capek P, Sasinkova V, Wellner N, Ebringerova A, Kac M (2000) FT-IR study of plant cell wall model compounds : pectic polysaccharides and hemicelluloses. 43:195–203.

Cejudo-Bastante MJ, Hermosín-Gutierrez I, Castro-Vasquez LI, Perez-Coello M (2011) Hyperoxygenation and Bottle Storage of Chardonnay White Wines : Effects on Color-Related Phenolics , Volatile Composition , and Sensory Characteristics. J Agric Food Chem 4171–4182. doi: 10.1021/jf104744q

Chamorro S, Viveros A, Alvarez I, Vega E, Brenes A (2012) Changes in polyphenol and polysaccharide content of grape seed extract and grape pomace after enzymatic treatment. Food Chem 133:308–314. doi: 10.1016/j.foodchem.2012.01.031

Cheynier V, Rigaud J, Souquet J-MM, Barillere JM, Moutounet M (1989) Effect of Pomace Contact and Hyperoxidation on the Phenolic Composition and Quality of Grenache and Chardonnay Wines. Am J Enol Vitic 40:36–42.

Coelho C, Julien P, Nikolantonaki M, Noret L, Magne M, Ballester J, Gougeon RD (2018) Molecular and Macromolecular Changes in Bottle-Aged White Wines Reflect Oxidative Evolution – Impact of Must Clarification and Bottle Closure. Front Chem 6:1–9. doi: 10.3389/fchem.2018.00095

Darias-Martín JJ, Diaz-Gonzalez D, Diaz-romero C (2004) Influence of two pressing processes on the quality of must in white wine production. J Food Eng 63:335–340. doi: 10.1016/j.jfoodeng.2003.08.005

Dizy M, Bisson L (2000) Proteolytic Activity of Yeast Strains During Grape Juice Fermentation. Am J Enol Vitic 155–167.

Downey MO, Harvey JS, Robinson SP (2003) Analysis of tannins in seeds and skins of Shiraz grapes throughout berry development. Aust J Grape Wine Res 15–27.

Stellenbosch University https://scholar.sun.ac.za

Du Plessis K (2017) The evaluation of the impact of microclimatic factors on grapevine berries in a vineyard setting through molecular profiling. Thesis, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

Esteruelas M, Kontoudakis N, Gil M, Fort MF, Canals JM, Zamora F (2011) Phenolic compounds present in natural haze protein of Sauvignon white wine. FRIN 44:77–83. doi: 10.1016/j.foodres.2010.11.010

Fang Z, Zhang M, Tao G, Sun Y, Sun J (2006) Chemical composition of clarified bayberry (Myrica rubra Sieb, et Zucc.) juice sediment. J Agric Food Chem 54:7710–7716. doi: 10.1021/jf0618980

Ferreira-lima NE, Burin VM, Caliari V (2016) Impact of Pressing Conditions on the Phenolic Composition , Radical Scavenging Activity and Glutathione Content of Brazilian Vitis vinifera White Wines and Evolution During Bottle Ageing. Food Bioprocess Technol 944–957. doi: 10.1007/s11947-016-1680-7

Fournand D, Vicens A, Sifhoum L, Souquet J, Moutounet M, Cheynier V (2006) Accumulation and Extractability of Grape Skin Tannins and Anthocyanins at Different Advanced Physiological Stages. J Agric Food Chem. doi: 10.1021/jf061467h

Fukui M, Yokotsuka K (2003) Content and Origin of Protein in White and Red Wines : Am J Enol Vitic 3:178–188.

Fulcrand H, Salas E, Cheynier V (2006) Phenolic Reactions during Winemaking and Aging. 3:289–297.

Gao Y (2016) Parameters involved in the enzymatic deconstruction of the Wine Grape cell wall matrix during winemaking.

Garrido J, Borges F (2013) Wine and grape polyphenols - A chemical perspective. Food Res Int 54:1844–1858. doi: 10.1016/j.foodres.2013.08.002

Gazzola D, Vincenzi S, Pasini G, Lomolino G, Curioni A (2015) Advantages of the KDS / BCA Assay over the Bradford Assay for Protein Quantification in White Wine and Grape Juice. 2:227–233. doi: 10.5344/ajev.2014.14076

Gomez-Miguez JM, Gonzalez-Miret LM, Hernanz D, Fernandez AM, Vicario IM, Heredia FJ (2007) Effects of prefermentative skin contact conditions on colour and phenolic content of white wines. J Food Eng 78:238–245. doi: 10.1016/j.jfoodeng.2005.09.021

González-Manzano S, Rivas-Gonzalo JC, Santos-Buelga C (2004) Extraction of flavan-3-ols from grape seed and skin into wine using simulated maceration. Anal Chim Acta 513:283–289. doi: 10.1016/j.aca.2003.10.019

Hanlin RL, Hrmova M, Harbertson JF, Downey MO (2010) Review: Condensed tannin and grape cell wall interactions and their impact on tannin extractability into wine. Aust J Grape Wine Res 16:173–188. doi: 10.1111/j.1755-0238.2009.00068.x

Hernanz D, Recamales ÁF, González-Miret ML, Gómez-Míguez MJ, Vicario IM, Heredia FJ (2007) Phenolic composition of white wines with a prefermentative maceration at experimental and industrial scale. J Food Eng 80:327–335. doi: 10.1016/j.jfoodeng.2006.06.006

Kammerer D, CLaus A, Carle R, Schieber A (2004) Polyphenol Screening of Pomace from Red and White Grape Varieties (Vitis vinifera L .) by HPLC-DAD-MS / MS. J Agric Food Chem 52:27–30.

Kammerer D, Claus A, Schieber A, Carle R (2005) A Novel Process for the Recovery of Polyphenols from Grape (Vitis vinifera L .) Pomace.

Lecas M, Brillouet J-M (1994) Cell wall compostion of grape berry skins. 35:1241–1243.

Lee J-H, Talcott ST (2002) Ellagic Acid and Ellagitannins Affect on Sedimentation in Muscadine Juice and

Stellenbosch University https://scholar.sun.ac.za

Wine. 3971–3976. doi: 10.1021/jf011587j

Liu J., Gallander JF, Wilker KL (1987) Effect of Juice Clarification on the Composition and Quality of Eastern US Table Wines. Am J Enol Vitic 38:147–150.

Maggu M, Winz R, Kilmartin PA, Trought MCT, Trought M, Nicolau L (2007) Effect of Skin Contact and Pressure on the Composition of Sauvignon Blanc Must. J Agric Food Chem 55:10281–10288.

Nunan K, Sims I, Bacic A (1998) Changes in cell wall composition during ripening of grape berries. Plant Physiol 118:783–792.

Ortega-Regules A, Romero-Cascales I, Ros-Garcia J., Lopez-Roca J, Gomez-Plaza E (2006) A first approach towards the relationship between grape skin cell-wall composition and anthocyanin extractability. 563:26–32. doi: 10.1016/j.aca.2005.12.024

Ough CS (1969) Substances Extracted during Skin Contact with White Musts. I. General Wine Composition and Quality Changes with Contact Time. Am J Enol Vitic 20:93–100.

Patel P, Herbst-Johnstone M, Lee SA, Gardner RC, Weaver R, Nicolau L, Kilmartin PA (2010) Influence of Juice Pressing Conditions on Polyphenols , Antioxidants , and Varietal Aroma of Sauvignon blanc Microferments. Agric Food Chem 58:7280–7288. doi: 10.1021/jf100200e

Pinelo M, Arnous A, Meyer AS (2006) Upgrading of grape skins : Significance of structural components and extraction techniques for phenol release. doi: 10.1016/j.tifs.2006.05.003

Raffo MD, Ponce NMA, Sozzi XGO, Vicente XAR, Stortz CA (2011) Compositional Changes in ‘ Bartlett ’ Pear (Pyrus communis L .) Cell Wall Polysaccharides As Affected by Sunlight Conditions. J Agric Food Chem 12155–12162.

Recamales F, Sayago A, Gonza ML, Hernanz D (2006) The effect of time and storage conditions on the phenolic composition and colour of white wine. 39:220–229. doi: 10.1016/j.foodres.2005.07.009

Romero-Cascales I, Ortega-Regules A, López-Roca JM, Fernández-Fernández JI, Gómez-Plaza E (2005) Differences in anthocyanin extractability from grapes to wines according to variety. Am J Enol Vitic 56:212–219.

Romero-Cascales I, Ros-Garcia J., Lopez-Roca J., Gomez-Plaza E (2012) The effect of a commercial pectolytic enzyme on grape skin cell wall degradation and colour evolution during the maceration process. Food Chem 130:626–631. doi: 10.1016/j.foodchem.2011.07.091

Rossello C, Teissedre P (2013) Characterization of Polyphenols and Antioxidant Potential of White Grape Pomace Byproducts (Vitis vinifera L.). doi: 10.1021/jf403168k

Singleton VL, Salgues M, Zaya J, Trousdale E (1985) Caftaric Acid Disappearance and Conversion to Products of Enzymic Oxidation in Grape Must and Wine. Am J Enol Vitic 36:50–56.

Su D, Zhang R, Hou F, Zhang M, Guo J, Huang F, Deng Y (2014) Comparison of the free and bound phenolic profiles and cellular antioxidant activities of litchi pulp extracts from different solvents. 1–10.

Szymanska-chargot M, Zdunek A (2013) Use of FT-IR Spectra and PCA to the Bulk Characterization of Cell Wall Residues of Fruits and Vegetables Along a Fraction Process. 29–42. doi: 10.1007/s11483-012-9279-7

Tapre AR, Jain RK (2014) Mini Review Pectinases : Enzymes for fruit processing industry. Int Food Res 21:447–453.

Tian B (2014) Pathogenesis-related proteins in Sauvignon Blanc grapes and the influence of their extraction on resultant juice composition and wine protein stability. Thesis, University of Auckland, New Zealand.

Stellenbosch University https://scholar.sun.ac.za

Tian B, Harrison R, Morton J, Jaspers M, Hodge S, Grose C, Trought M (2017) Extraction of Pathogenesis-Related Proteins and Phenolics in Sauvignon Blanc as Affected by Grape Harvesting and Processing Conditions. Molecules. doi: 10.3390/molecules22071164

van Jaarsveld FP Van, Blom M, Hattingh S, Marais J (2005) Effect of Juice Turbidity and Yeast Lees Content on Brandy Base Wine and Unmatured Pot-still Brandy Quality. South African J Enol Vitic 26:116–130.

Van Sluyter SC, McRae JM, Falconer RJ, Smith PA, Bacic A, Waters EJ, Marangon M (2015) Wine protein haze: Mechanisms of formation and advances in prevention. J Agric Food Chem 63:4020–4030. doi: 10.1021/acs.jafc.5b00047

Vidal S, Williams P, Neill MAO, Pellerin P (2001) Polysaccharides from grape berry cell walls . Part I : tissue distribution and structural characterization of the pectic polysaccharides.

Vincenzi S, Marangon M, Tolin S, Curioni A (2011) Protein evolution during the early stages of white winemaking and its relations with wine stability. 20–27. doi: 10.1111/j.1755-0238.2010.00113.x

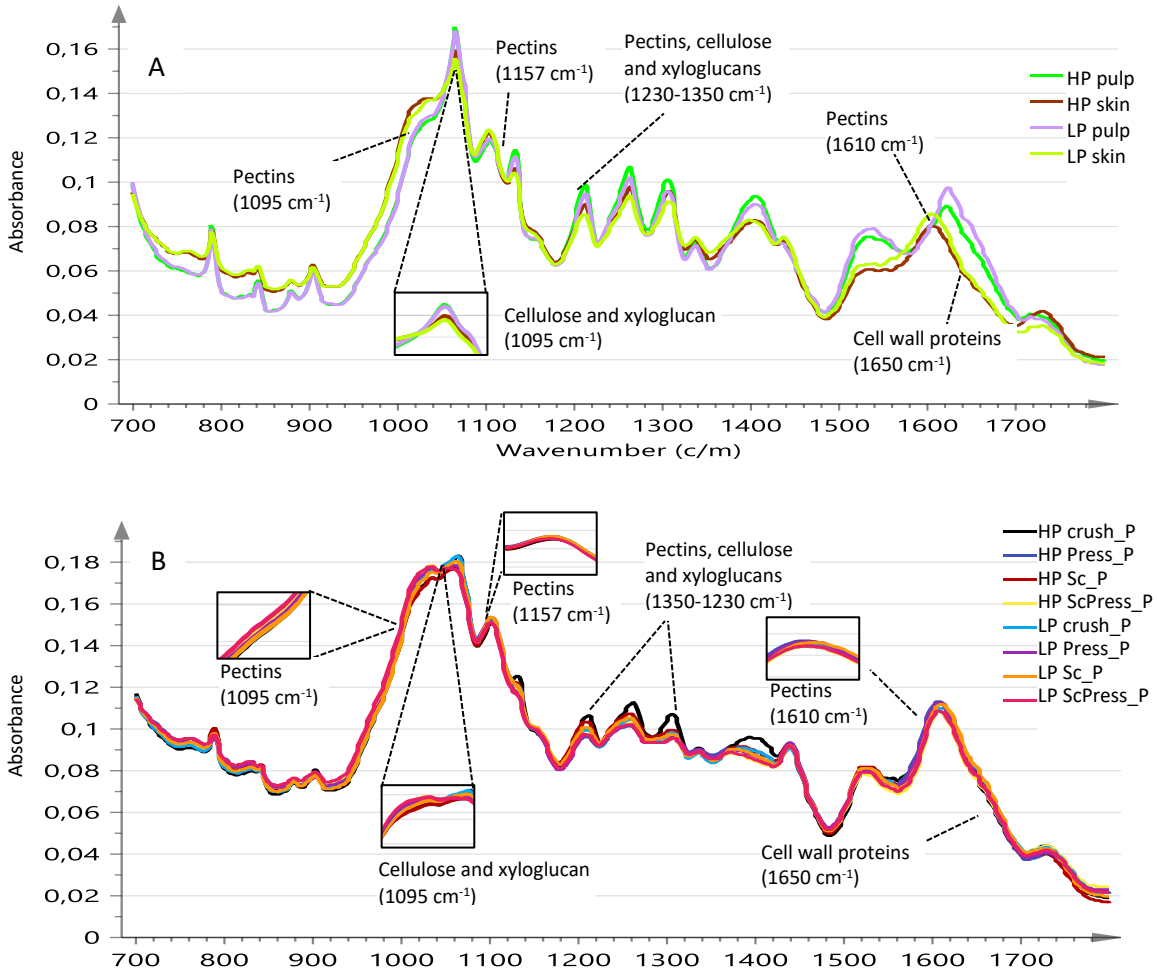
Waterhouse AL (2002) Wine Phenolics. Ann New York Acad Sci 36:21–36.

Zietsman AJJ, Moore JP, Fangel JU, Willats WGT, Trygg J, Vivier MA (2015a) Following the Compositional Changes of Fresh Grape Skin Cell Walls during the Fermentation Process in the Presence and Absence of Maceration Enzymes. doi: 10.1021/jf505200m

Zietsman AJJ, Moore JP, Fangel JU, Willats WGT, Vivier MA (2015b) Profiling the Hydrolysis of Isolated Grape Berry Skin Cell Walls by. doi: 10.1021/acs.jafc.5b02847

Stellenbosch University https://scholar.sun.ac.za

Supplementary data to Chapter 5



Supplementary Figure S5.1: FT-MIR spectra of low phenolic potential and high phenolic potential skin and pulp (A) cell walls of fresh grapes at harvest and pomace (B) at each stage of standard juice processing (crush or press), or with skin contact (Sc). Abbreviation: P (pomace)

Supplementary Table S5.1: A comparison of the total polyphenols of clarified juice and wine (mg/L).

	Standard juice and wine		Skin contact juice and wine		Sediment contact juice and wine	
	LP	**HP**	**LP**	**HP**	**LP**	**HP**
Juice	53.3	64.5	74.4	93.6	53.3	64.5
Wine	93.7	104.0	112.2	148.1	117.2	148.5
% increase	76	62	51	58	120	130

Abbreviations: LP (Low phenolic potential), HP (High phenolic potential)

Stellenbosch University https://scholar.sun.ac.za

Supplementary Table S5.2: The total protein concentration of the juices during processing and resulting wine.

	Concentration (albumin equivalent mg/L)
Juice	
LP Free run	18907.35 ± 1948.48 a
HP Free run	17146.17 ± 1278.88 ab
LP Press	13049.76 ± 1290.57 b
HP Press	15228.84 ± 1221.12 c
LP ScPress	16032.51 ± 2553.11 b
HP ScPress	16172.58 ± 3342.17 b
LP Clarify	909.77 ± 762.54 e
HP Clarify	1038.36 ± 457.18 e
LP ScClarify	2923.08 ± 927.60 e
HP ScClarify	3307.00 ± 152.34 d
Wine	
LP standard	1285.32 ± 201.22 ab
HP standard	1474.53 ± 329.82 a
LP Sc	1133.31 ± 124.93 bcd
HP Sc	1342.38 ± 161.96 ab
LP ScSed	918.27 ± 139.54 cde
HP ScSed	869.02 ± 117.16 bc
LP Sed	1033.54 ± 98.72 de
HP Sed	1208.40 ± 263.62 e

Abbreviations: Low phenolic potential (LP), high phenolic potential (HP), skin contact and pressed (ScPress, juice), skin contact and clarified (ScClarify, juice), skin contact (Sc, wine), sediment contact (Sed), skin contact and sediment contact (ScSed).

Different letters indicate statistical significance (One-Way ANOVA with Fisher's LSD post hoc test, $p<0.05$)

Stellenbosch University https://scholar.sun.ac.za

Chapter 6

General discussion and conclusions

Stellenbosch University https://scholar.sun.ac.za

Chapter 6- General discussion and conclusions

6.1. General discussion and conclusions

The polyphenol composition and content of grapes (Rodríguez Montealegre et al. 2006; Di Lecce et al. 2014; Dragana et al. 2016), juice (Maggu et al. 2007; Coetzee et al. 2013; Fracassetti et al. 2013) and wine (Gomez-Miguez et al. 2007; Cejudo-Bastante et al. 2011a; Ferreira-lima et al. 2016) of white cultivars, including Sauvignon Blanc, has been well established in literature. Manipulation of certain aspects of grape production and winemaking, e.g. the intensity of the light that reaches the grape bunches (microclimate manipulation) or contact time between the skin and juice, is well known to increase the polyphenol content of white grapes (Gregan et al. 2011; Šebela et al. 2017), juice and wine (Hernanz et al. 2007; Cejudo-Bastante et al. 2011a; Aleixandre-Tudo et al. 2015). Studying the effect of a microclimate treatment is often concluded at the grape matrix and few studies have focused on whether the induced changes on grape phenolic content persists in the derived juice (Honeth 2018) and wine (Ristic et al. 2006). This study aimed to determine the phenolic potential of Sauvignon Blanc grape tissues, as influenced by a high light (HL) microclimate (and in contrast to a low light, LL microclimate); how this potential affects the transitioning of polyphenols to the resulting juice and wine during wine making and how much of this potential remains in the winery waste matrices. The phenolic potential refers to the sum and variety of free and bound grape polyphenols that are available for extraction under wine making conditions. Furthermore, additional winemaking steps were implemented to study their effect on extractability of polyphenols into the juice and wine matrices. Grape-derived tissues or macromolecules that could potentially influence polyphenol release, stability or persistence in a specific matrix was also investigated. The analysis method that was employed within this study, high performance liquid chromatography (HPLC), was only able to consistently quantify and identify seven individual flavan-3-ols, three hydroxycinnamic acids and two flavonols. Therefore, for the sake of this study, the phenolic potentials are defined as the non-stilbenoid phenolic potential. Figure 6.1 provides a summary of the experimental layout and the main results of this study.

Distribution of phenolic compounds in low and high phenolic potential Sauvignon Blanc grape berry tissues

A HL microclimate, generated by bunch zone defoliation, was found to successfully increase the total polyphenol content, or potential of Sauvignon Blanc grapes compared to those harvested from a low light (LL) microclimate (no defoliation). Compared to the grape pulp and seeds, the polyphenol content of the skin was most affected by the HL microclimate and although all measured polyphenol groups accumulated in the skin, the most significant increase was seen for flavonols (4.3 $\log_2$ fold change). The pulp accumulated some hydroxycinnamic acids, whereas the seeds were unaffected. The variety of polyphenols found in the individual grape tissues appeared to be conserved and unaffected by the microclimates. In grape berries that ripened within an un-manipulated canopy, such as the LL microclimate of this study, a specific part of the

Stellenbosch University https://scholar.sun.ac.za

polyphenol biosynthesis pathway is presumed to be more active in each grape tissue which results in the abundant accumulation of a specific group of compounds. This accumulation implies a specified functional role for each phenolic group which is more readily required by a tissue. For example, the increased accumulation of flavonols in specifically the skin, in response to a HL microclimate, is compatible with the UV-absorptive properties and function as natural sunscreen reported for these compounds (Gregan et al. 2011; Agati et al. 2013).

The composition of the grape skin cell wall differed marginally between low and high phenolic potential samples, but these differences could not be conclusively linked to the HL microclimate. High phenolic potential skin had higher signals for xyloglucan but lower rhamnogalacturonan I (RGI) which might correspond with the firmness and low extractability of RGI previously reported in pears exposed to sunlight (Raffo et al. 2011). The cell wall composition of the pulp appeared to be unaffected by the increased exposure.

Transitioning of polyphenol compounds from LP and HP grapes to juice and wine.

The transitioning of polyphenols from the grapes to the juice occurred only partially as more than half of the polyphenols that were quantified within the grape berry were not detected in the juice. This was true for both LP and HP grapes. However, most of the polyphenols that were not transferred originated from the seeds, whereas the skin and pulp polyphenols were readily distributed to the juice, with the exception of flavonols.

A limitation of this study is that the juice yield per berry mass was not measured which prevents direct comparison between the polyphenol content of the grapes and juice. Catechin was the only phenolic compound that was noticeably affected by the phenolic potential of grapes and/or juice, more so with skin contact, whereas the other measured compounds were not necessarily affected. Compared to the phenolic potential of the grapes and skin contact treatment, the sequential steps of juice processing had the biggest influence on polyphenol content, but the profile was unaffected. The most noticeable effect was the decrease of the total hydroxycinnamic acids and flavan-3-ols from the free run to the clarified juice. However, individual polyphenols responded uniquely to the processing steps as caftaric acid and catechin decreased whereas coutaric and caffeic acid increased. The same phenomenon was observed by Maggu et al. (2007) in Sauvignon Blanc juice and they reported the reduction in caftaric acid to be as a result of oxidation, but did not provide an explanation for the increase in coutaric acid. Caftaric acid and catechin was the most abundant hydroxycinnamic acid and flavan-3-ol, respectively, and their decrease meant a decrease in total hydroxycinnamic acids, flavan-3-ols and ultimately total polyphenol content too. The skin contact treatment was effective at increasing the total polyphenol content of the pressed and clarified juices. Skin contact also enhanced total flavan-3-ol extraction to a greater extent in the high phenolic potential juices, which reflected as a higher total polyphenol content.

Stellenbosch University https://scholar.sun.ac.za

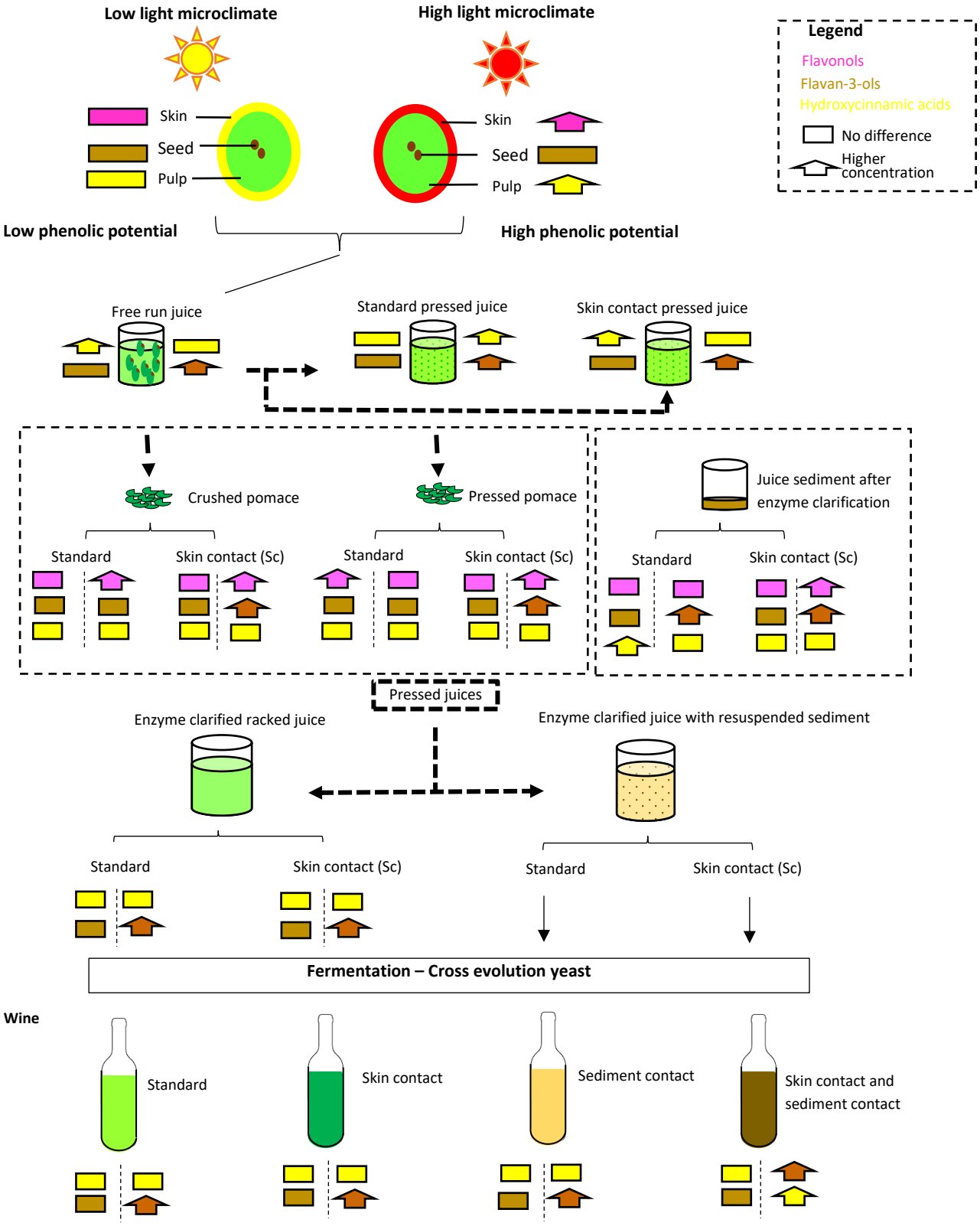


Figure 6.1: A model that summarizes the main results of this study from harvest to the final wine. The low light (LL) and highlight (HL) microclimates gave rise to grapes with a low (LP) and high phenolic potential (HP) respectively. The rectangles indicate lower content for a phenolic group and the arrow a higher content. Two squares indicate comparable content. The dotted lines separates the compounds measured in the LP and HP samples respectively. The dotted rectangle indicates sampling of the waste matrices (pomace and sediment) generated during juice processing.

Stellenbosch University https://scholar.sun.ac.za

Besides oxidation (Singleton et al. 1985; Maggu et al. 2007; Patel et al. 2010; Ferreira-lima et al. 2016), polymerization (Waterhouse 2002; Garrido and Borges 2013), or binding to cell wall material and other grape derived molecules and structures (Bindon et al. 2010; Hanlin et al. 2010; Vincenzi et al. 2011; Bindon and Smith 2013; Bautista-Ortín et al. 2015) can also reduce the concentration and availability of these compounds in the grape juice. With our data, we were unable to link the cell wall composition of the pomace with differences in polyphenol content between low and high phenolic potential juice, nor extraction or retention of polyphenols.

The total polyphenol concentrations (hydroxycinnamic acids and flavan-3-ols) of the wine was higher compared to the corresponding clarified juice. This could potentially be due to hydrolysis of hydroxycinnamic acids esters or pH mediated chemical hydrolysis of flavan-3-ol polymers to liberate hydroxycinnamic acids and catechin respectively (Kallithraka et al. 2009; Cejudo-Bastante et al. 2011b; Di Lecce et al. 2013). Polymeric polyphenols were not detected within the juice or wine but were present within the grape berries. These compounds were solely located within the seeds and it was assumed that seed polyphenols were not transferred to the juice, hence their absence in the juice and wine.

The wine making treatments exerted a stronger effect on wine polyphenol content than the defoliation treatment done in the vineyard. The skin and sediment contact treatments were employed to evaluate the effect of additional phenolic compound extraction steps employed. Under standard white winemaking conditions (without skin and/or sediment contact) the higher phenolic potential of the grapes was not noticeable in the wine. Individually, skin contact and sediment contact increased the total polyphenol levels of all wines, and to a greater extent in the high phenolic potential wine. Both treatments increased flavan-3-ol levels but sediment contact induced the most significant increase. The skin contact treatment was more successful at increasing total hydroxycinnamic acids in the wine, but only because coutaric acid was marginally higher (specifically in the high phenolic potential wine), while sediment contact appeared to attenuate caftaric acid levels.

Descriptive sensory analysis of the wines revealed that the perception of bitterness and astringency was largely dependent on the winemaking treatment that was applied. All wines received low scores for astringency which correlates with the undetected, potentially low levels, of polymeric flavan-3-ols. The perception of bitterness in wine correlated with the concentration of catechin as found in low and high phenolic potential wines that had sediment contact. The counterpart skin contact wine, which had lower levels of catechin, were less bitter and sour as well as being sweeter. It is interesting to note that wines which had both skin and sediment contact (ScSed) had the highest catechin content of all the wines but the skin contact treatment appeared to alleviate the bitter and sour attributes associated with the high catechin content. With regards to the standard wine, phenolic potential did not appear to affect the bitterness as high phenolic potential wine had a higher catechin concentration compared to the low potential wines but was

Stellenbosch University https://scholar.sun.ac.za

regarded as less bitter. Standard high phenolic potential wines had higher residual fructose which may have masked the perception of bitterness.

Residual polyphenols in waste matrices

The Sauvignon blanc pomace, consisting primarily of grape skins, had significantly lower residual polyphenols compared to the fresh grape skins at harvest. This potentially indicates that the majority of the polyphenols were extracted during juice processing. Alternatively, during juice processing the deconstruction of the cell wall exposes polymers that can potentially interact with the polyphenols, resulting in fewer polyphenols being extracted by the extraction solvent prior to analysis (Garrido and Borges 2013; Zietsman et al. 2015; Bindon et al. 2016; Gao 2016). The residual polyphenols found in the pomace are an indication of the fraction that is unextractable under winemaking conditions. Residual total hydroxycinnamic acids in the pressed pomace fractions were comparable between low and high phenolic potential samples, but flavonol levels were significantly higher in the latter, which reflected the significantly higher levels that were found in the fresh skin at harvest.

Based on the juice and wine data, skin contact increased polyphenol content, but this effect was not clearly visible in the pomace after pressing. The pomace can also be considered as a polyphenol trap in the juice matrix as residual flavonoid concentration increased after pressing, presumably by binding to exposed cell wall material (Bindon et al. 2010; Bindon and Smith 2013; Bindon et al. 2016). Skin contact also appeared to lower extractability of xyloglucan in the high phenolic potential pomace fractions collected after skin contact and pressing. In contrast, the standard pomace samples did not display any noticeable difference in cell wall composition.

The establishment of the polyphenol composition of the juice sediment, that was collected after clarification, is a novel result for white cultivars as it is yet to be reported in literature. The sediment was found to contain a variety of polyphenols which revealed that the majority of initially extracted polyphenols settled to the bottom after enzyme clarification. Sediment samples originating from juice which had skin contact contained higher levels of total hydroxycinnamic acids and flavan-3-ols correlating with the elevated levels observed in the corresponding juice and wine samples. Thus, the increase in extraction persisted to the final product, although a considerable amount was also trapped in the sediment. In addition to the extraction of more polyphenols with skin contact, more grape-derived compounds could have been extracted which could bind to polyphenols and facilitate precipitation in the sediment. Further investigation into the biochemical composition of the sediment is required to elucidate why more polyphenols were not reintroduced into the wine when fermentation occurred in the presence of the sediment, seeing as it contained a significant quantity and variety of polyphenols. One explanation could be that the fermentative conditions, referring to ethanol as extraction reagent and the duration of extraction, were not favourable to liberate greater quantities of bound polyphenols from the sediment. Final ethanol concentrations were

Stellenbosch University https://scholar.sun.ac.za

between 9.9% and 11.6% (v/v) for all wines which is much lower compared to the optimal extraction concentration between 60% and 70% (v/v) (Agustin-Salazar et al. 2014; Bosso et al. 2016).

A proportional relationship was found between total polyphenol and protein content of the juice during processing. Caftaric acid and catechin were the only polyphenols which showed a similar decline pattern as the total proteins. The likelihood that polyphenol and protein content was influenced by the same factor, rather than each other, was high as proteins are stable in the juice matrix and are unlikely to unfold and participate in binding (Van Sluyter et al. 2015). The phenolic potential of the grapes did not affect initial protein levels in the free run juice. An inverse relationship was observed between the protein concentration of wine and the polyphenol content and wine protein content was noticeably lower in wines which had skin or sediment contact, or a combination of the two treatments (ScSed) while these wines also had the highest total polyphenol content.

In conclusion, this study has shown that the polyphenol composition of the skin and pulp is important as these tissues arguably contribute the most toward the polyphenol composition of the juice. Grapes with a higher phenolic potential is not sufficient to noticeably increase the hydroxycinnamic acid or flavan-3-ol content of juice under standard white wine making conditions. We have shown that additional winemaking treatments, aimed at stimulating enhanced extraction from the skin, is more effective at increasing the catechin content in the juice. The holistic approach followed in this study elucidated that a considerable quantity of polyphenols initially extracted during juice processing was lost during enzyme clarification and can potentially be accounted for in the juice sediment. The analysis of the pomace on the other hand provided an indication of the efficacy of the extraction steps. Based on our findings, the majority of polyphenols present in enzyme clarified juice were distributed to the wine. The phenolic potential of grapes and/or juice can be further utilized through contact with the juice sediment during fermentation as an attempt to reintroduce polyphenols that have settled out. Our results indicate that sediment contact predominantly supplemented the catechin content of the wine. A higher phenolic content wine, where catechin was the abundant polyphenol due to sediment contact, did however increased the sensory perception of bitterness and astringency.

Sauvignon Blanc grapes are exposed to HL microclimates as a means of influencing the volatile composition of the juice and wines but with it also comes a concurrent increase phenolics. This study demonstrated that grapes with a higher phenolic potential, induced by a HL microclimate, only noticeably increases the content of certain individual polyphenols, even when skin contact, which is aimed at enhancing extraction, is applied. Our data shows that these increases will have little negative impact on the organoleptic properties of the wine, unless extensive sediment contact is allowed. Furthermore, this study also provides a better understanding of the transitioning of polyphenols from Sauvignon Blanc grapes to the juice and wine during winemaking.

Stellenbosch University https://scholar.sun.ac.za

6.1 References

Agati G, Brunetti C, Di M, Ferrini F, Pollastri S, Tattini M (2013) Plant Physiology and Biochemistry Functional roles of fl avonoids in photoprotection : New evidence , lessons from the past. Plant Physiol Biochem 72:35–45. doi: 10.1016/j.plaphy.2013.03.014

Agustin-Salazar S, Medina-Juárez LA, Soto-Valdez H, Manzanares-López F, Gámez-Meza N (2014) Influence of the solvent system on the composition of phenolic substances and antioxidant capacity of extracts of grape (Vitis vinifera L.) marc. Aust J Grape Wine Res 20:208–213. doi: 10.1111/ajgw.12063

Aleixandre-Tudo J, Weightman C, Panzeri V, Nieuwoudt HH, Toit WJ (2015) Effect of Skin Contact Before and During Alcoholic Fermentation on the Chemical and Sensory Profile of South African Chenin. South African J Enol Vitic 36:366–377.

Bautista-Ortín AB, Ruiz-García Y, Marín F, Molero N, Apolinar-Valiente R, Gómez-Plaza E (2015) Remarkable proanthocyanidin adsorption properties of monastrell pomace cell wall material highlight its potential use as an alternative fining agent in red wine production. J Agric Food Chem 63:620–633. doi: 10.1021/jf503659y

Bindon KA, Li S, Kassara S, Smith PA (2016) Retention of Proanthocyanidin in Wine-like Solution Is Conferred by a Dynamic Interaction between Soluble and Insoluble Grape Cell Wall Components. J Agric Food Chem 64:8406–8419. doi: 10.1021/acs.jafc.6b02900

Bindon KA, Smith PA (2013) Comparison of the affinity and selectivity of insoluble fibres and commercial proteins for wine proanthocyanidins. Food Chem 136:917–928. doi: 10.1016/j.foodchem.2012.08.016

Bindon KA, Smith PA, Holt H, Kennedy JA (2010) Interaction between Grape-Derived Proanthocyanidins and Cell Wall Material . 2 . Implications for Vinification. J Agric Food Chem 10736–10746. doi: 10.1021/jf1022274

Bosso A, Guaita M, Petrozziello M (2016) Influence of solvents on the composition of condensed tannins in grape pomace seed extracts. 207:162–169.

Cejudo-Bastante M, Castro-Vazquez L, Hermosín-Gutierrez MP-C (2011a) Combined Effects of Prefermentative Skin Maceration and Oxygen Addition of Must on Color-Related Phenolics , Volatile Composition , n White Wine and Sensory Characteristics of Air e. J Agric Food Chem 12171–12182. doi: 10.1021/jf202679y

Cejudo-Bastante MJ, Hermosín-Gutierrez I, Castro-Vasquez LI, Perez-Coello M (2011b) Hyperoxygenation and Bottle Storage of Chardonnay White Wines : Effects on Color-Related Phenolics , Volatile Composition , and Sensory Characteristics. J Agric Food Chem 4171–4182. doi: 10.1021/jf104744q

Coetzee C, Lisjak K, Nicolau L (2013) Oxygen and sulfur dioxide additions to Sauvignon blanc must : effect on must and wine composition. 155–167. doi: 10.1002/ffj.3147

Di Lecce G, Arranz S, Jáuregui O, Tresserra-rimbau A, Quifer-rada P, Lamuela-raventós RM (2014) Phenolic profiling of the skin , pulp and seeds of Albariño grapes using hybrid quadrupole time-of-flight and triple-quadrupole mass spectrometry. Food Chem 145:874–882. doi: 10.1016/j.foodchem.2013.08.115

Di Lecce G, Boselli E, D'Ignazi G, Frega NG (2013) LWT - Food Science and Technology Evolution of phenolics and glutathione in Verdicchio wine obtained with maceration under reductive conditions. LWT - Food Sci Technol 53:54–60. doi: 10.1016/j.lwt.2013.03.006

Dragana C, Davidovic SM, Todic SR, Bešlic ZS, Tešic L (2016) Identification and quantification of phenolic compounds in berry skin , pulp , and seeds in 13 grapevine varieties grown in Serbia. 211:243–252.

Stellenbosch University https://scholar.sun.ac.za

Ferreira-lima NE, Burin VM, Caliari V (2016) Impact of Pressing Conditions on the Phenolic Composition , Radical Scavenging Activity and Glutathione Content of Brazilian Vitis vinifera White Wines and Evolution During Bottle Ageing. Food Bioprocess Technol 944–957. doi: 10.1007/s11947-016-1680-7

Fracassetti D, Coetzee C, Vanzo A, Ballabio D, Toit WJ (2013) Oxygen Consumption in South African Sauvignon Blanc Wines : Role of Glutathione , Sulphur Dioxide and Certain Phenolics. 34:156–169.

Gao Y (2016) Parameters involved in the enzymatic deconstruction of the Wine Grape cell wall matrix during winemaking.

Garrido J, Borges F (2013) Wine and grape polyphenols - A chemical perspective. Food Res Int 54:1844–1858. doi: 10.1016/j.foodres.2013.08.002

Gomez-Miguez JM, Gonzalez-Miret LM, Hernanz D, Fernandez AM, Vicario IM, Heredia FJ (2007) Effects of prefermentative skin contact conditions on colour and phenolic content of white wines. J Food Eng 78:238–245. doi: 10.1016/j.jfoodeng.2005.09.021

Gregan SM, Wargent JJ, Liu L, Shinkle J, Hofmann R, Winfield C, Trough M, Jordan B (2011) Effects of solar ultraviolet radiation and canopy manipulation on the biochemical composition of Sauvignon Blanc grapes. Aust J Grape Wine Res. doi: 10.1111/j.1755-0238.2012.00192.x

Hanlin RL, Hrmova M, Harbertson JF, Downey MO (2010) Review: Condensed tannin and grape cell wall interactions and their impact on tannin extractability into wine. Aust J Grape Wine Res 16:173–188. doi: 10.1111/j.1755-0238.2009.00068.x

Hernanz D, Recamales ÁF, González-Miret ML, Gómez-Míguez MJ, Vicario IM, Heredia FJ (2007) Phenolic composition of white wines with a prefermentative maceration at experimental and industrial scale. J Food Eng 80:327–335. doi: 10.1016/j.jfoodeng.2006.06.006

Honeth. C (2018) Evaluating the effects of UVB radiation on field grown grape berry metabolites under high and low light microclimates and the consequent impacts on the resulting wines. Thesis, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

Kallithraka S, Salacha MI, Tzourou I (2009) Changes in phenolic composition and antioxidant activity of white wine during bottle storage : Accelerated browning test versus bottle storage. Food Chem 113:500–505. doi: 10.1016/j.foodchem.2008.07.083

Maggu M, Winz R, Kilmartin PA, Trought MCT, Trought M, Nicolau L (2007) Effect of Skin Contact and Pressure on the Composition of Sauvignon Blanc Must. J Agric Food Chem 55:10281–10288.

Patel P, Herbst-Johnstone M, Lee SA, Gardner RC, Weaver R, Nicolau L, Kilmartin PA (2010) Influence of Juice Pressing Conditions on Polyphenols , Antioxidants , and Varietal Aroma of Sauvignon blanc Microferments. Agric Food Chem 58:7280–7288. doi: 10.1021/jf100200e

Raffo MD, Ponce NMA, Sozzi XGO, Vicente XAR, Stortz CA (2011) Compositional Changes in ‘ Bartlett ’ Pear (Pyrus communis L .) Cell Wall Polysaccharides As Affected by Sunlight Conditions. J Agric Food Chem 12155–12162.

Ristic R, Downey MO, Iland PG, Bindon K, Francis L, Herderich M, Robinson S (2006) Exclusion of sunlight from Shiraz grapes alters wine colour ,. Aust J Grape Wine Res 53–65.

Rodríguez Montealegre R, Romero Peces R, Chacón Vozmediano JL, Martínez Gascueña J, García Romero E (2006) Phenolic compounds in skins and seeds of ten grape Vitis vinifera varieties grown in a warm climate. J Food Compos Anal 19:687–693. doi: 10.1016/j.jfca.2005.05.003

Šebela D, Turóczy Z, Olejníčková J, Kumšta M, Sotolář R (2017) Effect of Ambient Sunlight Intensity on the Temporal Phenolic Profiles of Vitis Vinifera L . Cv . Chardonnay During the Ripening Season – A Field

Stellenbosch University https://scholar.sun.ac.za

Study. South African J Enol Vitic 38:94–102.

Singleton VL, Salgues M, Zaya J, Trousdale E (1985) Caftaric Acid Disappearance and Conversion to Products of Enzymic Oxidation in Grape Must and Wine. Am J Enol Vitic 36:50–56.

Van Sluyter SC, McRae JM, Falconer RJ, Smith PA, Bacic A, Waters EJ, Marangon M (2015) Wine protein haze: Mechanisms of formation and advances in prevention. J Agric Food Chem 63:4020–4030. doi: 10.1021/acs.jafc.5b00047

Vincenzi S, Marangon M, Tolin S, Curioni A (2011) Protein evolution during the early stages of white winemaking and its relations with wine stability. 20–27. doi: 10.1111/j.1755-0238.2010.00113.x

Waterhouse AL (2002) Wine Phenolics. Ann New York Acad Sci 36:21–36.

Zietsman AJJ, Moore JP, Fangel JU, Willats WGT, Trygg J, Vivier MA (2015) Following the Compositional Changes of Fresh Grape Skin Cell Walls during the Fermentation Process in the Presence and Absence of Maceration Enzymes. doi: 10.1021/jf505200m