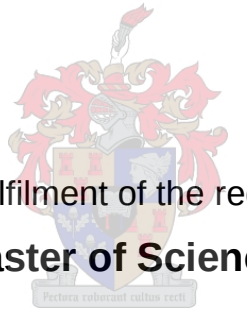
Extraction and bioconversion of aroma impact compounds from Sauvignon Blanc grapes to wine matrices during white wine production

by

Isabel Greyling



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Co-supervisors: Dr Philip Young and Dr Anscha Zietsman

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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, with the exception of the contributions made by others as stated below. The study was conceived by Professor Melané Vivier who along with Dr Philip Young and Dr Anscha Zietsman contributed critical evaluation of the results throughout the study. Dr Anscha Zietsman helped with logistical preparation for the winemaking and sampling strategy. Dr Philip Young and Ms Anke Berry performed the vineyard treatments. Ms Anke Berry provided support in the winemaking process, as well as integration and analysis of chemical data. Dr Hans Eyeghe-Bickong assisted in the UPLC, HPLC and GC-MS analysis. The wine sensory evaluation was supervised by Ms Jeanne Brand from the Sensory Laboratory of the Department of Viticulture and Oenology. The thesis was drafted and completed by myself, with inputs from all supervisors on the content and interpretation of results.

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Summary

The phenotypic response of Sauvignon Blanc grapes grown in high light (HL) conditions has been shown to acclimate to stress conditions via photoprotective responses that includes the upregulation of specific grape metabolites. These metabolites are typically concentrated in the berry skins and include aroma compounds/precursors, photosynthetic pigments and polyphenolic compounds which contribute to the distinctive aroma of the resulting wines. The fate of these aroma impact metabolites from the grape tissues during juice processing and fermentation up until the final wine has been studied and revealed a highly dynamic environment involving metabolite bioconversions and/or interaction.

The aim of this study was to profile selected aroma-related metabolites in Sauvignon Blanc grape berries that were exposed to high light (HL) and low light (LL) microclimates and to follow the fate of the metabolites throughout the key processing stages of winemaking. Two winemaking procedures that could potentially affect the extraction of aroma-related metabolites from grape skins and juice sediment (particulate grape debris) were evaluated. The impact of the various factors (vineyard microclimate, skin contact and fermentation with grape sediment) on the sensory profile of the wine was also determined.

Berries grown in the HL environment contained higher concentrations of grape derived aroma-linked metabolites (including IBMP, limonene, linalool, 6-MHO and hexyl formate) confirming previously published results. These berries were therefore characterised as having higher aromatic potential (HAP) compared to the berries from the LL microclimate with lower aromatic potential (LAP). During juice processing there was a dramatic decrease in the aromatic potential in both HAP and LAP juices and the chemical analysis of the sediment could account for many of the “lost” compounds. Thus, we established that the sediment represents a reservoir of untapped aromatic potential. The distinction between HAP and LAP berries persisted throughout juice processing up to the final wines when prepared by standard winemaking (Std) procedures. The main difference between HL-Std and LL-Std wines where in the concentration of grape derived aroma compounds with the former containing significantly higher concentrations of IBMP, hotrienol, linalool and β-damascenone. Confirming previous studies, the LL-Std wines had a vegetative sensorial character and the HL-Std wines were fruity.

Modulations to the standard winemaking procedure to investigate enhanced extraction of aroma metabolites included a skin contact treatment before pressing and fermentation in contact of the sediment formed during juice clarification. Skin contact (Sc) mitigated the loss in aroma potential seen during juice processing and increased hexyl formate, hotrienol and IBMP concentration in both LAP and HAP juice. The corresponding skin contact wines contained higher concentrations of

linalool, IBMP and 3-MH. Interestingly LL-Sc wines had a similar chemical and especially sensory profile to HL-Std and HL-Sc wines. This demonstrated that the aromatic potential of LAP berries (with additional extraction) is adequate to alter the aromatic potential of wine. Wines fermented in contact with the sediment were enriched by the metabolites that were detected in the sediment and this enrichment effect was stronger than what was achieved with skin contact. These wines had significantly higher concentrations of hexyl formate, IBMP and 3MH, but had lower ester levels and malodours were perceived during sensorial analysis.

This study provided insights into the transfer and fate of the aromatic potential of Sauvignon Blanc from the grapes, throughout juice processing onto the final wine. It highlights the impacts of viticultural manipulations and winemaking steps that aims to improve aroma compound extraction on the aromatic potential and sensory profile of the wine.

Opsomming

Studies het gewys dat Sauvignon Blanc druiwe wat in ‘n hoë-lig (HL) mikroklimaat gekweek is ‘n sekere fenotiepiese respons toon om by streskondisies aan te pas. Die respons sluit fotobeskermende meganismes in wat tot die ooruitdrukking van spesifieke druifmetaboliete lei. Hierdie metaboliete versamel hoofsaaklik in die druiwedoppe en sluit aroma komponente en hul voorgangers, fotosintetiese pigmente en polifenoliese verbindings wat almal bydra tot die kenmerkende aroma van die wyne wat daarvan geproduseer word. Die roete en uiteindelike lot van hierdie aroma impak-metaboliete is bestudeer in die druiwe, tydens sap verwerking en fermentasiestappe, tot en met die finale wyn. Hierdie benadering het die hoogs dinamiese aard van die wynmaakproses, gekenmerk deur metaboliet bio-omskakelings en/of interaksies, uitgewys.

Die doel van hierdie studie was om ‘n profiel te verkry van gekose aroma verwante metaboliete in Sauvignon Blanc druiwe, wat afkomstig was van twee verskillende mikroklimate, naamlik ‘n hoë lig (HL) en lae lig (LL) mikroklimaat. Die aroma verwante metaboliete is bepaal in die druiwe en daarna “gevolg” tydens die hoof stappe van die wynmaak proses. Twee wynmaakprosedures is geëvalueer op grond van hulle vermoë om die ekstraksie van aroma verwante metaboliete vanuit druiwedoppe, asook sapsediment (druifmateriaal wat uitsak na ensiem geïnduseerde sapverheldering) te beïnvloed. Die invloed van die verskillende faktore (wingerd-mikroklimaat, dopkontak en gisting in die teenwoordigheid van sapsediment) op van die wyn se sensoriese profiel is ook bepaal.

Druifkorrels afkomstig vanaf die HL-mikroklimaat, het hoër konsentrasies van aroma-verwante metaboliete (insluitend IBMP, limoneen, linalool, 6-MHO en heksielformaat) bevat, in lyn met voorheen gepubliseerde resultate. Die HL druifkorrels het dus 'n hoër aromatiese potensiaal (HAP) gehad, in kontras met dié van die LL-mikroklimaat wat 'n laer aromatiese potensiaal (LAP) vertoon het. Tydens die verwerking van sap was daar 'n merkwaardige afname in die aromatiese potensiaal van beide HAP- en LAP-sappe. Chemiese ontleding van die sediment het die teenwoordigheid van baie van hierdie “verlore” metaboliete aangedui wat uitwys dat die sediment 'n moontlike “reservoir” vir onbenutte aromatiese potensiaal verteenwoordig. Tydens standaard wynmaak prosedures (Std) het die verskille tussen HAP- en LAP-druiwe, van sap tot die finale wyne, behoue gebly. Die grootste verskil (in terme van aroma-verwante metabolitee) tussen die samestelling van HL-Std en LL-Std wyne, was dat eersgenoemde aansienlik hoër konsentrasies IBMP, hotrienol, linalool en β-damascenoon bevat het. Sensoriese analiese het bevestig wat in vorige studies gevind is: die LL-Std wyn het ‘n vegetatiewe karakter gehad, terwyl die HL-Std wyne beskryf is met ‘n “vrugtige” sensoriese karakter, ondanks die relatief hoë IBMP-vlakke in die wyne.

Aanpassings in die standaard wynmaakprosedure is gemaak met die doel om die ekstraksie van aroma-verwante metaboliete te versterk, en het dopkontak (voor pars) en ‘n sediment kontak behandeling (tydens fermentasie) ingesluit. Dopkontak (Sc) het die verlies aan aroma potensiaal wat tydens sapverwerking gesien is, tot ‘n sekere mate teengewerk en die heksielformaat-, hotrienol- en IBMP-konsentrasie in beide LAP- en HAP-sap verhoog. Die ooreenstemmende dopkontakwyne het voorts hoër konsentrasies van linalool, IBMP en 3-MH bevat. Interessant genoeg het LL-Sc-wyne 'n soortgelyke chemiese en veral sensoriese profiel as HL-Std- en HL-Sc wyne gehad. Dit het getoon dat die aromatiese potensiaal van LAP-druiwe (met ekstra ekstraksie) voldoende is om die aromatiese potensiaal van die wyn te verander. Wyn wat in kontak was met die sediment tydens fermentasie, was verryk met die metaboliete wat in dié matriks gevind word. Hierdie verrykingseffek was groter as dit wat met dopkontak verkry is. Die sedimentkontakwyne het merkwaardige hoër konsentrasies van heksielformaat, IBMP en 3MH gehad, maar laer konsentrasies van esters, en afgeure is ook bespeur tydens sensoriese analise.

Hierdie studie het gelei tot waardevolle insigte in die oordrag en “lot” van die aromatiese potensiaal van Sauvignon Blanc-druiwe, vanaf die druif, deur die wynmaak prosedure tot in die finale wyn. Dit werp lig op die gevolge van wingerdmanipulasies, asook aanpassings in wynmaakstappe wat daarop gemik is om die ekstraksie van aroma verwante metaboliete te verbeter, veral op die uituidelike impakte op die aromatiese potensiaal en sensoriese profiele van die finale wyne.

“If you don’t have talent, then you’ve got to have tenacity” – Alex Honnold

This thesis is dedicated to my father, who always encouraged and supported me in chasing my dreams and to my mother who is always there to provide support and love as only a mother can.

Biographical sketch

Isabel Greyling was born in Nelspruit, South Africa and matriculated from Rob Ferreira High School in White River in 2012. Isabel obtained BSc- and BScHonours degrees in Food Science from the University of Pretoria in 2015 and 2016. On completion of these degrees, she broadened her study field by enrolling for an MSc-degree in Wine Biotechnology.

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Preface

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

Chapter 1 **General Introduction, literature overview and project aims**

Chapter 2 **Research results:**
The release and fate of Sauvignon Blanc aroma compounds in a grape-to-juice-to-wine analysis

Chapter 3 **General discussion and conclusions**

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Chapter 1
Introduction, literature overview and project aims

1.1. Introduction

The origins of wine aroma cannot be easily defined. The difficulty is that an almost endless number of factors contribute to, or can modulate the aroma of a wine. The aroma of wine also "evolves" over time, emphasising the dynamic nature of wine and the complexity of describing and studying wine quality impact factors, such as aroma.

The topic of this study is the aroma of Sauvignon Blanc (SB) and particularly the origins and the ultimate fate of the grape-derived aroma compounds of this cultivar. The term "aromatic potential of the grapes" will be used when discussing the grape-derived aroma compounds that are linked to the cultivar itself (typical of SB). These compounds can be modulated by the specific meso- and micro-climatic factors of the site as well as the management practices implemented in the vineyard. This grape-derived aromatic potential and the extractability thereof into the wines will be studied in an integrated manner that will incorporate several matrices such as the grape, the juice and sediment as well as the wine. A concise literature overview of the importance of the SB cultivar and the origins of the aroma compounds typically linked to the wines will be provided before the study aims will be presented and contextualised.

1.2. Literature Overview

1.2.1. Sauvignon Blanc as an international cultivar

Sauvignon Blanc is considered an economically important varietal with wide consumer appeal. Sauvignon Blanc is the 9th most planted grape variety in the world (OIV, 2017), with plantings in all major wine-producing countries (Table 1.1). In terms of hectares planted, France, New Zealand and Chile are in the top three positions; but New Zealand is the country that has made SB its major focus, with 60% of all vines planted being this cultivar. In South Africa, SB contributes 10% to all vine plantings, but when considering only white varietals, SB makes up 18% of all planted white varieties (Figure 1.1). SB in South Africa is largely used for the production of varietal table wines (Floris, 2018). It was the 2nd most exported South African white wine in terms of volume in 2018, with 47% sold as bottled wine, and delivering a total of R997 million in income (SAWIS, 2018).

Table 1.1: The total plantings (ha) of Sauvignon Blanc (SB) in some of the major wine-producing countries and the percentage contribution to the total vine plantings per country (Anderson & Aryal, 2016; OIV, 2017)

Country	SB plantings in Ha	Percentage of total plantings
France	30 000	4%
New Zealand	21 400	60%
Chile	15 000	7%
South Africa	9 277	10%
USA	6 584	3%
Australia	6 000	4%
Romania	6 000	3%
Total global	**110 138**	**2.4%**

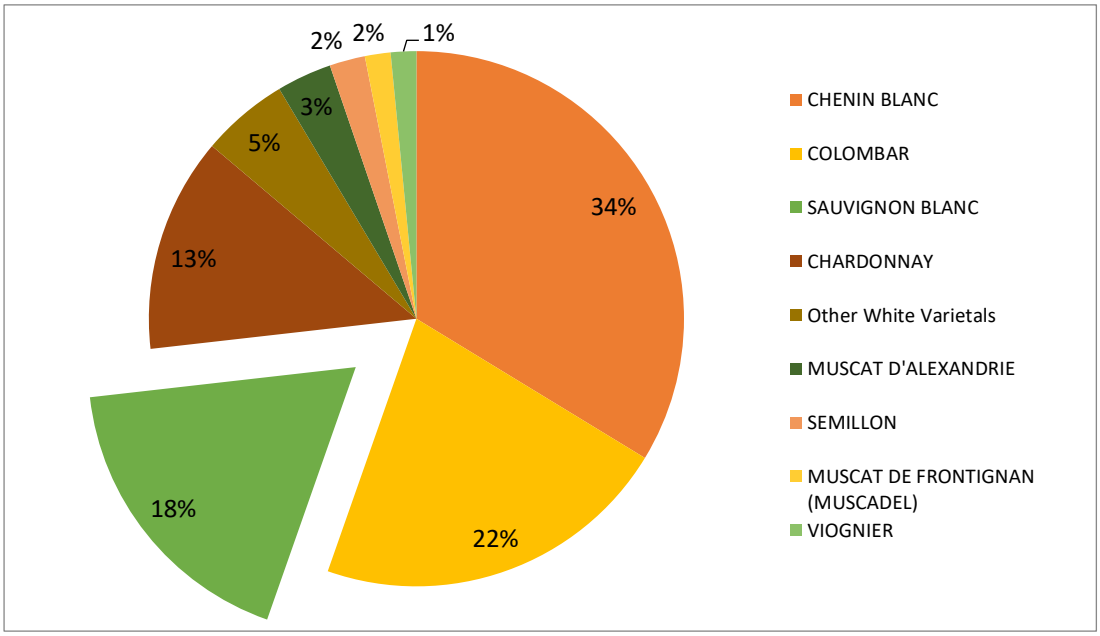


Figure 1.1: Pie graph showing the proportions of the different white wine grape plantings (ha) in South Africa. Proportion was calculated by dividing the total number of plantings per varietal by the total number of white wine plantings (ha). Data obtained and adapted from SAWIS (2018).

The popularity of SB amongst consumers can be attributed to the distinct sensory profile of SB that can be produced in a variety of styles ranging from tropical/fruity to green/herbaceous, depending on the region where the wine is produced and specific viticultural and oenological practices.

1.2.2. Sauvignon Blanc wine styles, typicity and impact compounds

All wines reflect (in a complex way) the specific vineyard site, the cultivar and rootstock, the climatic impacts, as well as the management practices and winemaking techniques used in the production of the grapes. Sauvignon Blanc is very responsive to environmental factors, leading to significant phenotypic plasticity in this cultivar. Phenotypic plasticity in plants can be defined as the ability of a plant to modify certain characteristics (phenotype) in response to different environmental conditions (Bradshaw, 1965).

In general, SB is considered better suited to cooler climatic regions. In South Africa, SB is planted in all the wine-growing areas (Figure 1.2), where the conditions could vary between cool coastal climates and warmer, drier inland climates. Sauvignon Blanc produced in the coastal regions (for example Elgin) tends to be crisp and acidic, with green and vegetative aromas, whereas SB produced more inland (e.g. Stellenbosch) tends to be more fruit-forward.

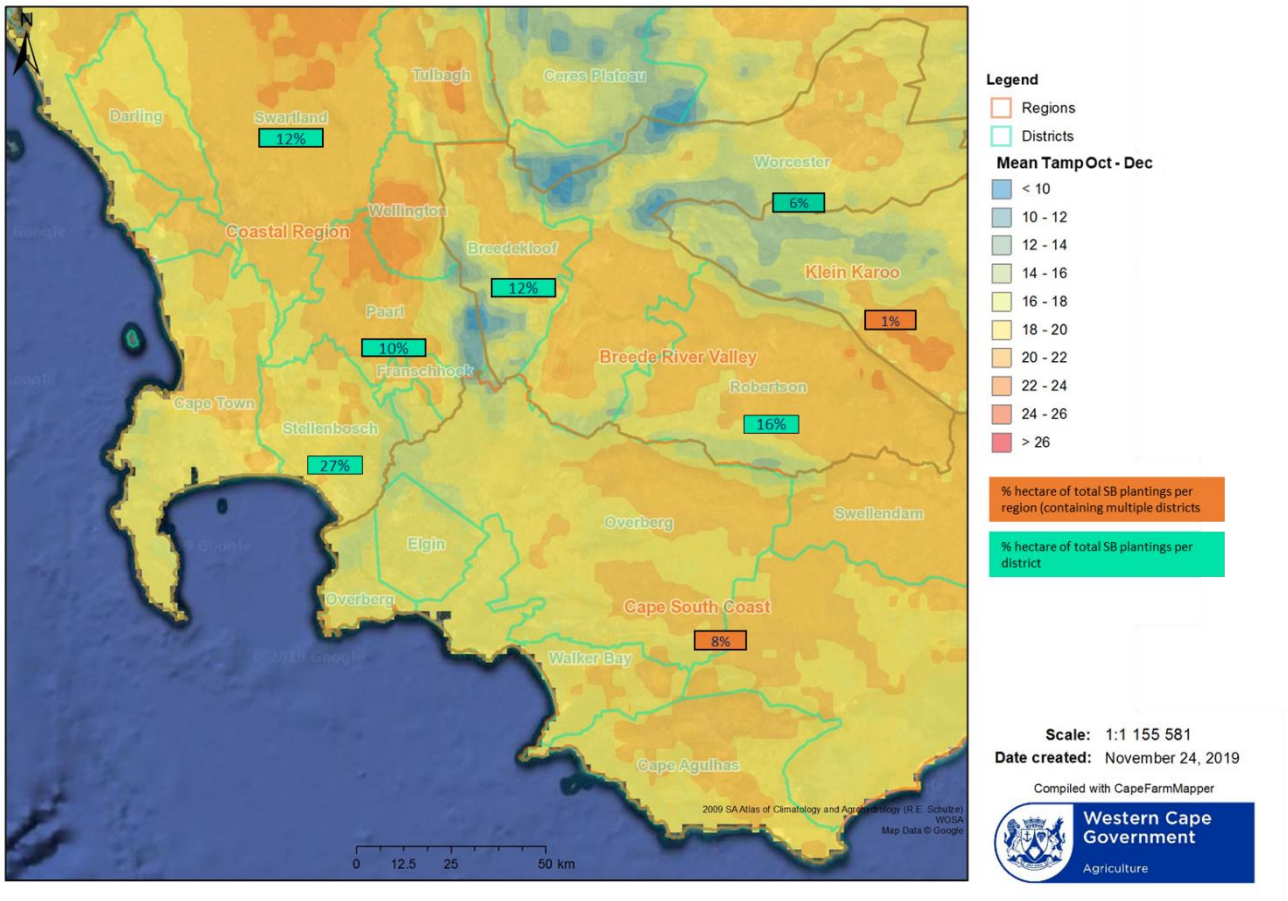


Figure 1.2: Map of the mean annual temperatures of the different wine regions and districts of South Africa., and the significant % of hectares of SB planted (Northern Cape (0.3%) and Olifants Rivier (6%) not shown). Remaining 11.7%, include all other regions and districts including Franschhoek and Cape Town. Map generated with CapeFarm mapper and data obtained from SAWIS, 2018.

Several studies have also shown that different styles of SB wines can be produced from a single vineyard site by manipulating the microclimatic factors in the bunch zone (Marais *et al.*, 1999; Šuklje *et al.*, 2014; Joubert *et al.*, 2016; Martin *et al.*, 2016). Moreover, SB wine styles, makes it ideal for aroma studies, particularly when studying the linking of grape-derived to wine aroma compounds.

There is no clear definition for wine style found in literature. Wine styles are established under unique climatic and politico-socio-economic environments, resulting in countries/areas with their own unique sense of “wine style” (Jackson, 2000a). The definition of cultivar-based wine styles can therefore vary between different counties and contexts (Jackson, 2009). Due to the variable nature of wine, fuelled by the wide range of environmental factors and cultivars, as well as production methods, there is little commonality by which to classify different wines (Jackson, 2009).

Traditionally, wines are classified into different styles by simple, easily detectable features such as colour, sweetness, fortification, geographical and/or varietal origin and presence/absence of effervescence. Broadly there are three main wine styles: sparkling wine, still table wine (including red, rosé and white wines) and fortified wine (Jackson, 2009). However, within each of these overarching styles, certain emphasis could be placed on the sensory profile of the cultivar from which the wine style is produced. For example, in the production of SB (still table wine), wines with both green/herbaceous and fruity aromas are also considered to be different "styles" of SB. Based on inferences made from various literature that referred to wine style (Parr *et al.*, 2007; Styger *et al.*, 2011; Herbst-Johnstone *et al.*, 2013; Jiang *et al.*, 2013; Yang *et al.*, 2013; Jackson, 2009; Pinu *et al.*, 2014; Cheng *et al.*, 2015; Drappier *et al.*, 2019), a generalised definition could be: Wine styles refer to the manner and conditions under which wine is produced, both at the vineyard and wine processing levels.

Wine typicity, on the other hand, refers to how accurately the sensory profile of the wine reflects its varietal origin. Wine typicity furthermore demonstrates signature characteristics of the specific grape cultivar and desired style (Robinson & Harding, 2014). The typicity of wine is more concerned with the specific aroma nuances and can be subjective. This will have a large influence on the perceived quality of the wine (Hopfer & Heymann, 2014). Wine quality is notoriously difficult to define, but the "perceived quality" is considered to be linked to a combination of the appearance, taste, aroma, mouthfeel and even the price of the wine (Hopfer & Heymann, 2014). Based on the definitions mentioned above, both "green" and "fruity" styles fall within the typicity of SB wines, and several major aroma impact compounds that define these styles have been characterised and will be briefly summarised in the sections below.

1.2.3. Aroma impact compounds of Sauvignon Blanc and their origins

Aroma compounds can roughly be divided into three groups based on their origin: Primary aroma compounds (grape-derived), secondary aroma compounds (produced during winemaking from non-volatile precursors) or tertiary aroma compounds (derived during ageing) (Robinson *et al.*, 2014; González-Barreiro *et al.*, 2015a). The most important aroma impact compounds of SB are presented in Table 1.2. Aroma impact compounds can be defined as aroma compounds that will have a significant impact on the perceived aroma of wine (Ferreira, 2010), and often contribute to the varietal typicity.

1.2.3.1. Primary aroma compounds

Grape-derived aromatic compounds, such as methoxypyrazines and volatile monoterpenes (Figure 1.3), originate in the grape, and do not undergo additional metabolic changes throughout processing, and can be found unchanged in the wine, (Dunlevy *et al.*, 2009; Styger *et al.*, 2011; Darriet, 2012a; Robinson *et al.*, 2014). The varietal aroma of SB is mainly due to the presence, profile and levels of specific methoxypyrazines and volatile thiols (Dunlevy *et al.*, 2009; Coetzee & du Toit, 2012; Darriet, 2012a; Robinson *et al.*, 2014; González-Barreiro *et al.*, 2015a).

The composition of grape berries includes a wide spectrum of chemical compounds, each with its own function within the berry. Figure 1.3 summarises different classes of chemical constituents in grape berries and their roles in berry development and subsequent wine fermentation as well as any possible linked to wine aroma and/or general wine quality. Grape metabolites all have physiological functions within the berry, for example, towards cell growth (nitrogen and sugars), light-harvesting (chlorophylls and carotenoids), or as antioxidants (glutathione, carotenoids and monoterpenes) (Coombe, 1987; Dokoozlian, 2000; Kennedy, 2002; Conde *et al.*, 2007; Jackson, 2007; Keller, 2015; Deloire, 2015). Grape metabolites are however not equally distributed throughout the grape berry tissues. There are three main types of tissues in berries: the exocarp (skin), mesocarp (flesh) and endocarp (seeds) (Figure 1.3), each with unique chemical compositions. The distribution of the various chemical compounds in the different berry tissues have been extensively reviewed by Conde *et al.* (2007) and Jackson (2007). The seeds for example contain high levels of phenolic compounds (such as tannins) (Figure 1.3). The mesocarp on the other hand contains mostly water, sugars, organic acids (Coombe, 1987), making up the majority of the berry and wine volume (Figure 1.3). Finally, the exocarp contain phenolic compounds (tannins and pigments), as well as the majority of grape-derived aroma precursors such as carotenoids, glycosidically bound terpenes, lipids, volatile thiol precursors, as well as volatiles such as methoxypyrazines, monoterpenes, C_6 compounds, and C_{13}-norisoprenoids, as reviewed by Coetzee *et al.* (2012), Conde *et al.* (2007) and Jackson (2007).

Table 1.2: Table summarising selected cultivar-derived, as well as general wine aroma compounds typical of Sauvignon Blanc

Compound Group	Examples of specific compound(s)	Associated Aroma	Aroma threshold (ug/L)	Varietal/General wine aroma compound	Compound origin	References
Methoxypyrazines	3-isobutyl-2-methoxypyrazine **(IBMP)**	green pepper, asparagus	0.001	Aroma impact compound	Grape derived	(Seifert *et al.*, 1972; Allen *et al.*, 1991; Marais, 1998; Darriet, 2012a)
	3-isopropyl-2-methoxypyrazine **(IPMP)**	earthy, pea	0.002			
	3-sec-butyl-2-methoxypyrazine **(SBMP)**	earthy	0.001			
Volatile Thiols	4-mercapto-4-methylpentan-2-one **(4MMP)**	box tree, passion fruit, broom, black current	0.0008	Aroma impact compound	Grape derived	(Coetzee & du Toit, 2012)
	3-mercaptohexan-1-ol **(3MH)**	passion fruit, grapefruit, citrus	0.06			
	3-mercaptohexyl acetate **(3MHA)**	passion fruit, grapefruit, citrus	0.004			
Norisoprenoids	β-damascenone, β-ionone	floral and fruity	0.05	General aroma	Grape derived	(Ferreira *et al.*, 2002a; Zelena *et al.*, 2009; Styger *et al.*, 2011)
Monoterpenes	linalool, geraniol, hotrienol, limonene and α-terpineol	floral and fruity	60 - 66000	General aroma	Grape derived	(Ribéreau-Gayon *et al.*, 1975; Moreno *et al.*, 2005; Palomo *et al.*, 2007; Darriet, 2012a; Jiang *et al.*, 2013)
C_6-Compounds	hexanol and hexanal	green, leafy and grassy	14 - 8000	General aroma	Grape derived	(Ferreira *et al.*, 2002a; Culleré *et al.*, 2004; Mendez-Costabel *et al.*, 2013)
Esters	ethyl acetate, ethyl butyrate ethyl hexanoate, hexyl acetate, isoamyl acetate, isobutyl acetate and phenylethyl acetate	sweet and fruity	20 - 494000	General aroma	Fermentation derived	(Rodríguez-Bencomo *et al.*, 2002; Swiegers *et al.*, 2005; Robinson *et al.*, 2014)
Higher Alcohols	phenylethyl alcohol, isoamyl and isobutyl alcohol	sweet and fruity	10000 - 40000	General aroma	Fermentation derived	(Ferreira *et al.*, 2002a; Francis & Newton, 2005; Swiegers *et al.*, 2005)
Fatty Acids	acetic acid, hexanoic acid, octanoic acid and decanoic acid	fresh flavour		General aroma	Fermentation derived	(Ferreira *et al.*, 2002b; Coetzee & Du Toit, 2015)

The aroma impact compounds play a critical role in the flavour of SB, but other compounds can also contribute to the aroma profile and style of SB. In the following section the aroma impact compounds and general aroma compounds of SB will be briefly discussed.

Methoxypyrazines

Methoxypyrazines are nitrogen heterocyclic compounds belonging to the pyrazine group, residing mostly in the skin of the berry. Among the various methoxypyrazines present in plants, alkylated and highly volatile methoxypyrazines are of special interest in SB grapes (Darriet, 2012a). Noteworthy, methoxypyrazines present in high quantities in SB are 3-isobutyl-2-methoxypyrazine (IBMP – “green pepper”, “asparagus”), 3-isopropyl-2-methoxypyrazine (IPMP – “earthy”, “pea”) and 3-sec-butyl-2-methoxypyrazine (SBMP – “earthy”) (Allen *et al.*, 1991). Due to their low aroma thresholds (Marais, 1998), the odour impact of methoxypyrazines are usually very high, making them significant contributors to SB aroma and wine style. They are very prominent in SB wines (Lacey *et al.*, 1991), producing wines with distinctive “green”/”herbaceous” aromas.

Volatile Thiols

Volatile thiols contribute significantly to the varietal aroma of SB, with characteristic fruity aromas. Thiols (also known as mercaptans) are sulphur containing organic compounds . (Coetzee & du Toit, 2012). The three most prominent thiols present and contributing to Sauvignon Blanc aroma are 4-mercapto-4-methylpentan-2-one (4MMP – “box tree”, “passion fruit”, “broom” and “blackcurrent bud”), 3-mercaptohexan-1-ol and 3-mercaptohexyl acetate (3MH & 3MHA - “passion fruit”, “grapefruit” and “citrus”). Precursors of volatile thiols have been identified as the cysteinylated [S-3-(hexan-1-ol)-L-cysteine (Cys-3MH) and S-4-(4-methylpentan-2- one)-L-cysteine (Cys-4MMP)] and glutathionylated [S-3-(hexan-1-ol)- glutathione (Glut-3MH) and S-4-(4-methylpentan-2-one)-glutathione (Glut-4MMP)] compounds (Coetzee & du Toit, 2012). The different precursors have unique distributions in the grape tissue. For example the precursor Cys-4MMP was found to be equally distributed between the pulp and the skin at harvest, while most of the glutathionylated precursor of 4MMP was located in the skin (Coetzee & du Toit, 2012). The Glut-3MH precursor showed relatively equal distribution between skin and pulp whereas the Cys-3MH was found to be mostly in the skin.

It is thought that these precursors form aromatic thiols predominantly by the cleavage of the carbon sulphur linkage by the carbon–sulphur β-lyase enzyme during yeast metabolism (Coetzee & du Toit, 2012). Other proposed mechanisms include the esterification of 3MH with acetic acid to form 3MHA, but precise mechanism remains unknown (Coetzee & du Toit, 2012). However, it has been found that these precursors only account for a small portion of the total amount of thiols found in the wine (Darriet, 2012a). Although it thought that aromatic thiols are formed through the cleavage of the respective precursors by the yeast, they do not undergo bioconversion during fermentation, and are therefore still considered to be grape-derived aroma compounds in this study.

Monoterpenes

Monoterpenes are a large and important group of compounds as they not only contribute to the aroma in grapes (and wines), but they also function as antioxidants in berry stress responses (Reynolds & Wardle, 1989) (Figure 1.3). They are found in both free and glycosidically bound forms predominantly in the skin of the grape berries (Wilson *et al.*, 1986). Unlike methoxypyrazines, monoterpenes do not contribute significantly to the varietal characteristic of SB, but SB. As the monoterpene aromas have a synergistic effect, they can have a significant influence on the aromatic profile of wine (Ribéreau-Gayon *et al.*, 1975). The most important monoterpenes in terms of aroma are monoterpene alcohols and oxides (including linalool, geraniol, hotrienol, limonene and α-terpineol), contributing floral and fruity aromas to SB (Darriet, 2012a).

Norisoprenoids and C_6 compounds

Many important grape-derived aroma compounds are produced from oxidative reactions during harvesting and berry crushing. Norisoprenoids are produced from the oxidative degradation and enzymatic cleavage of carotenoids in grape skins that occur during harvesting and crushing(Darriet, 2012b). The C_{13}-norisoprenoids usually contribute floral and fruity notes to wine with the most pronounced being β-damascenone and β-ionone. (Zelena *et al.*, 2009; Styger *et al.*, 2011). C_6 compounds contribute to the green, leafy and grassy odour characteristics in SB. They are the products of lipoxygenase enzymes and aerobic oxidation of polyunsaturated fatty acids (producing important aroma compounds like hexanol and hexanal). They are usually formed during juice processing (crushing and destemming), when the cell structure is disrupted and are known as green leaf volatiles (Mendez-Costabel *et al.*, 2013).

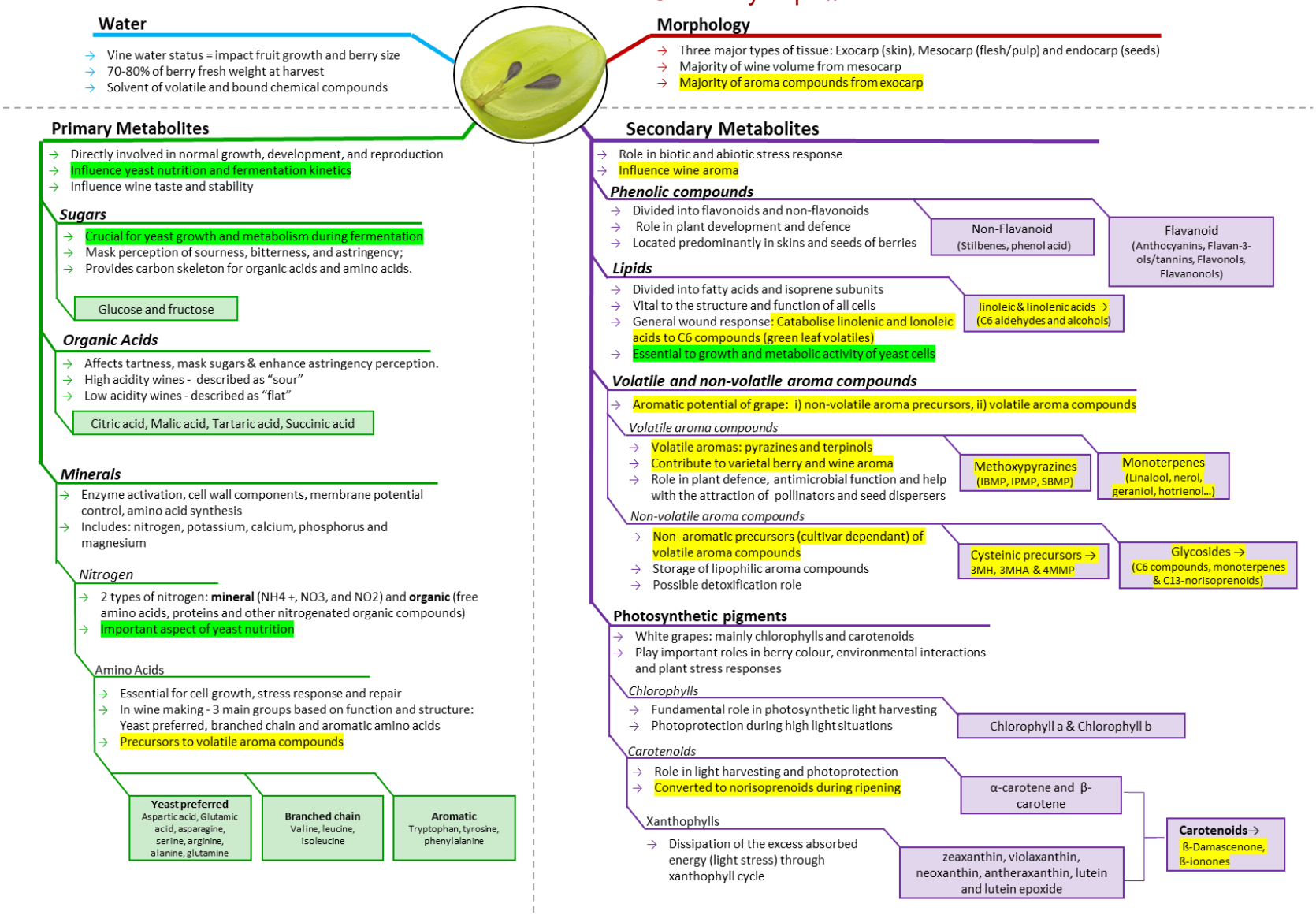


Figure 1.3: Summary of the different classes and roles of chemical constituents in grape berries. Green colour highlights functions indirectly linked to aroma compound production like yeast nutrition. Yellow highlights aromatically important compounds and characteristics. (Coombe, 1987; Dokoozlian, 2000; Kennedy, 2002; Conde *et al.*, 2007; Jackson, 2007; Keller, 2015; Deloire, 2015).

1.2.3.2. Secondary aroma compounds

Aroma precursors are odourless compounds that, upon processing or yeast metabolism, are cleaved or oxidised and converted to odoriferous compounds of aromatic importance, for example: esters, higher alcohols, norisoprenoids and volatile thiols (Dunlevy *et al.*, 2009; Coetzee & du Toit, 2012; Darriet, 2012a; Roland *et al.*, 2012). These precursors include glycosylated precursors, fatty acids, amino acids and carotenoids (Figure 1.3).

Esters and higher alcohols

Esters and higher alcohols contribute to the general vinous base aroma of wine; depending on the type and quantity present. Esters and higher alcohols are both derived from sugar and amino acid metabolism (Swiegers *et al.*, 2005; Robinson *et al.*, 2014). Esters represent the highest concentration of volatile compounds in most alcoholic beverages. They are known to contribute to, and enhance, sweet and fruity aromas in wines (Robinson *et al.*, 2014). The most important acetates and esters in wine are considered to be fatty acid esters and acetates (including phenylethyl acetate, ethyl acetate, ethyl hexanoate, ethyl butyrate) (Rodríguez-Bencomo *et al.*, 2002; Swiegers *et al.*, 2005).

Higher alcohols are mostly produced by yeast from sugar metabolism producing α-keto acid precursors from pyruvate and acetyl-CoA via the tricarboxylic acid (TCA) cycle (Bell & Henschke, 2005; Swiegers *et al.*, 2005). Alternatively some higher alcohols are produced when amino acids are catabolised by yeast via the Ehrlich pathway, producing branched chain higher alcohols (isoamyl and isobutyl alcohol) from branched chain amino acids and aromatic alcohols (phenylethyl alcohol – which is considered to play an important role in white wine aroma) from aromatic amino acids (Francis & Newton, 2005).

Aroma compounds can be manipulated, either at point of origin (as the result of vineyard treatments which cause the up or down-regulation of these compounds within the grapevine) or during physical winemaking processes such as yeast fermentation.

1.2.4. The influence of viticultural practices on Sauvignon Blanc aroma impact compounds

The chemical composition of grapes is the result of the grape genotype (cultivar), the environment and the vineyard management practices implemented throughout grape development (Jackson *et al.*, 1993).The composition of berries changing in response to variations in these conditions. The compositional change is not random. Berries react in specific ways to biotic and abiotic conditions, modulating their composition in response to these changing conditions. These adaptations can be used to change the berry composition at a vineyard level to yield a berry that will deliver a specific

style of wine (Ferrandino & Lovisolo, 2014). Figure 1.4 presents a summary of various abiotic factors, their impacts on aroma compounds (or precursors) and the corresponding viticultural manipulations that can be implemented to mimic/mitigate certain abiotic stress factors. Specific focus is placed on natural/induced microclimatic factors and mechanical harvesting effects.

1.2.4.1. Changing bunch microclimatic factors through viticultural treatments

Studies have found that grapevines acclimate in response to changes in the grapevine microclimate. The grapevine microclimate can change due to various reasons, including changes facilitated through viticultural treatments like leaf and lateral shoot removal. The application of these treatments at various stages of berry development; before flowering (Ryona *et al.*, 2008; Sivilotti *et al.*, 2016), after flowering (Reynolds *et al.*, 1986; Sivilotti *et al.*, 2016), at peppercorn stage (Šuklje *et al.*, 2014; Joubert *et al.*, 2016; Mosetti *et al.*, 2016), pre-véraison (Gregan *et al.*, 2012, 2017; Gregan & Jordan, 2016; Šuklje *et al.*, 2016) and after véraison (Reynolds & Wardle, 1989; Gregan & Jordan, 2016; Gregan *et al.*, 2017) result in various microclimatic changes. These changes are usually a combination of increased temperature and light (Reynolds *et al.*, 1986; Ryona *et al.*, 2008; Song *et al.*, 2015; Gregan & Jordan, 2016; Mosetti *et al.*, 2016; Šuklje *et al.*, 2016), or exclusive changes in light quantity and quality (Gregan *et al.*, 2012; Šuklje *et al.*, 2014; Song *et al.*, 2015; Joubert *et al.*, 2016, Young et al., 2016, Du Plessis, et al., 2017). The changes in microclimate facilitated the modulation of various chemical and biochemical processes within the grape berry, changing the subsequent berry composition (Reynolds *et al.*, 1986; Ryona *et al.*, 2008; Gregan *et al.*, 2012; Song *et al.*, 2015; Gregan & Jordan, 2016; Joubert *et al.*, 2016; Martin *et al.*, 2016; Mosetti *et al.*, 2016; Sivilotti *et al.*, 2016; Šuklje *et al.*, 2016; Young et al, 2016; Du Plessis et al., 2017).

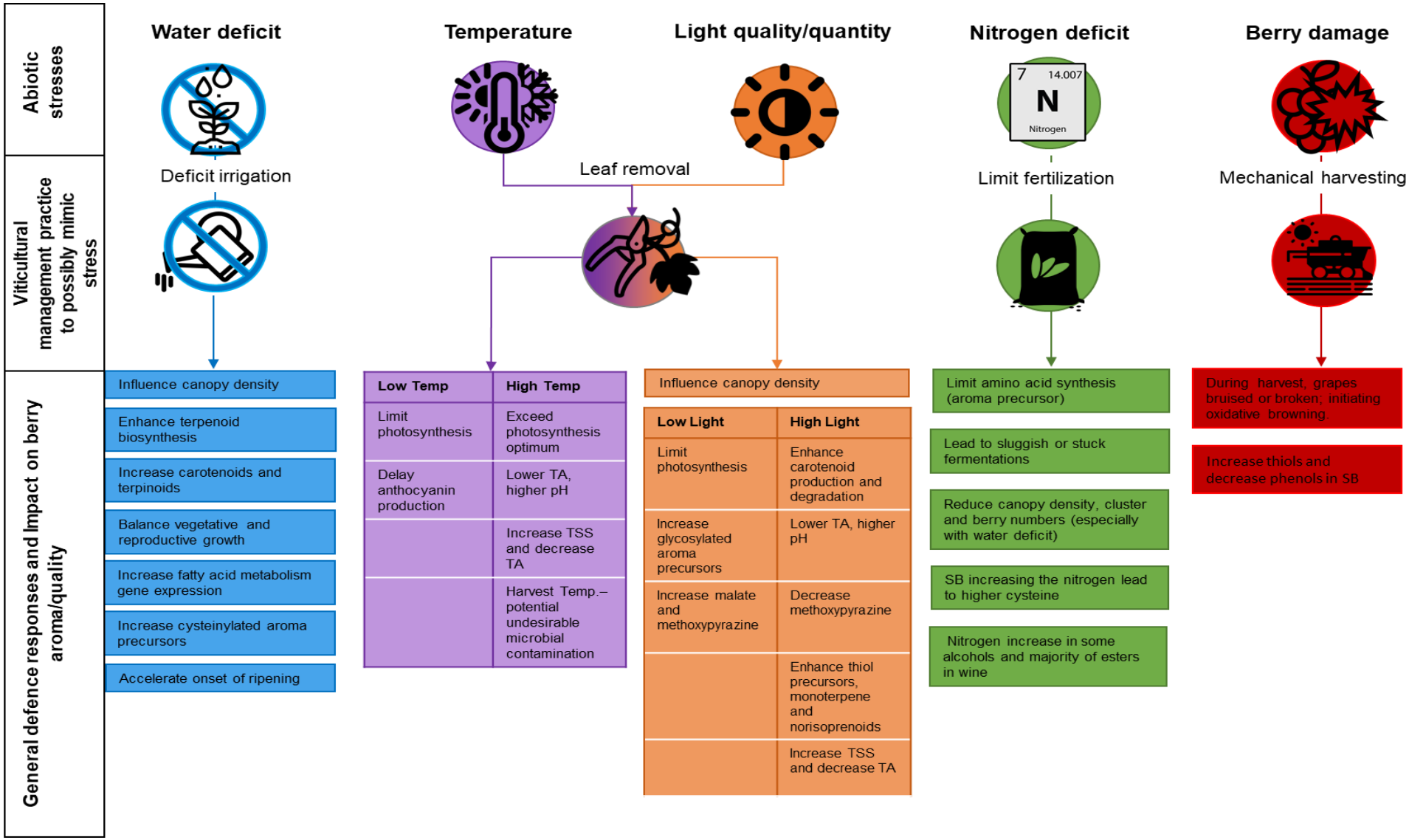


Figure 1.4: A - Overview of direct and indirect berry responses to abiotic stress factors and viticultural management practices that may be used to mimic the stress (Des Gachons *et al.*, 2005; Cramer, 2010; Ferrandino & Lovisolo, 2014; González-Barreiro *et al.*, 2015b)

The effect of temperature on berry composition.

Temperature has been found to have a large impact on berry composition. Elevated temperatures often lead to higher total soluble solids (TSS) and lower titratable acidity (TA), both of which have significant influences on wine quality (Coombe, 1987). Furthermore it has been established that grapevine temperature has a significant effect on the various types and concentrations of aroma compounds produced (as reviewed by Drappier *et al.*, 2019b) in the grape berries, and the subsequent wine. Many of these phenotypic changes are acclimation responses to heat shock experienced by the grapevine (Figure 1.4). The aromatic profile of white grapes and subsequent wine quality is particularly sensitive to high temperatures. However, the influence of temperature on the subsequent wine are often reported by comparing wine attributes between warm and cool seasons within a region, or wine attributes from different regions with contrasting climates (Ashenfelter *et al.*, 1995; Jones & Davis, 2000; Grifoni *et al.*, 2006; Soar *et al.*, 2008); with very few evaluating the effect of temperature alone on the aroma of berries (Spayd *et al.*, 2002; Sadras, Moran, *et al.*, 2013). Furthermore this indirect approach is inconclusive as temperature is often correlated with other climatic factors, such as solar radiation, which also has an influence on grape and wine composition (Figure 1.4). Sadras *et al.* (2013) conducted a study on the effect of elevated temperature on the grapevine, juice pH and TA and wine sensory attributes in a controlled field-like environment, to isolate temperature effects. Different grape cultivars (Cabernet Franc, Chardonnay, Semillon and Shiraz) were exposed to either elevated temperatures (achieved through a heating system) or ambient temperatures (control) over two growing seasons. It was found that juice pH and TA responses were cultivar dependent and responded in one of three ways: (1) In Cabernet Franc and Chardonnay pH increased, whereas the TA decreased; (2) in Shiraz no response was noted and, (3) in Semillon the pH increased whilst the TA was unaffected. Furthermore, elevated temperatures resulted in Semillon wines with reduced green aromas, enhanced mouthfeel and tropical aromas over both seasons, whereas the response of the Shiraz and Cabernet Franc were season dependent (Sadras *et al.*, 2013).

The effect of temperature and light on berry and wine

With leaf removal treatments, it is often difficult to separate temperature and radiation effects, both of which have an influence on berry composition. Methoxypyrazine concentrations in berries are sensitive to both light and temperature. Gregan *et al*. (2016), as well as Mosetti *et al*. (2016), have found that increased light and temperature exposure through leaf removal had a significant impact on methoxypyrazines concentration, though this impact was dependant on when during berry development the treatment was applied. Pre-véraison leaf removal led to a significant decrease in methoxypyrazines concentration, whereas post-véraison leaf removal had no effect. Furthermore it has been found that fruit zone shading resulted in lower total soluble solid and anthocyanidin concentrations (Chorti *et al.*, 2010). A study conducted by Lee *et al.* (2007) on the impact of light exposure on the on the concentration of C_{13}-norisoprenoid concentration in Cabernet Sauvignon

grapes and wine. These compounds have also been identified in SB grapes and wines. The study found that the C_{13}-norisoprenoids responded differentially to leaf removal and vine microclimate. Leaf removal was positively linearly correlated with increasing levels of some C_{13}-norisoprenoids (excluding β-damascenone), whereas the concentration of β-Damascenone was highest in the controls.

The effect of light quantity and quality on berry composition

A number of recent studies have demonstrated that not only light quantity influences phenolic and aroma compounds in grape berries, but also light quality (Gregan *et al.*, 2012; Gil *et al.*, 2013; Šuklje *et al.*, 2014; Joubert *et al.*, 2016; Young *et al.*, 2016). It was found that increased UV-B radiation had a larger effect on Tempranillo grapes than on Viura grapes, resulting in increased concentrations of carotenoids (Núñez-Olivera *et al.*, 2006). The effect of increased UV radiation on phenolic compounds in the skins of the Tempranillo grape berries were studied and it was found that increased UV radiation resulted in increased levels of flavanols and anthocyanins (Carbonell-Bejerano *et al.*, 2014; Martínez-Lüscher *et al.*, 2014). Furthermore it was also found that increased UV radiation of Carignan and Grenache grapes resulted in increased concentrations of anthocyanins (De Oliveira *et al.*, 2015). Gregan *et al.* (2012) found that most of the amino acids and methoxypyrazines in the grape berries (at harvest) did not respond to increased UV radiation, whereas Gil *et al.* (2013) found that some of the volatile organic compounds (monoterpenes, alcohols, aldehydes and ketones) were augmented with increased UV-B radiation. It is suggested that these compounds (mainly monoterpenes) are produced by the plant to protect the tissues from radiation damage (Gil *et al.*, 2013). Similar results were obtained in South African studies on SB by Young *et al.* (2016) and Joubert *et al.* (2016), who found that bunch exposure and UV-B radiation led to higher concentrations of volatile terpenoids (monoterpenes and norisoprenoids) within the later ripening stages of the grape berries. Šuklje *et al.* (2014) furthermore, confirmed the effect of bunch exposure on the aromatic and final sensory profile of SB berries and wine. A twofold effect was observed: a decrease in greener aromas and elevated fruity aromas. The effect therefore of grapevine microclimate is not only on methoxypyrazines, (which are important for SB typicity), but also on other aroma compounds like volatile thiols and monoterpenes.

The effect of mechanical harvesting on Sauvignon Blanc berry and wine composition

Hand harvesting is traditionally preferred over mechanical harvesting of grapes, as mechanical harvesting often leads to quality problems. These are usually associated with bruising and damaging of grapes and higher phenolic content (Jackson, 2008). However, some studies indicate that mechanical harvesting may be beneficial to SB aroma.

Capone *et al.* (2012), measured various compounds in machine harvested SB grapes at certain time points after harvest. It was found that increased berry storage time led to higher concentrations of

volatile thiol precursors and C_6 alcohols. Whereas prolonged post-harvest berry storage led to decreased concentrations of (E)-2-hexenal. Only berries and juice were studied however, and the compound concentrations were not followed to the wine. Another study was done on the effect of mechanical harvesting on the protein and phenolic concentrations of SB juice and wine (Tian *et al.*, 2013). In this study it was found that juice from mechanically harvested grapes had a lower concentration of proteins ; although no significant differences were detected in phenolic concentrations (Tian *et al.*, 2013). In the wine made from mechanically harvested grapes lower protein concentrations were found when compared to wine made from hand harvested grapes (Tian *et al.*, 2013). Furthermore Herbst-Johnstone *et al.* (2013) did a study of the effect of mechanical harvesting on SB aroma and found similar concentrations of methoxypyrazines, fatty acids, terpenes, ethyl esters, higher alcohols and their acetate esters, regardless of the harvesting technique. Mechanical harvesting resulted in higher concentrations of C_6-alcohols, such as hexanol and *cis*-3-hexenol, their associated acetate esters as well as volatile thiols.

1.2.5. The effect of winemaking steps on Sauvignon Blanc aroma composition of the wine (bioconversion and manipulation)

Major biochemical changes occur during winemaking with the transformation of must to wine through alcoholic fermentation. Although significant changes in composition occur during fermentation, the process of winemaking can also have an influence on the aroma composition of SB. In this section, an overview is given of some winemaking steps that have been shown to influence the aromatic composition of SB wine, as well as a summary of the typical bioconversions of the major aroma linked metabolites during wine processing (Figure 1.5).

1.2.5.1. Juice processing and winemaking

Crushing and pressing

During berry crushing, grapes and grape cell walls are ruptured, bringing solutes in contact with enzymes. Contact with air may lead to oxidation of hydroxycinnamates and reduction of glutathione. This leads to an increase in the oxidation potential of the final wine that may cause unsightly browning and/or off-flavours (Kritzinger *et al.*, 2015). Reynolds *et al.* (1993) found that the concentration of free volatile terpenes (FVT) of some cultivars (Kerner) increased with pressing, whilst pressing had no effect on FVT in other grape cultivars (such as Gewürztraminer, Müller-Thurgau and Muscat Ottonel). Later Roland *et al.* (2011) found that during an industrial pressing cycle of SB grapes, the extraction of volatile thiol precursors increased as pressing pressure increased. Patel *et al.* (2010) in a complementary study on SB found that pressed juices and wines exhibited a more rapid decline in glutathione content, more progressive polyphenol oxidation and exhibited lower acidity values. The final SB wines contained less than half the concentration of

volatile thiols. These studies however did not include the analysis of grapes, and no links could be made between the vineyard and grape aromatic potential to the various analyses.

Yeast nutrition, strain and fermentation temperature

Yeast species, strain nutrition and fermentation temperature are all major contributing factors to wine aroma through the production of secondary aroma metabolites and have been researched extensively (Jolly et al., 2003; Swiegers et al., 2005, 2006, 2009; Molina et al., 2007; Sadoudi et al., 2012).

Yeast assimilable nitrogen (YAN) is an essential nutrient for yeast growth and development and inadequate levels lead to sluggish or stuck fermentation and consequently a decrease in wine aroma quality and taste (Rapp & Versini, 1995; Keller, 2015). Ammonium and amino acids make up 40 – 60% of YAN in grape must (Crépin *et al.*, 2012). Not all amino acids metabolised at the same rate – some are metabolised more readily than others and are known as yeast preferred amino acids (Jiranek *et al.*, 1995; Rapp & Versini, 1995; Crépin *et al.*, 2012). Furthermore the metabolism of other amino acids namely the branched chain amino acids (BCAA) and aromatic amino acids (AAA) by yeast will result in the production of various volatile aromatic compounds (esters, higher alcohols, volatile fatty acids), all ultimately contributing to the final sensory perception of the wine (Rapp & Versini, 1995; Swiegers *et al.*, 2006; Hazelwood *et al.*, 2008). Amino acids can therefore be divided into four groups: yeast preferred amino acids (Aspartic acid, Glutamic acid, Asparagine, Serine, Arginine, Alanine, Glutamine), BCAA (Valine, Leucine, Isoleucine), AAA (Tryptophan, Tyrosine, Phenylalanine) and other amino acids (including the sulphur containing amino acids methionine and cysteine) (Šuklje *et al.*, 2016).

Lipids are another essential nutrient that is important for yeast nutrition and performance. The lipids enable the yeast to tolerate high ethanol concentrations by maintaining the plasma membrane of the yeast and limits the risk for sluggish and stuck fermentations (Casalta *et al.*, 2016). A study on the effect of yeast nutrition on fermentation kinetics found that lipid concentrations had a major influence on yeast nutrition and fermentation performance (Houtman, A. C., & Du Plessis, 1986). Prolonged contact with grape tissues (including skins and sediment) may lead to the increased extraction of lipids. However, an abundance of unsaturated fatty acids may lead to the underproduction of some esters, influencing the aroma of wine.

Molina et al. (2007) found that fermentation temperature influenced the type of aroma compounds produced during fermentation: at 15°C, higher concentrations of aroma compounds associated with fruit was formed whereas more compounds related to flowery aromas were formed at higher fermentation temperature (28°C).

Swiegers *et al.* (2009) determined the effect of different *Saccharomyces cerevisiae* wine yeast strains on volatile thiols and fermentation metabolite concentrations in SB wines. The results of the study indicated that the yeast strains varied significantly in terms of their ability to produce volatile and fermentation metabolites and the choice of strain, therefore, contributed significantly to the varietal characteristics of SB. The authors furthermore concluded that whilst the "green" characters of SB could be determined and manipulated in the vineyard through various vineyard practices, the fruity characters appear to be to some extent dependant on the strain of yeast during fermentation. This indicated that there is potential to modulate wine aroma profiles by using specific yeast and thus "tailor" wine styles to consumer demands (Swiegers *et al.*, 2009).

Skin and sediment contact

The degree to which compounds (specifically aroma compounds) are extracted during winemaking is by no means exhaustive, as the waste matrices (pomace and sediment) potentially contain high concentrations of residual compounds (including sugars, amino acids, organic acids, aroma compounds and aroma compound precursors). The waste matrices include pomace after pressing (skin, seeds and some pulp) and sediment after clarification (residues of skin, seeds and pulp). White winemaking (like SB) in particular has very limited extraction as traditionally the must is only in contact with the pomace for a very short amount of time (Jackson, 20014).

It is well-known that the majority of SB aroma compounds (monoterpenes, methoxypyrazines and C_{13}-norisoprenoids) and aroma compound precursors (carotenoids, glycosylated terpenes and cysteinylated thiol precursors) reside in the skin of the grape berry (reviewed by Coetzee *et al.* (2012), Conde *et al.* (2007) and Jackson (2007)), confirming the skin as a major source of aromatic potential of grape berries. However, the skin is also rich in polyphenolic compounds (including tannins) which would contribute undesirable bitterness and astringency to SB wine. Traditionally skin contact time is limited in white winemaking to minimise the extraction of phenolic compounds, however, this also limits the extraction of potential aroma compounds.

Few studies have been done on the effect of prolonged skin contact time on the aroma and quality of white wine (Cabaroglu *et al.*, 1997; Marais, 1998; Selli *et al.*, 2006) and even less on the effect of prolonged sediment contact on wine (Houtman & Du Plessis, 1981; Ancín *et al.*, 1996; Nicolini *et al.*, 2011). Generally, prior studies found that longer skin contact led to an unwanted increased extraction of phenolic compounds and a wanted increase in the extraction of aroma compounds. However, the increase of phenolic compound extraction depended on the pressing pressure, and the use of reductive winemaking techniques. A SB study conducted by Maggu *et al*. (2007) found that prolonged skin contact (32 h) resulted in a greater release of varietal aroma precursors (3MH-S-cys) and aroma compounds (IBMP) into the juice. Furthermore, the concentration was increased by pressing at higher pressure. However, the increase in extraction of the varietal aroma compounds

and precursors was offset by the increase in the oxidative potential of the juice. This could possibly lead to browning and/or of varietal aromas of the must or resulting wine.

The composition of a white must sediment (i.e. the small grape-derived particles from static settling) consist (as percentage of dry weight) mostly out of 72% total sugars, 8% lipids, 5.5% minerals, 5.2% pectin, and ~2.6% nitrogen. The authors concluded that the solid particles were mostly composed of cell wall fragments (Alexandre *et al.,* 1994, Casalta *et al.,* 2016). Since the sediment of wine contains vital reserves of lipids for fermentation, highly filtered juice tends to lead to sluggish or stuck fermentation (Houtman et al., 1980; Nicolini et al., 2011). Lipids are essential for yeast cell membrane health which protects the yeast from various stresses like ethanol toxicity (Casalta *et al*., 2016). A study done by Nicolini *et al.* (2011) found that a moderate increase in juice turbidity led to increased fermentation rates. The increase in fermentation rate could be linked to the increase of nutrient availability during sediment contact. With increasing juice turbidity, the concentrations of certain aroma compounds increase (like C6 compounds and alcohols), however this also resulted in the decrease of esters and fatty acids. These increases could be due to the extraction of fatty acids from the grape particulates, which serve as precursor for higher alcohol production (Casalta et al., 2016).

Bioconversion of aroma-linked metabolites during winemaking

Various grape-derived aroma-related precursors undergo conversions during winemaking, either through enzymatic reactions, due to physical conditions, or metabolism linked to yeasts (Pinheiro *et al.*, 2002; Swiegers *et al.*, 2005; Rodríguez-Bustamante & Sánchez, 2007; Styger *et al.*, 2011; Robinson *et al.*, 2014; González-Barreiro *et al.*, 2015a). Figure 1.5 provides a summary of the major biochemical conversions and the resulting products that occur during the production of wine.

Some compounds are produced via enzymatic reactions (monoterpenes and C_6 compounds – Figure 1.5) and oxidation upon rupturing of the cell walls during crushing and pressing. Most of the biochemical changes in the must medium occur due to yeast metabolism (impacting, amongst others, thiols, esters and higher alcohols). For this reason the yeast strain and yeast nutrition used is particularly important (Swiegers *et al.*, 2005; Styger *et al.*, 2011). Some of the aroma and flavour compounds are directly related to the main carbon metabolism pathway of yeast (for example ethanol, glycerol and acetaldehyde). However many aroma compounds are secondary metabolites and are the result of amino- and fatty acid metabolism by the yeast (Styger *et al.*, 2011). The bioconversion of precursors to form volatile thiols (Figure 1.5) during the production of SB is important, as these thiols are responsible for the fruity characteristics that are typical of SB. The bioconversion of other compounds (like amino acids to esters – Figure 1.5), although not specifically contributing to the varietal characteristic of SB, can contribute other aromas (like esters) which could

influence the perceived sensory attributes (van Wyngaard, 2013; Coetzee & Du Toit, 2015; Wilson, 2017).

It has been found that there is an antagonistic interaction between thiols (3MH - tropical attributes) and esters (linalool - floral attributes) in partially dearomatized Chenin Blanc wines (Wilson, 2017). Campo *et al*. (2005) found similar results with SB wines where the floral character of linalool and 2-phenyl acetate was suppressed in conditions with high concentrations of thiols. Conversely, when the thiols were present in moderate to high concentrations, the esters enhanced the thiol perception (Campo *et al.*, 2005; King *et al.*, 2011). Methoxypyrazines on the other hand, reduced the tropical character of the thiols, and thiols have also been found to reduce the green character of methoxypyrazines, resulting in a mutual suppression (van Wyngaard, 2013). Similar interactions (antagonistic and synergistic) between different aroma compounds in SB have been found in other interaction studies, extensively reviewed by Coetzee *et al.* (2015).

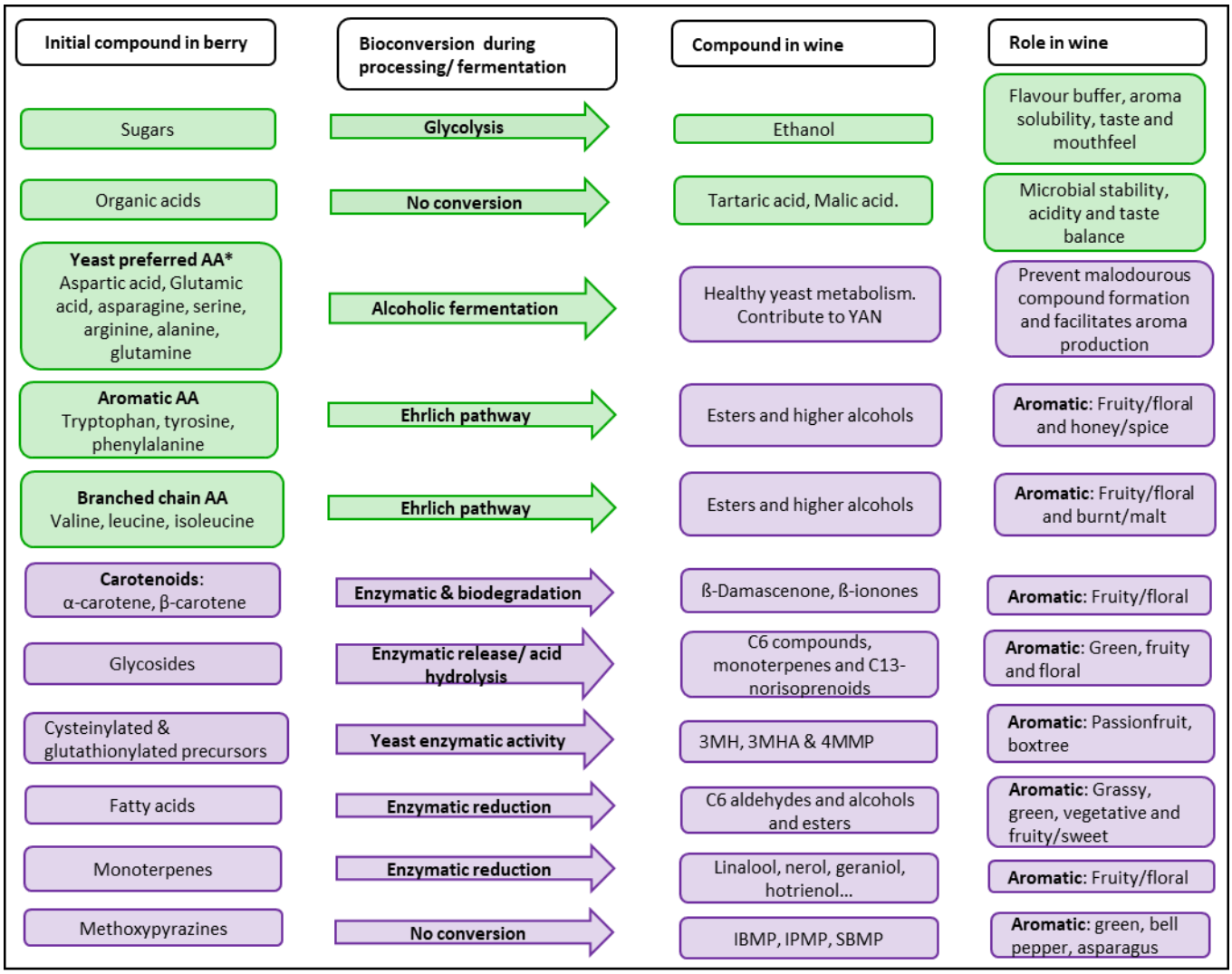


Figure 1.5: Summary of the biochemical reactions occurring during SB juice processing and alcoholic fermentation and the subsequent impacts on wine quality/aroma composition. Information summarised from (Jackson, 20014; Darriet, 2012; Robinson *et al.*, 2014). Different colours depict compound origins and physiological function in berry: **Green** = Primary metabolites, **Purple** = secondary metabolites**.**

Wine flavour, aroma and quality are thus the result of complex interactions between many different aroma compounds. Furthermore, the differences between wine typicity and different styles produced from the same cultivar can be the result of different aroma compounds and compound precursors in grapes. This may also depend on the method of juice processing and the liberation of compound precursors during fermentation. It is therefore important to consider the role and fate of aroma compounds throughout processing to determine whether some aromatic potential is underutilised and whether increased extraction treatments can be used to produce more complex, higher quality wines, with distinctive styles.

1.3. Aims and Objectives

The central question of this study is: To what extent is the aromatic potential of SB grapes extracted from berries and carried over to juice and wine matrices during winemaking, and how will extraction treatments impact SB wine aroma?

Approach and resources available.

The assumption is that important insights could be gained by studying the aromatic potential of SB grapes and wines throughout various matrices of the winemaking process. This will be done by determining the concentrations and distribution of aroma-related compounds at different stages of the winemaking process, to determine if there is an increase/decrease of specific aroma compounds in the juice and wine matrices. The aromatic potentials will be theoretically modulated by the addition of modified winemaking steps, with the purpose to increase extraction of aroma compounds. Furthermore the effect of these modified steps on the sensory profile of finished wine will be determined.

This study will benefit from previous work done on a model vineyard in Elgin, South Africa. During a previous foundational study, Young *et al.* (2016) validated the modulation of light exposure over multiple seasons by removing leaves in the bunch zone after berry set. It was established that there were no significant differences in bunch temperatures. It was shown that through leaf removal in the bunch zone, a high light (HL) microclimate was obtained. Moreover it was verified through metabolite and molecular profiling that the major impacts of increased light exposure on berry metabolite composition were on amino acids and secondary metabolites like pigments and other aroma compounds/precursors (Young *et al.*, 2016; Du Plessis, 2017). Furthermore, it was found that the phenolic composition of berries grown in the two microclimates were significantly different. Samples grown in increased light exposure conditions having significantly higher concentrations of phenolic compounds(Williams, 2019). This finding was further supported by analysing the genes encoding enzymes of the phenylpropanoid pathway which found that there were different gene expression patterns between LL and HL samples (Du Plessis, 2017).

A study by Šuklje *et al.* (2014), conducted in the same model vineyard, included the reduction of UVB radiation as a treatment to determine the impact of both light quantity and quality on the composition of SB wine. Both increased light exposure (light quantity) and UVB radiation (light quality) led to significant changes in both chemical composition and perceived sensory profiles of wines (Šuklje *et al.*, 2014). However, no chemical analyses were conducted on the berries in this study.

Another study conducted in the same viticulture plot by Joubert *et al.* (2016) also included the UVB mitigation treatment, resulting in four microclimates. This study, however, followed a field-omics approach in investigating the effects of modulated light quantity and quality throughout four berry development stages and the winemaking process (including three juice processing stages) on the composition and sensory profile of SB wine. Berries in the green developmental stage being photosynthetically active, responded differentially to exposure and UVB attenuation signals. The berries modulated the metabolic profile in response to these signals, displaying metabolic plasticity to specifically photosynthesis-related metabolites (as these were the main metabolites affected). Ripe berries in contrast also responded to the four microclimates by altering the metabolic profile. The main acclimation response was the formation of volatiles and phenols that had photoprotective and antioxidant abilities (Joubert *et al.*, 2016). Williams (2019) studied the effect of a low light or high light microclimate on the phenolic potentials of grape and wine matrices and confirmed that the HL microclimate yielded increased phenolic potential in the berries. , Interestingly the total polyphenol levels in both LL and HL wines decreased significantly as juice processing proceeded, with most of the polyphenols “lost” to the pomace and sediment fractions with standard white winemaking methods (Williams, 2019).

Against this background, this study aims to perform an integrative analysis of SB aroma compounds, where the focus will be placed on the transitioning of aroma compounds throughout winemaking, from berries to wine, and the evaluation of possible methods to increase extraction of aroma compounds during winemaking and the ultimate impact on the sensory profile of the wines.

Specific objectives of the study:

1. Quantify aroma compounds and certain aroma compound precursors of grapes harvested from LL and HL microclimates from the SB model vineyard to and confirm aromatic potentials of LL and HL berries.

2. Determine the impacts of juice processing, cold, pre-fermentative skin contact, sediment contact and skin + sediment contact during fermentation as modified wine-making steps by quantifying primary and secondary metabolites in juices (during juice processing), in juice sediments (after enzyme clarification), and in wines.

3. Evaluate the effect of increased aroma compound extraction on the aroma profiles on the sensory profiles of the wines made from the different treatments.

The research results obtained from these objectives are presented and contextualised in Chapter two of this thesis, followed by general discussion and conclusions presented in Chapter 3.

1.4. References

Allen, M.S., Lacey, M.J., Harris, R.L. & Brown, W.V., 1991. Contribution of methoxypyrazines to Sauvignon blanc wine aroma. Am. J. Enol. Vitic. 42(2), 109–112.

Ancín, C., Ayestarán, B., Corroza, M., Garrido, J. & González, A., 1996. Influence of prefermentation clarification on the higher alcohol contents of wines. Food Chem. 55(3), 241–249.

Anderson, K. & Aryal, N.R., 2016. Which winegrape varieties are grown where? A global empirical picture. University of Adelaide Press.

Ashenfelter, O., Ashmore, D. & Lalonde, R., 1995. Bordeaux wine vintage quality and the weather. Collect. Investments High Net Worth Invest. 2480(1995), 233–244.

Bell, S.J. & Henschke, P. a, 2005. Implications of nitrogen nutrition for grapes, fermentation and wine. Aust. J. Grape Wine Res. 11(3), 242–295.

Bradshaw, A.D., 1965. Evolutionary Significance of Phenotypic Plasticity in Plants. Adv. Genet. 13(C), 115–155.

Cabaroglu, T., Canbas, A., Baumes, R., Bayonove, C., Lepoutre, J.P. & Günata, Z., 1997. Aroma composition of a white wine of Vitis vinifera L. cv. Emir as affected by skin contact. J. Food Sci. 62(4), 680–683.

Campo, E., Ferreira, V., Escudero, A. & Cacho, J., 2005. Prediction of the wine sensory properties related to grape variety from dynamic-headspace gas chromatography-olfactometry data. J. Agric. Food Chem. 53(14), 5682–5690.

Capone, D.L., Black, C.A. & Jeffery, D.W., 2012. Effects on 3-Mercaptohexan-1-ol precursor concentrations from prolonged storage of sauvignon blanc grapes prior to crushing and pressing. J. Agric. Food Chem. 60(13), 3515–3523.

Carbonell-Bejerano, P., Diago, M.P., Martínez-Abaigar, J., Martínez-Zapater, J.M., Tardáguila, J. & Núñez-Olivera, E., 2014. Solar ultraviolet radiation is necessary to enhance grapevine fruit ripening transcriptional and phenolic responses. BMC Plant Biol. 14(1), 183.

Cheng, G., Liu, Y., Yue, T.X. & Zhang, Z.W., 2015. Comparison between aroma compounds in wines from four Vitis vinifera grape varieties grown in different shoot positions. Food Sci. Technol. 35(2), 237–246.

Chorti, E., Guidoni, S., Ferrandino, A. & Novello, V., 2010. Effect of different cluster sunlight exposure levels on ripening and anthocyanin accumulation in Nebbiolo grapes. Am. J. Enol. Vitic. 61(1), 23–30.

Coetzee, C. & du Toit, W.J., 2012. A comprehensive review on Sauvignon blanc aroma with a focus on certain positive volatile thiols. Food Res. Int. 45(1), 287–298.

Coetzee, C. & Du Toit, W.J., 2015. Sauvignon blanc wine: Contribution of ageing and oxygen on aromatic and non-aromatic compounds and sensory composition: A review. South African J. Enol. Vitic. 36(3), 347–365.

Conde, C., Silva, P., Fontes, N., Dias, A.C.P., Tavares, R.M., Sousa, M.J., Agasse, A., Delrot, S. & Gerós, H., 2007. Biochemical changes throughout grape berry development and fruit and wine quality. Food 1 1–22.

Coombe, B.G., 1987. Distribution of solutes within the developing grape berry in relation to its morphology. Am. J. Enol. Vitic. 38(2), 120–127.

Cramer, G.R., 2010. Abiotic stress and plant responses from the whole vine to the genes. Aust. J. Grape Wine Res. 16(SUPPL. 1), 86–93.

Crépin, L., Nidelet, T., Sanchez, I., Dequin, S. & Camarasa, C., 2012. Sequential use of nitrogen compounds by saccharomyces cerevisiae during wine fermentation: A model based on kinetic and regulation characteristics of nitrogen permeases. Appl. Environ. Microbiol. 78(22), 8102–8111.

Culleré, L., Escudero, A., Cacho, J. & Ferreira, V., 2004. Gas chromatography-olfactometry and chemical quantitative study of the aroma of six Premium quality Spanish aged red wines. J. Agric. Food Chem. 52(6), 1653–1660.

Darriet, P., 2012. Aroma and aroma precursors in grape berry. Biochem. Grape Berry 111–136.

Deloire, A., 2010. Grapevine berry morphology and composition: A review. Wineland Magazine. YearBook. 104-119.

Dokoozlian, N., 2000. Grape berry growth and development. Raisin Prod. Man. 30–37.

Drappier, J., Thibon, C., Rabot, A. & Geny-Denis, L., 2019. Relationship between wine composition and temperature: Impact on Bordeaux wine typicity in the context of global warming—Review. Crit. Rev. Food Sci. Nutr. 59(1), 14–30.

Dunlevy, J.D., Kalua, C.M., Keyzers, R.A. & Boss, P.K., 2009. The production of flavour & aroma compounds in grape berries. In: Grapevine Mol. Physiol. Biotechnol. Second Ed. 293–340.

Ferrandino, A. & Lovisolo, C., 2014. Abiotic stress effects on grapevine (Vitis vinifera L.): Focus on abscisic acid-mediated consequences on secondary metabolism and berry quality. Environ. Exp. Bot. 103 138–147.

Ferreira, V., Ortín, N., Escudero, A., López, R. & Cacho, J., 2002a. Chemical characterization of the aroma of Grenache rosé wines: Aroma extract dilution analysis, quantitative determination, and sensory reconstitution studies. J. Agric. Food Chem. 50(14), 4048–4054.

Ferreira, V., Ortín, N., Escudero, A., López, R. & Cacho, J., 2002b. Chemical characterization of the aroma of Grenache rosé wines: Aroma extract dilution analysis, quantitative determination, and sensory reconstitution studies. J. Agric. Food Chem. 50(14), 4048–4054.

Floris, B., 2018. SAWIS industry statistics.

Francis, I.L. & Newton, J.L., 2005. Determining wine aroma from compositional data. Aust. J. Grape Wine Res. 11(2), 114–126.

Des Gachons, C.P., Van Leeuwen, C., Tominaga, T., Soyer, J.P., Gaudillère, J.P. & Dubourdieu, D., 2005. Influence of water and nitrogen deficit on fruit ripening and aroma potential of Vitis vinifera L cv Sauvignon blanc in field conditions. J. Sci. Food Agric. 85(1), 73–85.

Gil, M., Bottini, R., Berli, F., Pontin, M., Silva, M.F. & Piccoli, P., 2013. Volatile organic compounds characterized from grapevine (Vitis vinifera L. cv. Malbec) berries increase at pre-harvest and in response to UV-B radiation. Phytochemistry 96 148–157.

González-Barreiro, C., Rial-Otero, R., Cancho-Grande, B. & Simal-Gándara, J., 2015a. Wine Aroma compounds in grapes: A critical review. Crit. Rev. Food Sci. Nutr. 55(2), 202–218.

Gregan, S.M. & Jordan, B., 2016. Methoxypyrazine accumulation and O-Methyltransferase gene expression in Sauvignon blanc grapes: The role of leaf removal, light exposure, and berry development. J. Agric. Food Chem. 64(11), 2200–2208.

Gregan, S.M., Wargent, J.J., Liu, L., Shinkle, J., Hofmann, R., Winefield, C., Trought, M. & Jordan, B., 2012. Effects of solar ultraviolet radiation and canopy manipulation on the biochemical composition of

Sauvignon Blanc grapes. Aust. J. Grape Wine Res. 18(2), 227–238.

Gregan, S.M., Winefield, C. & Jordan, B., 2017. Amino acid metabolism and accumulation in 'Sauvignon Blanc' grapes – investigating berry composition in response to canopy manipulation. Acta Hortic. 1188 9–14.

Grifoni, D., Mancini, M., Maracchi, G., Orlandini, S. & Zipoli, G., 2006. Analysis of Italian wine quality using freely available meteorological information. Am. J. Enol. Vitic. 57(3), 339–346.

Hazelwood, L.A., Daran, J.M., Van Maris, A.J.A., Pronk, J.T. & Dickinson, J.R., 2008. The Ehrlich pathway for fusel alcohol production: A century of research on Saccharomyces cerevisiae metabolism (Applied and Environmental Microbiology (2008) 74, 8, (2259-2266)). Appl. Environ. Microbiol. 74(12), 3920.

Herbst-Johnstone, M., Araujo, L.D., Allen, T.A., Logan, G., Nicolau, L. & Kilmartin, P.A., 2013. Effects of mechanical harvesting on "Sauvignon blanc" aroma. In: Acta Hortic. Vol. 978 179–186.

Hopfer, H. & Heymann, H., 2014. Judging wine quality: Do we need experts, consumers or trained panelists? Food Qual. Prefer. 32(PA), 221–233.

Houtman, A.C. & Du Plessis, C.S., 1981. The effect of juice clarity and several conditions promoting yeast growth on fermentation rate, the production of aroma components and wine quality. South African J. Enol. Vitic. 2(2), 71–81.

Houtman, A.C., Marais, J. & Du Plessis, C.S., 1980. Factors affecting the reproducibility of fermentation of grapes jiuce and of the aroma composition of wines. I. Grape maturity, sugar, inoculum concentration, aeration, juice turbidity and ergosterol. Vitis 54(19), 37–54.

Jackson, D.I., Lombard, P.B. & Kabinett, L.Q., 1993. Environmental and management practices affecting grape composition and wine quality - A review. Am. J. Enol. Vitic. 44(4), 409–430.

Jackson, R.S., 2014. Wine science : principles, practice, perception. Fourth edition. Academic Press.

Jackson, R.S., 2007. Chemical constituents of grapes and wine. In: Wine Sci. 232–280.

Jackson, R.S., 2009. Wine Tasting - A Professional Handbook. (Second Edition) Academic Press.

Jiang, B., Xi, Z., Luo, M. & Zhang, Z., 2013. Comparison on aroma compounds in Cabernet Sauvignon and Merlot wines from four wine grape-growing regions in China. Food Res. Int. 51(2), 482–489.

Jiranek, V., Langridge, P. & Henschke, P.A., 1995. Amino acid and ammonium utilization by Saccharomyces cerevisiae wine yeasts from a chemically defined medium. Am. J. Enol. Vitic. 46(1), 75–83.

Jolly, N.P., Augustyn, O.H.P. & Pretorius, I.S., 2003. The effect of Non-Saccharomyces yeasts on fermentation and wine quality. South African J. Enol. Vitic. 24(2), 55–62.

Jones, G. V & Davis, R.E., 2000. Climate influences on grapevine phenology, grape composition, and wine production and quality for Bordeaux, France. Am. J. Enol. Vitic. 51 (3)(3), 249-261.

Joubert, C., Young, P.R., Eyeghe-Bickong, H.A. & Vivier, M.A., 2016. Field-grown grapevine berries use carotenoids and the associated xanthophyll cycles to acclimate to UV exposure differentially in high and low light (shade) conditions. Front. Plant Sci. 7(June), 786.

Keller, M., 2015. The Science of Grapevines: Anatomy and Physiology. Second Edition. Academic Press

Kennedy, B.Y.J., 2002. Understanding grape berry development. Pract. Winer. Vineyard 1–5.

King, E.S., Osidacz, P., Curtin, C., Bastian, S.E.P. & Francis, I.L., 2011. Assessing desirable levels of sensory properties in Sauvignon Blanc wines - consumer preferences and contribution of key aroma compounds. Aust. J. Grape Wine Res. 17(2), 169–180.

Kritzinger, E.C., Bauer, F.F. & Du Toit, W.J., 2015. Role of glutathione in winemaking. J. Agric. Food Chem. 61(May), 269–277.

Lacey, M.J., Allen, M.S., Harris, R.L.N. & Brown, W.V., 1991. Methoxypyrazines in Sauvignon blanc Grapes and Wines. Am. J. Enol. Vitic. 42(2), 103–108.

Lee, S.H., Seo, M.J., Riu, M., Cotta, J.P., Block, D.E., Dokoozlian, N.K. & Ebeler, S.E., 2007. Vine microclimate and norisoprenoid concentration in Cabernet Sauvignon grapes and wines. Am. J. Enol. Vitic. 58(3), 291–301.

Marais, J., 1998. Effect of grape temperature, oxidation and skin contact on Sauvignon blanc juice and wine composition and wine quality. South African J. Enol. Vitic. Stellenbosch 19(1), 10–16.

Marais, J., Hunter, J.J. & Haasbroek, P.D., 1999. Effect of canopy microclimate , season and region on Sauvignon blanc grape composition and wine quality. South African J. Oenology Vitic. 20(1), 1–30.

Martin, D., Grose, C., Fedrizzi, B., Stuart, L., Albright, A. & McLachlan, A., 2016. Grape cluster microclimate influences the aroma composition of Sauvignon blanc wine. Food Chem. 210 640–647.

Martínez-Lüscher, J., Sánchez-Díaz, M., Delrot, S., Aguirreolea, J., Pascual, I. & Gomès, E., 2014. Ultraviolet-B radiation and water deficit interact to alter flavonol and anthocyanin profiles in grapevine berries through transcriptomic regulation. Plant Cell Physiol. 55(11), 1925–1936.

Mendez-Costabel, M.P., Wilkinson, K.L., Bastian, S.E.P., McCarthy, M., Ford, C.M. & Dokoozlian, N., 2013. Seasonal and regional variation of green aroma compounds in commercial vineyards of vitis vinifera L. Merlot in California. Am. J. Enol. Vitic. 64(4), 430–436.

Molina, A.M., Swiegers, J.H., Varela, C., Pretorius, I.S. & Agosin, E., 2007. Influence of wine fermentation temperature on the synthesis of yeast-derived volatile aroma compounds. Appl. Microbiol. Biotechnol. 77(3), 675–687.

Moreno, J.A., Zea, L., Moyano, L. & Medina, M., 2005. Aroma compounds as markers of the changes in sherry wines subjected to biological ageing. Food Control 16(4), 333–338.

Mosetti, D., Herrera, J.C., Sabbatini, P., Green, A., Alberti, G., Peterlunger, E., Lisjak, K. & Castellarin, S.D., 2016. Impact of leaf removal after berry set on fruit composition and bunch rot in “Sauvignon blanc”. Vitis - J. Grapevine Res. 55(2), 57–64.

Nicolini, G., Moser, S., Román, T., Mazzi, E. & Larcher, R., 2011. Effect of juice turbidity on fermentative volatile compounds in white wines. Vitis - J. Grapevine Res. 50(3), 131–135.

Núñez-Olivera, E., Martínez-Abaigar, J., Tomás, R., Otero, S. & Arróniz-Crespo, M., 2006. Physiological effects of solar ultraviolet-B exclusion on two cultivars of Vitis vinifera L. from La Rioja, Spain. Area 4 441–448.

OIV, 2017. Distribution of the world’s grapevine varieties.

De Oliveira, A.F., Mercenaro, L., Del Caro, A., Pretti, L. & Nieddu, G., 2015. Distinctive anthocyanin accumulation responses to temperature and natural UV radiation of two field-grown (Vitis vinifera L.) cultivars. Molecules 20(2), 2061–2080.

Palomo, E.S., Díaz-Maroto, M.C., Viñas, M.A.G., Soriano-Pérez, A. & Pérez-Coello, M.S., 2007. Aroma profile of wines from Albillo and Muscat grape varieties at different stages of ripening. Food Control 18(5), 398–403.

Parr, W. V., Green, J.A., White, K.G. & Sherlock, R.R., 2007. The distinctive flavour of New Zealand Sauvignon blanc: Sensory characterisation by wine professionals. Food Qual. Prefer. 18(6), 849–861.

Patel, P., Herbst-Johnstone, M., Lee, S.A., Gardner, R.C., Weaver, R., Nicolau, L. & Kilmartin, P.A., 2010. Influence of juice pressing conditions on polyphenols, antioxidants, and varietal aroma of sauvignon blanc microferments. J. Agric. Food Chem. 58(12), 7280–7288.

Pinheiro, C., Rodrigues, C.M., Schäfer, T. & Crespo, J.G., 2002. Monitoring the aroma production during wine-must fermentation with an electronic nose. Biotechnol. Bioeng. 77(6), 632–640.

Pinu, F.R., Edwards, P.J.B., Jouanneau, S., Kilmartin, P.A., Gardner, R.C. & Villas-Boas, S.G., 2014. Sauvignon blanc metabolomics: Grape juice metabolites affecting the development of varietal thiols and other aroma compounds in wines. Metabolomics 10(4), 556–573.

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

Rapp, A. & Versini, G., 1995. Influence of nitrogen compounds in grapes on aroma compounds of wines. Dev. Food Sci. 37(C), 1659–1694.

Reynolds, A.G. & Wardle, D. a., 1989. Impact of various canopy manipulation techniques on growth, yield, fruit composition, and wine quality of Gewürztramine. Am. J. Enol. Vitic. 40(2), 121–129.

Reynolds, A.G., Pool, R.M. & Mattick, L.R., 1986. Influence of cluster exposure on fruit composition and wine quality of Seyval blanc grapes. VITIS - J. Grapevine Res. 25(2), 85.

Reynolds, A.G., Wardle, D. & Dever, M., 1993. Terpene response to pressing , harvest date , and skin contact in Vitis vinifera. HortScience 28(9), 920–924.

Ribéreau-Gayon, P., Boidron, J.N. & Terrier, A., 1975. Aroma of Muscat grape varieties. J. Agric. Food Chem. 23(6), 1042–1047.

Rienth, M., Torregrosa, L., Luchaire, N., Chatbanyong, R., Lecourieux, D., Kelly, M.T. & Romieu, C., 2014. Day and night heat stress trigger different transcriptomic responses in green and ripening grapevine (vitis vinifera) fruit. BMC Plant Biol. 14 108.

Rienth, M., Torregrosa, L., Sarah, G., Ardisson, M., Brillouet, J.M. & Romieu, C., 2016. Temperature desynchronizes sugar and organic acid metabolism in ripening grapevine fruits and remodels their transcriptome. BMC Plant Biol. 16(1), 1–23.

Robinson, A.L., Boss, P.K., Solomon, P.S., Trengove, R.D., Heymann, H. & Ebeler, S.E., 2014. Origins of grape and wine aroma. Part 1. Chemical components and viticultural impacts. Am. J. Enol. Vitic. 65(1), 1–24.

Robinson, J. & Harding, J., 2014. The oxford companion to wine, third edition. Oxford University Press, Incorporated.

Rodríguez-Bencomo, J.J., Conde, J.E., Rodríguez-Delgado, M.A., García-Montelongo, F. & Pérez-Trujillo, J.P., 2002. Determination of esters in dry and sweet white wines by headspace solid-phase microextraction and gas chromatography. J. Chromatogr. A 963(1–2), 213–223.

Rodríguez-Bustamante, E. & Sánchez, S., 2007. Microbial production of C13-norisoprenoids and other aroma compounds via carotenoid cleavage. Crit. Rev. Microbiol. 33(3), 211–230.

Roland, A., Schneider, R., Charrier, F., Cavelier, F., Rossignol, M. & Razungles, A., 2011. Distribution of varietal thiol precursors in the skin and the pulp of Melon B. and Sauvignon Blanc grapes. Food Chem. 125(1), 139–144.

Roland, A., Cavelier, F. & Schneider, R., 2012. How organic and analytical chemistry contribute to knowledge of the biogenesis of varietal thiols in wine. A review. Flavour Fragr. J. 27(4), 266–272.

Ryona, I., Pan, B.S., Intrigliolo, D.S., Lakso, A.N. & Sacks, G.L., 2008. Effects of cluster light exposure on 3-isobutyl-2-methoxypyrazine accumulation and degradation patterns in red wine grapes (Vitis vinifera L. Cv. Cabernet Franc). J. Agric. Food Chem. 56(22), 10838–10846.

Sadoudi, M., Tourdot-Maréchal, R., Rousseaux, S., Steyer, D., Gallardo-Chacón, J.J., Ballester, J., Vichi, S., Guérin-Schneider, R., Caixach, J. & Alexandre, H., 2012. Yeast-yeast interactions revealed by aromatic profile analysis of Sauvignon Blanc wine fermented by single or co-culture of non-Saccharomyces and Saccharomyces yeasts. Food Microbiol. 32(2), 243–253.

Sadras, V.O., Moran, M.A. & Bonada, M., 2013. Effects of elevated temperature in grapevine. I Berry sensory traits. Aust. J. Grape Wine Res. 19(1), 95–106.

Sadras, V.O., Petrie, P.R. & Moran, M.A., 2013. Effects of elevated temperature in grapevine. II juice pH, titratable acidity and wine sensory attributes. Aust. J. Grape Wine Res. 19(1), 107–115.

Seifert, R.M., Buttery, R.G., Guadagni, D.G., Black, D.R. & Harris, J.G., 1972. Synthesis and Odor Properties of Some Additional Compounds Related to 2-Isobutyl-3-methoxy pyrazine. J. Agric. Food Chem. 20(1), 135–137.

Selli, S., Canbas, A., Cabaroglu, T., Erten, H., Lepoutre, J.P. & Gunata, Z., 2006. Effect of skin contact on the free and bound aroma compounds of the white wine of Vitis vinifera L. cv Narince. Food Control 17(1), 75–82.

Sivilotti, P., Herrera, J.C., Lisjak, K., Baša Česnik, H., Sabbatini, P., Peterlunger, E. & Castellarin, S.D., 2016. Impact of leaf removal, applied before and after flowering, on anthocyanin, tannin, and methoxypyrazine concentrations in Merlot' (Vitis vinifera L.) grapes and wines. J. Agric. Food Chem. 64(22), 4487–4496.

Soar, C.J., Sadras, V.O. & Petrie, P.R., 2008. Climate drivers of red wine quality in four contrasting Australian wine regions. Aust. J. Grape Wine Res. 14(2), 78–90.

Song, J., Smart, R., Wang, H., Dambergs, B., Sparrow, A. & Qian, M.C., 2015. Effect of grape bunch sunlight exposure and UV radiation on phenolics and volatile composition of Vitis vinifera L. cv. Pinot noir wine. Food Chem. 173 424–431.

Spayd, S.E., Tarara, J.M., Mee, D.L. & Ferguson, J.C., 2002. Separation of sunlight and temperature effects on the composition of Vitis vinifera cv. Merlot berries. Am. J. Enol. Vitic. 3(December 2001), 171–182.

Styger, G., Prior, B. & Bauer, F.F., 2011. Wine flavor and aroma. J. Ind. Microbiol. Biotechnol. 38(9), 1145–1159.

Šuklje, K., Antalick, G., Coetzee, Z., Schmidtke, L.M., Baša Česnik, H., Brandt, J., du Toit, W.J., 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. 20(2), 223–233.

Šuklje, K., Antalick, G., Buica, A., Langlois, J., Coetzee, Z.A., Gouot, J., Schmidtke, L.M. & Deloire, A., 2016. Clonal differences and impact of defoliation on Sauvignon blanc (Vitis vinifera L.) wines: A chemical and sensory investigation. J. Sci. Food Agric. 96(3), 915–926.

Swiegers, J.H., Bartowsky, E.J., Henschke, P.A. & Pretorius, I.S., 2005. Yeast and bacterial modulation of wine aroma and flavour. Aust. J. Grape Wine Res. 11(2), 139–173.

Swiegers, J.H., Willmott, R., Hill-Ling, A., Capone, D.L., Pardon, K.H., Elsey, G.M., Howell, K.S., de Barros Lopes, M.A., Sefton, M.A., Lilly, M. & Pretorius, I.S., 2006. Modulation of volatile thiol and ester aromas by modified wine yeast. Dev. Food Sci. 43(C), 113–116.

Swiegers, J.H., Kievit, R.L., Siebert, T., Lattey, K.A., Bramley, B.R., Francis, I.L., King, E.S. & Pretorius, I.S., 2009. The influence of yeast on the aroma of Sauvignon Blanc wine. Food Microbiol. 26(2), 204–211.

Tian, B., Harrison, R., Morton, J., Jaspers, M., Grose, C. & Trought, M., 2013. Effects of mechanical harvesting and grape processing on the composition of "Sauvignon blanc" juice and wine. In: Acta Hortic. Vol. 978 187–190.

Torregrosa, L., Bigard, A., Doligez, A., Lecourieux, D., Rienth, M., Luchaire, N., Pieri, P., Chatbanyong, R., Shahood, R., Farnos, M., Roux, C., Adiveze, A., Pillet, J., Sire, Y., Zumstein, E., Veyret, M., Le Cunff, L., Lecourieux, F., Saurin, N., Muller, B., Ojeda, H., Houel, C., Péros, J.P., This, P., Pellegrino, A. & Romieu, C., 2017. Developmental, molecular and genetic studies on grapevine response to temperature open breeding strategies for adaptation to warming. Oeno One 51(2), 155–165.

Williams, D.L., 2019. Linking the phenolic potential of grape berries with polyphenol distribution during wine making : A Sauvignon Blanc case study. (MSc), Stellenbosch University, Stellenbosch, South Africa.

Wilson, B., Strauss, C. & Williams, P., 1986. The distribution of free and glycosidically-bound monoterpenes among skin, juice, and pulp fractions of some white grape varieties. Am. J. Enol. Vitic. 37(2), 107–111.

Wilson, C., 2017. Chemical evaluation and sensory relevance of thiols in South African Chenin Blanc wines. (MSc), Stellenbosch University, Stellenbosch, South Africa.

van Wyngaard, E., 2013. Volatiles playing an important role in South African Sauvignon blanc wines. (MSc), Stellenbosch University, Stellenbosch, South Africa.

Yang, Y.X., Li, X.L., Wang, L., Han, S.Y., Zhang, Y.R., Pratheeshkumar, P., Wang, X., Lu, J., Yin, Y.Q., Sun, L.J., Budhraja, A., Hitron, A.J. & Ding, S.Z., 2013. Anti-apoptotic proteins and catalase-dependent apoptosis resistance in nickel chloride-transformed human lung epithelial cells. Int. J. Oncol. 43(3), 936–946.

Young, P., Eyeghe-Bickong, H.A., du Plessis, K., Alexandersson, E., Jacobson, D.A., Coetzee, Z.A., Deloire, A. & Vivier, M.A., 2016. Grapevine plasticity in response to an altered microclimate: Sauvignon Blanc modulates specific metabolites in response to increased berry exposure. Plant Physiol. 170(March), 1235–1254.

Zelena, K., Hardebusch, B., Hülsdau, B., Berger, R.G. & Zorn, H., 2009. Generation of norisoprenoid flavors from carotenoids by fungal peroxidases. J. Agric. Food Chem. 57(21), 9951–9955.

Chapter 2
The release and fate of Sauvignon Blanc aroma compounds in a grape-to-juice-to-wine analysis

2.1. Introduction

Berry composition is determined by the cultivar, the developmental progression of the berries and stage of ripening, as well as several environmental factors, including temperature (Coombe, 1987; Sadras *et al.*, 2013; Drappier *et al.*, 2019), light (Gregan *et al.*, 2012; Šuklje *et al.*, 2014; Young *et al.*, 2015; Joubert *et al.*, 2016; Mosetti *et al.*, 2016; Šuklje *et al.*, 2016), and water availability (Des Gachons *et al.*, 2005; Kobayashi *et al.*, 2011) that can all modulate the levels and profile of berry compounds, and by extension wine quality impact factors. Grape berries consist out of three main tissue types: the exocarp (skin), mesocarp (pulp) and endocarp (seeds), all of which have unique compositions (Jackson, 2014). The skins in particular accumulate the majority of the aroma compounds as well as the anthocyanin pigments that are present in red, but not white grapes. The basic berry composition is similar in most white grape types, but varietal typicity is determined by the profile and levels of compounds that are distinctive to a specific grape cultivar. These compounds are found in grapes prior to fermentation in both volatile and non-volatile forms (the latter in the case of bound aromas and precursors that are only released during processing and wine fermentation) (Lanaridis *et al.*, 2002).

Sauvignon Blanc (SB) is a cultivar with well-defined typicity and wine style characteristics - SB wines are associated with “green” aromas (“vegetative”, “grassy”, “herbaceous”, “asparagus”, “green pepper”, “capsicum”, “tomato leaf”) as well as tropical aromas (“grapefruit”, “gooseberry”, and “passion fruit”) (Swiegers *et al.*, 2009). Some of the aroma-active compounds of SB are grape-derived that have been shown to be sensitive to environmental factors such as temperature and light quantity (Marais *et al.*, 1999; Coetzee & du Toit, 2012; Šuklje *et al.*, 2014). There are ample reports that have shown that SB berries subjected to specific viticultural management practices, such as early leaf removal in the bunch zone, can lead to increased aroma-linked compounds in the ripe berries (Reynolds *et al.*, 1986; Ryona *et al.*, 2008; Gregan *et al.*, 2012; Song *et al.*, 2015; Gregan & Jordan, 2016; Joubert, Young, Eyeghe-Bickong, *et al.*, 2016; Martin *et al.*, 2016; Mosetti *et al.*, 2016; Sivilotti *et al.*, 2016; Šuklje, Antalick, Buica, Langlois, *et al.*, 2016). It has also been shown that in a single vineyard of SB, two distinct wine styles could be achieved (Šuklje *et al.*, 2014; Joubert, Young, Eyeghe-Bickong, *et al.*, 2016; Young *et al.*, 2016). Grapes from a high light (HL) microclimate had different and higher concentrations of aroma-related metabolites and precursors (more “fruity”) compared to grapes from a low light (LL) microclimate (fewer aroma-compounds, more “green” and

"herbaceous" notes) (Šuklje *et al.*, 2014; Young *et al.*, 2015; Joubert, *et al.*, 2016; du Plessis *et al.*, 2017). Despite the large number of reports that confirmed that it is possible to manipulate the aromatic potential of the SB berries in the vineyard, limited information is available on the distribution, transfer and/or fate of grape-derived aroma compounds throughout the processing steps (from the grape berries to grape juice to fermenting must and finally the wine). In previous studies, the impact of the viticultural treatments and associated microclimatic impacts on berry and wine composition were evaluated and used a standardised white wine making procedure with all samples (Šuklje *et al.*, 2014). The results lead us to the following question: How much (more) grape-derived aroma compounds could potentially be extracted and what would the influence be on the sensory perception in the wines?

From a winemaking perspective, contact time with tissues (like skins) that contain high levels of aromatic compounds and precursors would determine their levels in the subsequent matrices such as juice and fermenting must and ultimately wine. During white winemaking this contact occurs during the first two steps of wine making, namely: crushing and pressing. These two steps physically disrupt the structure of the berry tissue, facilitating the extraction of grape metabolites into the grape juice. The degree of extraction of aroma-linked metabolites from grape tissue to juice is not exhaustive, and the contact time and temperature between juice and macerated tissue during traditional white wine making possibly limits the extraction of quality-associated compounds.

Enhanced contact time (cold maceration or pre-fermentative skin contact) enhance the extraction of certain aroma-linked metabolites, and also phenolic compounds (Maggu *et al.*, 2007; Darriet, 2012; Gawel *et al.*, 2014). Prolonged skin contact, however, is not a routine practice in white winemaking. High(er) concentrations of phenolic compounds result in increased astringency and bitterness which is undesirable in the sensory profile of white wines. Selli *et al.* (2006) conducted a study on the increased extraction of aroma compounds associated with floral and fruity aromas, and found that although there was an increase in these compounds with skin contact, that this effect was cultivar dependant. Cabaroglu *et al.* (1997) found that skin contact resulted in higher levels of free volatiles (including monoterpenes and C_6 compounds); however, there was no sensory differences between the wines in terms of an increase in "green" aromas in the wines. Furthermore, it was concluded that the increase in these volatiles could partially be due to increased concentrations of either the compounds themselves (e.g. monoterpenes) or precursors of the compounds that were extracted during skin contact. Marais (1998) did a similar study on Sauvignon Blanc in South Africa and found that there were increased levels of methoxypyrazine (IBMP) in skin contact samples versus the free run (control) samples. While an increase in the duration of skin contact can improve aroma compound extraction, it can also increase extraction of other compounds that can influence the yeast performance. Examples include pesticides which could inhibit yeast growth, increased nitrogen concentrations which result in quicker fermentation, and introduction of possible competitive

microorganisms. These factors could lead to specific yeast responses, possibly changing the composition of the final wine.

Occasionally during white wine production, some of the precipitate obtained after clarification (hereafter referred to as sediment) may be kept in contact with the fermenting must, permitting vital yeast nutrients like sterols and unsaturated fatty acids to remain available during fermentation (Jackson, 2014). Houtman, Marais & Du Plessis (1980) tested the effect of juice turbidity on fermentation rate and ester production. By reintroducing sediment to clarified juice (either at levels of 1%, 2%, 5%, 10%, 20%, 50%, or 100%), it was found that fermentation of slightly turbid juice (5%) had higher levels of ester production as well as increased fermentation rates. This agreed with other studies which also found that fermentations made with highly filtered juice proceeded slower (Houtman & Du Plessis, 1981; Ferrando *et al.*, 1998; Nicolini *et al.*, 2011), and did not complete fermentation unless fermentation stimulants were added. It was also found that with high juice turbidity there was also an increase of higher alcohol production, causing the formation of malodours (Houtman *et al.*, 1980b; Ancín *et al.*, 1996). This is possibly due to the high fermentation rate of the yeast resulting in the production of the off-odours and early yeast death due to ethanol toxicity.

Here we describe a set of experiments to evaluate the potential extraction of SB grape-derived aroma compounds and precursors through standard or adjusted juice and wine processing steps. We were interested to explore the concept of berry aromatic potential and evaluate how much of this potential is used (or discarded) when we employ standard white-wine making where skins (the source of most aromatic compounds) are only briefly in contact with the juice and must matrices, compared to winemaking procedures which would potentially enhance extraction.

2.2. Materials and Methods

Vineyard site, experimental layout, harvesting and sampling

For the experiment, a commercial model *Vitis vinifera* L. cv. Sauvignon Blanc vineyard, situated in the Elgin region (34°9952.1999S; 19°0957.4899E) in the Western Cape of South Africa was used (2016/2017). A detailed description of the vineyard and experimental design, as well as prior work achieved from this vineyard can be found in Šuklje *et al.* (2014); Young *et al.* (2015); Joubert *et al.* (2016) and du Plessis *et al.* (2017). To contextualise this follow-up study, a brief summary is provided here (refer to Figure 2.1). The vineyard owners performed an early, pre-fruit set leaf-removal treatment throughout the vineyard at the beginning of the 2017 season. This process was not part of the previously described experiments (Joubert, Young, Eyeghe-Bickong, *et al.*, 2016; Young *et al.*, 2016; Honeth, 2018). For the purpose of our experiments, leaves and laterals were removed in

the bunch zone (on the East-facing side of the canopy that receives morning sun exposure) at Eichorn-Lorenz (EL) 29 according to the experimental layout shown in Figure 2.1. The leaf removal was performed in alternating panels, leading to panels where bunches were more exposed to light than where leaves were not removed, therefore creating two distinct microclimates in the bunch zones, a low light (LL) and high light (HL) microclimate. Grape bunches from the HL and LL panels were monitored for progression of ripeness and harvesting occurred on 10 March 2017 when the grapes reached 19-21°B.

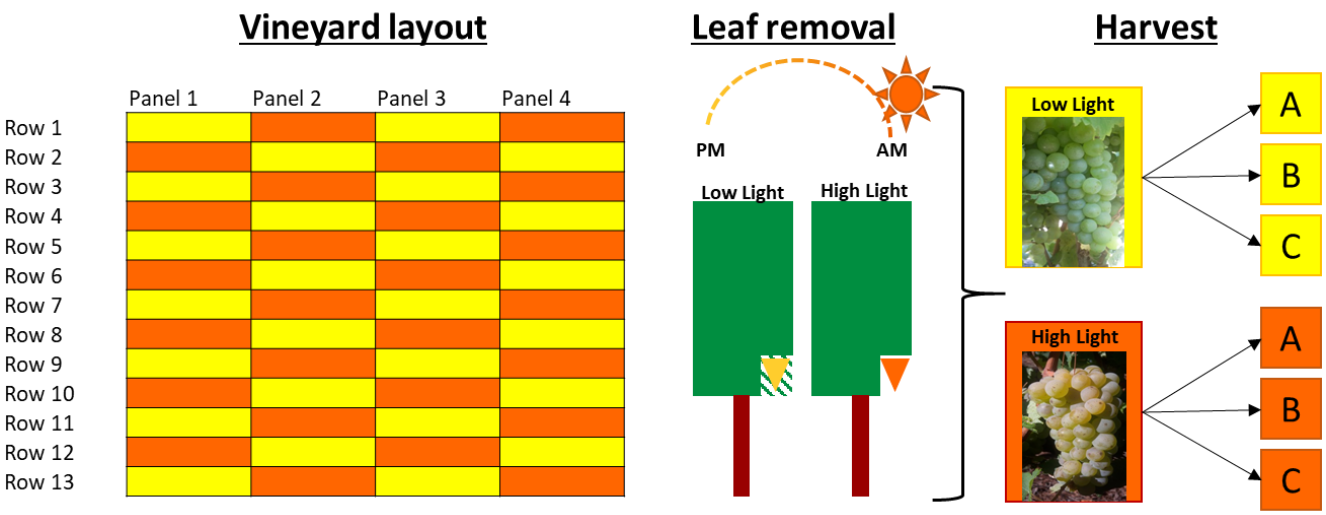


Figure 2.1: Layout of experimental vineyard and harvesting strategy. Leaves were removed on the East side which received morning sun. Grapes were harvested from the LL and HL panels, respectively, and from those pools, split into 3 biological repeats per microclimate (A, B and C). **Key**: Yellow, Low Light panels; Orange, High Light panels.

During harvesting, special attention was paid with regards to bunches chosen for harvesting – only bunches that were completely in the shade were considered representative of the LL microclimate, and conversely only exposed bunches were harvested from the HL micro-climate. From the harvested grapes, three lots per microclimate were randomised to yield three biological repeats/microclimates for subsequent winemaking (refer to Figure 2.1). From each biological repeat, berries (n=48) were randomly sampled and flash frozen in liquid nitrogen and stored at -80°C until further analysis. The frozen berries were homogenised and subjected to chemical analysis for selected primary and secondary metabolites. The harvested grapes were subjected to juice processing and winemaking as outlined below.

Standard and adjusted grape/juice processing and winemaking steps to potentially allow increased extraction from grape matrices

Harvested grapes were cooled overnight at 4°C and crushed the following morning. Figure 2.2 outlines the experimental design implemented to make wines from grapes from the two

microclimates, as well as which steps in the winemaking process were subjected to experimentation how samples for further analysis were generated and which analyses were performed on the samples.

For winemaking the three biological repeats per microclimate (Figure 2.1) were kept separate throughout standard and adjusted winemaking. A standard white winemaking procedure consisted of the following steps: de-stemming and crushing, pressing, enzyme clarification, inoculation with yeast and fermentation to dryness, cold shock and stabilisation, and finally bottling of the wines (Figure 2.2). Samples were also obtained from the free-run juice to compare with the pressed and clarified juice from each microclimate. The standard wine making procedure was modulated to evaluate increased extraction (of aromatic compounds) from the grape and fermentation matrices (Figure 2.2). The following treatments were included: extended skin contact, prolonged contact with the sediment during fermentation, as well as a combination of skin contact and sediment contact. To evaluate the effect that prolonged contact with grape skins would have on the aroma-related compounds grapes were either pressed immediately after crushing, or after a 24 h cold maceration period (skin contact), in the presence of dry ice to prevent oxidation. To evaluate the effect of sediment on the aromatic compounds, after crushing and pressing, the juice was allowed to clarify (Rapidase Clear [1 ml/hL], Oenobrands, Montpellier) overnight at 4°C before being racked from the sediment and inoculated with *Saccharomyces cereviseae* (Cross Evolution, Lallemand [0.25g/L]) for alcoholic fermentation, or inoculated immediately in the presence of the sediment (sediment contact) without clarification. SO_2 and dry ice were used throughout in an attempt to keep the system as reductive as possible. A combined treatment of skin and sediment contact was also included for each microclimate. Fermentation was monitored by measuring the weight loss. Once the weight loss stabilised/plateaued, a random sample was taken and the residual sugar levels measured. If the sugar concentration was less than 5 g/L, fermentation was considered complete and stopped by addition of 50 ppm SO_2 and stored at -4°C for 2 weeks (cold stabilisation). Fermented wine was racked from the lees, free SO_2 adjusted to 40 ppm, before being bottled and stored at 15°C for 6 months prior to sensory analysis.

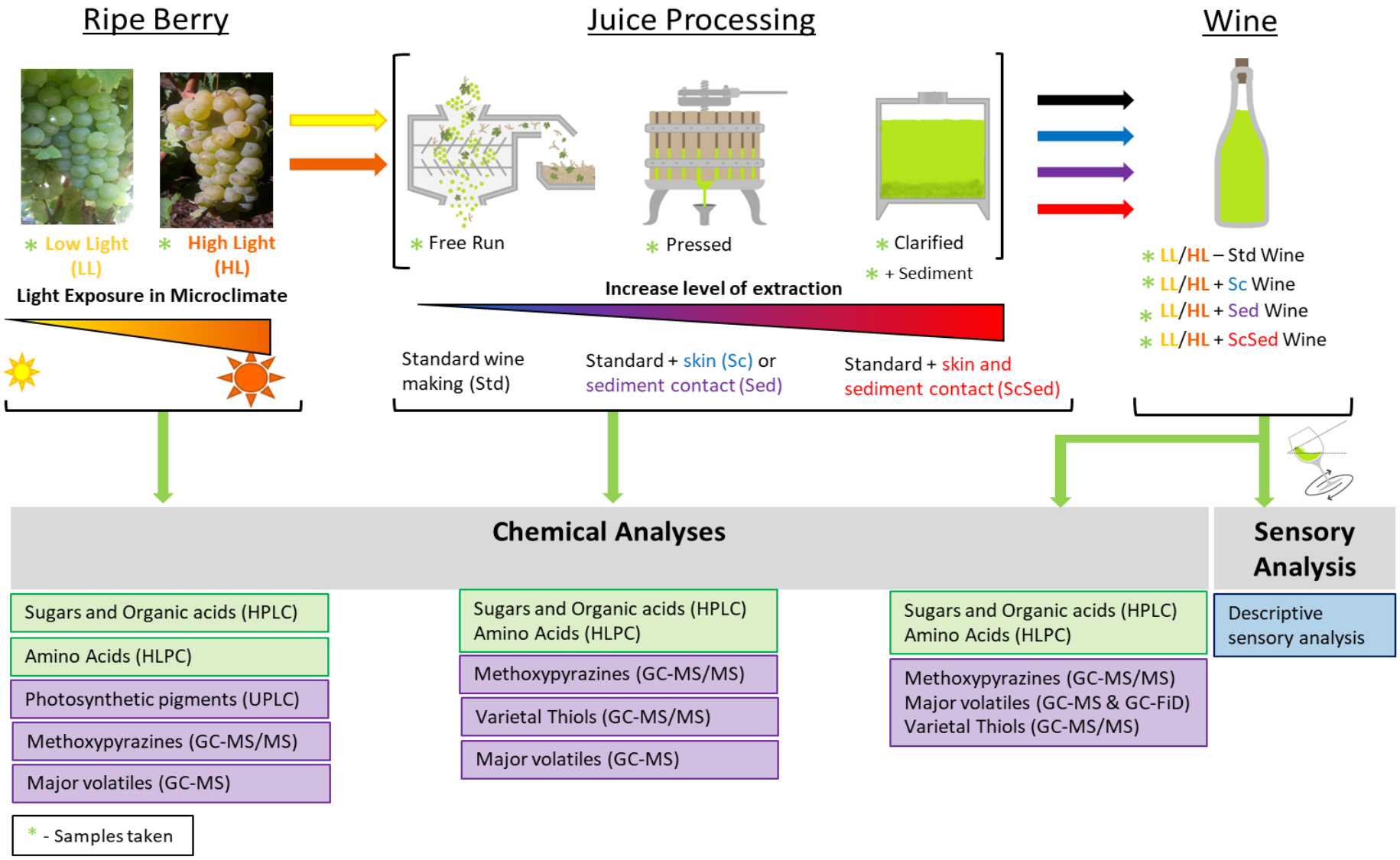


Figure 2.2: Diagram showing the winemaking process, the experimental design used to implement standardised and adjusted juice and wine-processing steps to evaluate extractability of aroma compounds from Sauvignon Blanc grapes for a high light (HL) and low light ((LL) microclimate from a model vineyard in the Elgin area of South Africa. Green asterisks indicate when sampling occurred or subsequent analysis.

Chemical Analyses of berries, juice, sediment and wine samples

Chemical analysis was performed at six distinct sampling points along the winemaking process (Figure 2.2). All samples were flash-frozen with liquid nitrogen and stored at -80°C until analysis. The samples were thawed at 4°C and different analyses were conducted concurrently to evaluate the metabolic composition of the different matrices broadly described as: berries, juice, sediment and wine. For each of these matrices the following compound groups were analysed (where applicable for the matrix in question): sugars, organic acids, amino acids, varietal thiols, photosynthetic pigments, major volatiles and methoxypyrazines. For chemical analyses of the sediment samples, two different approaches were used. For the analysis of the sugars and organic acids in the sediment fraction, the sediment was centrifuged, and the pellet was used, whereas the uncentrifuged sediment slurry was used for volatile headspace analyses.

Sugars and organic acids

Major sugars (glucose and fructose) and organic acids (citric acid, malic acid, tartaric acid and succinic acid) as berry ripeness parameters and as basic juice and wine parameters were analysed via high performance liquid chromatography (HPLC), described in Eyéghé-Bickong *et al.* (2012). The sample preparation for this analysis proceeded as follows for the different matrices:

Berry tissue and sediment solid samples:

The extraction of berry and sediment tissues were done as per the published method (Eyéghé-Bickong *et al.*, 2012).

Juice and wine samples:

The liquid samples (200 µL), were mixed with 600 µL MilliQ water and 1000 µL internal standard (4 g/l Adipic acid and Ribitol). The samples were filtered through 0.22 µm cellulose acetate filters (Lasec, Cape Town, South Africa) into a clear glass vial and capped with metal clamp caps. An Agilent1100 series HPLC system (Agilent Technologies©, Palo Alto, California, USA) equipped with diode array detector (DAD) coupled to a refractive index detector (RID) was used to simultaneously analyse sugars and organic acids. The sugars were detected with the RID and the organic acids with the DAD at 210 nm. An Aminex HPX-87H column (300 mm x 7.8 mm) with sulfonated divinyl dibenzene-styrene co-polymer matrix was used for the column protected with a Bio-Rad guard cartridge (30 mm × 4.6 mm) Injection volume was 10 µL. Standard calibration, data acquisition and peak integration was done with ChemStation Rev. A.10.02 software (Agilent Technologies©). Unless otherwise indicated, all chemicals and standards were HPLC grade and purchased from Sigma Aldrich Chemie (Steinheim, Germany). Concentrations of metabolites were determined by external standard curve (serial dilution of major sugars and organic acids) and normalised to the internal standards; fresh weights and the dilution factor was taken into consideration and expressed as mg/g FW or mg/L. Data presented are the means of three biological repeats.

Amino Acids

Amino acids were quantified in whole berries, juice, sediment and wine matrices with HPLC, according to the method described in du Plessis et al. (2017). The sample preparation for this analysis proceeded as follows for the different matrices:

Berry tissues:

Tissue (200 mg) samples were mixed with 1 ml internal standard/ methanol (70% - 100 mg/l) solution. Norvaline and Sacrosine were used as internal standards. Samples were sonicated and centrifuged (3 min at 1200 rpm). The supernatant was filtered through 0.22 µm cellulose acetate filters (Lasec, Cape Town, South Africa) into a clear glass vial and capped with metal crimp caps

Juice and wine samples:

1 ml Juice and wine samples were mixed at a 1:1 ratio with 70% methanol (100 mg/l). Norvaline (100 mg/l) was used as internal standard. Samples were sonicated and centrifuged (3 min at 1200 rpm). The supernatant was filtered through 0.22 µm cellulose acetate filters (Lasec, Cape Town, South Africa) into a clear glass vial and capped with metal clamp caps to amber glass vials with inserts, capped with metal crimp caps and analysed. An Agilent1100 series HPLC system (Agilent Technologies©, Palo Alto, California, USA) equipped with ChemStation Rev. A.10.02 software (Agilent Technologies©) for peak intergration, standard calibration and data acquisition. A Proshell HPH-C_{18} columm (4.6 x 150 mm, 2.7 µm) was used with UHPLC Guard 3PK Poroshell HPH-C_{18} as guard column and a flow rate of 1 mL/min (Mobile phase A: 10mmol L−1 sodium tetraborate, 10 mmol L−1 sodium phosphate and 5 mmol L−1 sodium azide pH8.2 and Mobile phase B: methanol:acetonitrile:water 45:45:10 (v/v). Concentrations of metabolites were determined by extrapolating from a standard curve (serial dilution of all amino acids measured: Aspartic acid, Glutamic acid, Cysteine, Asparagine, Serine ,Glutamine, Histidine, Glycine, Threonine, Arginine, Alanine, Tyrosine, Cys-Cys, Valine, Methionine, Triptophane, Phenylalanine, Isoleucine, Ornithine, Leucine, Lysine, Hydroxy proline, Proline) and normalised to the internal standard and by the fresh weight or dilution factor (where applicable), expressed as mg/g FW or mg/L. Data presented are the means of three biological repeats.

Photosynthetic Pigments

Carotenoids (β-carotene), xanthophylls (zeaxanthin, violaxanthin, neoxanthin, antheraxanthin, lutein and lutein epoxide) and chlorophylls (chlorophyll a and chlorophyll b) of the berries were analysed using ultra performance liquid chromatography (UPLC).

For sample preparation, the extraction was per the published method (Lashbrooke *et al.*, 2010). The analysis of major carotenoids and chlorophylls was done on a Waters AQUITY UPLC system

(Waters®, Stainleys) that was equipped with a diode array scanner (DAD) that could scan from 280 nm to 700 nm. Data acquisition and control of the system was done using Empower 2 software from Waters®. Different pigments were separated using a Waters UPLC BEH Shield RP18 (2.1 mm x 100 mm, 1.7 µm) column and a Waters UPLC BEH (2.1 mm x100 mm, 1.7um) was used for the guard cartridge. The major carotenoids and chlorophylls were simultaneously separated on a non-linear gradient solvent system (at 55°C). Relative concentrations of metabolites were determined by extrapolating from a standard curve and normalised by the internal standard and the fresh weight used, expressed as mg/g FW. Data presented are the means of three biological repeats.

Methoxypyrazines

The three main methoxypyrazines associated with Sauvignon Blanc aroma (3-isobutyl-2-methoxypyrazine (IBMP), 3-isopropyl-2-methoxypyrazine (IPMP) and 3-sec-butyl-2-methoxypyrazine (SBMP) were analysed through gas chromatography mass spectrophotometry (GC-MS/MS). Analyses were conducted at the Central Analytical Facility laboratory (Stellenbosch university). Sample preparation proceeded as follows:

Berry tissue samples:

The tissue samples (5 g) was mixed with 5 ml 20% NaCl solution and 100 µL internal standard (IBMP-d3 and IPMP-d3 in methanol) (100 ppb) was placed in a solid-phase-micro-extraction (SPME) vial and mixed thoroughly. The vials were places on an autosampler (Thermo Scientific TriPlus RSH) for 10 minutes at 50°C.

Juice, sediment slurry and wine samples:

Liquid samples (10 ml) was mixed with 2.5 ml 20% NaCl solution and 100 µL internal standard (IBMP-d3 and IPMP-d3 in methanol) (100 ppb) was placed in a solid-phase-micro-extraction (SPME) vial and mixed thoroughly. Thereafter the vials were places on an autosampler (Thermo Scientific TriPlus RSH) for 10 minutes at 50°C.

Analysis of methoxypyrazines was performed using a Thermo Scientific trace 1300 gas chromatograph (Anatech, coupled to a Thermo Scientific TSQ 8000 Triple Quadrupule Mass (Anatech Instruments (Pty) Ltd, RSA. A polar Zebron ZB-Wax (30 m, 0.25 mm ID, 0.25 µm film thickness) capillary column was used and the MS detector set for acquisition in single reaction monitoring (SRM) mode. After incubation a pink 65 µm Polydimethylsiloxane/Divinylbenzene/ (PDMS/DVB/) stableflex SPME fiber (Supelco, Belafonte, PA, USA) was exposed to the headspace for 15 minutes at the same temperature whereafter the fibre was inserted (in spitless mode) and left for ten minutes in order to allow desorption of methoxypyrazines. The chromatographic program was set at 35°C. After 6 min, it was raised to 60°C at 4°C/min for a 5 min. After 5 min the temperature was raised to 150°C at 8°C/min for 5 min. The temperature was further (and finally) raised to 240 °C

at 20°C/min, and held for 2 min. The injector and transfer line temperatures were maintained at 250°C. Helium at 1 mL/min flow rate was used carrier gas and emission current of 50 µA was used with argon collision gas. Relative concentrations of metabolites were normalised by fresh weight (for tissue samples) or by the dilution factor (liquid samples) expressed as ng/g FW or as ng/L, respectively. Data presented are the means of three biological repeats analysed in duplicate (n=3)

Major Volatiles

Major volatile organic compounds (VOC) of the whole berries, juice, sediment and wine, were analysed using head-space solid-phase microextraction (HS-SPME), according to the method described in Joubert *et al.*(2016). Unless otherwise specified, all standards were obtained from Sigma Aldrich (Steinheim, Germany). Furthermore, specifically esters, acids and higher alcohols in wine were analysed using gas chromatography with flame ionisation detection (GC/FID) as described in Mollendorff *et al.* (2013).

VOC analysis (HS-SPME-GC/MS)

Berry tissue samples:

Tissue samples (5 g) was mixed with 5 ml extraction buffer (5 g/l tartaric acid, 2 g/l ascorbic acid, and 1 g/l sodium azide, pH 3.2), 1 g NaCl and 20 µL internal standard (Anisol-d8 & 3-octanol (0.5 mg/L) in a SPME vial, vortexed and capped. VOCs in samples were identified according to elution times and mass spectra with those of pure standard compounds (when available) using the SCAN (50-350) mode. These elution times and mass spectra was compared to the spectra in the library data (Wiley Library 275). Concentrations were determined by extrapolating from standard curves from serial dilution on selected VOC compounds (including limonene, 1-hexanol, Propanoic acid, β-ionone) Data was normalized to the internal standard and fresh weight and expressed as ng/g FW. Data presented are the means of three biological repeats analysed in duplicate (n=3).

Juice, sediment slurry and wine samples:

Liquid samples (5 ml) were mixed with 5 ml extraction buffer (5 g/l tartaric acid, 2 g/l ascorbic acid, and 1 g/l sodium azide, pH 3.2), 1 g NaCl and 20 µL internal standard (Anisol-d8 and 3-octanol (0.5 mg/L)in a SPME vial, vortexed and capped. Major VOC's (Including monoterpenes, C13 norisoprenoids, carbonyl (C_6) aldehydes and ketones, alcohols and acids) were extracted with HS-SPME using a 50/30 µm grey Divinylbenzene /Carboxen/ Polydimethylsiloxane (DVB/CAR/PDMS) fibre (Supelco, Bellefonte, PA). GC/MS analysis was done on a 30 m × 250 µm ID, 0.25 µm polar, free fatty acid phase (FFAP, Zebron) 7HG-G009-11 capillary column (Phenomenex, Torrance, USA). Concentrations were determined by extrapolating from standard curves from serial dilution on selected VOC compounds (Cineol, p-cymene, 6-methyl-5-hepten-2-one (6-MHO), 1-hexanol, linalool, β-damascenone, p-mentha-8-thiol and β-ionone). Data was normalized to the internal

standard and dilution factor and expressed as ng/L. Data presented are the means of three biological repeats (n=3).

Esters and Higher alcohols (GC/FID)

For sample preparation 100 µL internal standard (4-methyl-2-pentanol) and 1 ml diethyl ether was mixed with 5 ml wine sample, capped, shaken and sonicated. Thereafter samples were centrifuged (3 min at 4000 rpm). The top phase of the ether was removed, dried on anhydrous Na_2SO_4 and transferred to a vial and capped. Instrumental parameters were used as described in Mollendorff *et al.* (2013). A fast GC-FID method was used to measure 39 compounds which was validated by Malherbe et al. (2011). The column used was a J&W DB-FFAP column with dimension 60 m x 0.32 mm i.d. x 0.5 µm film thickness (Agilent, Little Falls, Wilmington USA). A flame ionisation detector (FID) (Agilent, Little Falls, Wilmington USA) with temperature 250°C was used. Volatile compound peak integration was done using HP Chemstation software (Rev.B01.03 [204]). Volatile compounds were quantified using the ratio of the peak area and internal standard peak area. Data presented are the means of three biological repeats and expressed in mg/L.

Volatile Thiols

For sample preparation, 50 ml of wine, 500 µL butylated hydroxy anisole (BHA, 2mM), 500 µL ethylpropiolate (ETP) and 50 µL Internal standard (4MMP at a concentration of 300 ng/L in ethanol) was mixed together. Samples were loaded at 1 drop/sec flow rate in pre-conditioned SPME cartridges. Cartridges were washed with MilliQ water and vacuum-dried, the analytes eluted with dichloromethane (DCM) (again at 1 drop/s flow) and dried with anhydrous Na_2SO_4. Analytes were filtered through glass wool and evaporated at 30°C under a constant flow of N_2 gas until a volume of approximately 100 µL was transferred to vials with inserts and capped with metal crimp caps. The volatile thiols in wine samples were analysed using gas chromatography (Agilent Technologies 7890A) coupled with a mass spectrophotometric detector (Agilent Technologies 5975C upgraded with a Triple-Axis Detector, Agilent, Santa Clara, CA, USA). An HP-Innowax column (60 m x 0.25 mm x 0.25 µm) was used. Helium was used as the carrier gas at a flow rate of 0.6 mL/min. Instrumental setup and parameters were used as described in Coetzee (2014). Each biological repeat was analysed in duplicate (n=3) and expressed in ng/L.

Sensory analysis

Descriptive analysis (DA) was performed on the wine after 6 months of bottle ageing at 15°C. DA was undertaken by a trained panel consisting of 10 panelists (all female; 25 – 45 years of age). Sensory training consisted of three 1 h sessions per week, where the panelists were trained using wines produced in this study. Training included panelists individually generating descriptors which were then discussed in the group until consensus was reached concerning the predominant descriptors. Thereafter panelists were trained to accurately identify the descriptors; the identified

descriptors were compared with reference standards (consisting of actual examples of the descriptor) and discussed in the group as final confirmation of descriptors. Following descriptor confirmation, the panel was trained in recognition and discrimination using a blind test using the same reference standards presented in amber bottles. All panel members received re-calibration training in terms of basic tastes (sweet, sour and bitter) with a serial dilution of the main taste descriptors. Intensity scaling followed descriptor identification, with each attribute rated for intensity on a 10 cm unstructured line scale ranging from 0 to 10 (0 = not present; 10 = intense). Each biological repeat of the wine were evaluated in triplicate and each biological repeat was evaluated two times per assessor. Each fermentation repeat was evaluated on separate days, with two sessions per day. Wines were assigned a randomised three-digit number for identification. Wines were presented in black ISO tasting glasses (to exclude colour bias) and covered with lids. Wines were served at room temperature. The presentation order of samples were randomised across judges. Tasting commenced in a well-ventilated sensory laboratory with separate tasting booths.

Statistical analysis

Standard statistical analyses were performed in Microsoft Excel (v 360) and Statistica (v 13.5). Multivariate data analysis was conducted using SIMCA (Version 16 from MKS Umetrics AB). Unsupervised principle component analysis (PCA) was conducted to investigate and visualise any trends, groupings and outliers in the data. A supervised orthogonal partial least square – discriminant analysis (OPLS-DA) was used to analyse the data of all four matrices to correlate aroma impact metabolites measured (X, variables) with the applied treatments (Y, factors e.g. light exposure, skin contact, sediment contact, specified as a class).

Statistical significance of the differences in measured metabolites in response to the experimental factors (light exposure, skin contact and sediment contact within ripe grape berries, juice from different processing stages (free run, press and clarified) and wine) was determined using repeated measures, factorial and one-way analysis of variance (ANOVA), using Tibco Statistica (version 13, Oklahoma, USA). Fisher's least square difference (LSD) post hoc test was conducted to identify metabolites that responded statistically significant to a treatment(s), with significant differences interpreted at a 5% level of significance ($p<0.05$).

Furthermore, the significance of each compound in response to a particular experimental factor (i.e. light exposure, skin contact, sediment contact and juice processing), individually and in combination, was ranked using a repeated measure ANOVA. The repeated measures ANOVA was used to determine the experimental factors that drove sample differences, and specific compounds that responded particularly strong to the experimental factor. The results of a repeated measures ANOVA are reported as F-values; where the F-statistic is the ratio of two variances (and larger values represent greater dispersion of the data from the mean).

Hierarchical clustering analysis of metabolites was conducted using Expander (Developed at Ron Shamir's Computational Genomics group, Tel Aviv University, version 7.2 (2017) http://acgt.cs.tau.ac.il/expander/).

2.3. Results

2.3.1. Metabolite profiling of whole, grounded Sauvignon Blanc berries from a LL and HL microclimate

The grape matrix was analysed, and 61 metabolites were quantified belonging to the following compound classes: sugars (n = 2), organic acids (n = 4), chlorophylls (n = 2), carotenoids (n = 6), amino acids (n = 23), methoxypyrazines (n = 2) and major volatiles (n =24). A PCA analysis of all the analytical data from the ground berry tissues was conducted and shown in Figure 2.3, whereas the complete sets of analytical data and the statistical analysis thereof are presented in Tables 2.1, 2.2, 2.3, as well as Supplementary Table S2.1.

The PCA analysis showed that the berry samples separated according to light exposure ((from left to right; HL or LL) (Figure 2.3 A), explaining 68% of variance on PC1. The loadings (Figure 2.3 B) showed which important aroma-linked metabolites responded to light exposure, driving the separation of the compounds. The coefficients plot (Figure 2.3 C) showed the importance of the variation of the samples. HL samples had higher concentrations of sugars, monoterpenes and xanthophylls, whereas LL samples were enriched in chlorophylls, organic acids and yeast preferred amino acids (Table 2.1, 2.2, 2.3 and Figure 2.3B).

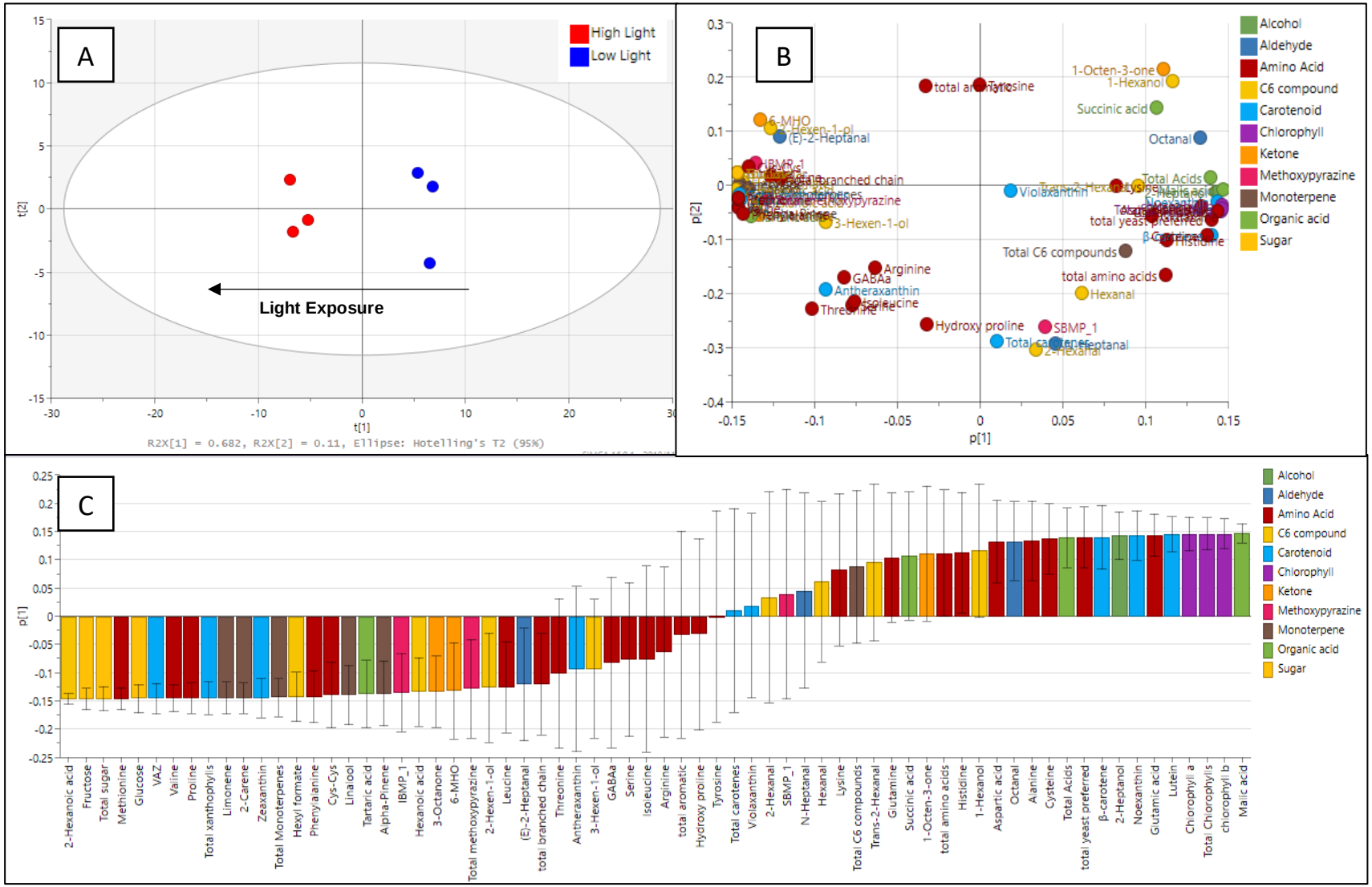


Figure 2.3: PCA score (A), loadings scatter plot (B) of aroma linked metabolites in ground up whole berries from low light and high light microclimate respectively. The loadings coefficient plot (C) is added to clearly visualise the compounds responding to light exposure and driving the separation of ground whole berry samples.

Ripeness monitoring in the experimental panels indicated that the HL and LL panels were similar in their ripeness progression with berries from the HL and LL microclimates differing by approximately 1-1.5 degree Brix (based on juice present in the berries, analysed by a refractometer). The more detailed analysis of samples taken from the harvested berries showed that the ground tissues of the whole berries (a combination of skins, pulp and seeds) of the HL berries accumulated slightly more sugars than the LL samples, while differences in the organic acids were also evident (Table 2.1). The basic parameters measured were statistically different between the treatments.

Table 2.1: Concentration of sugars and organic acids (mg/gFW) from ground, whole berries of Sauvignon Blanc harvested from either a high light (HL) or low light (LL) microclimate. Abbreviations: N.D.; not detected. One way ANOVA conducted per compound (row) between different samples (columns). Row-wise, different letters indicate statistical difference (Fisher's LSD, $p<0.05$). n=3

Compound	**Microclimates**	
	LL	**HL**
Citric acid	N.D.	N.D.
Malic acid	5.99±0.14^{a}	7.1±0.39^{b}
Tartaric acid	3.94±0.23 ^{a}	1.62±0.12 ^{b}
Succinic acid	1.76±0.41 ^{a}	1.15±0.16 ^{b}
Glucose	83.91±1.23 ^{a}	95.2±0.61 ^{b}
Fructose	88.09±1.17 ^{a}	101.16±0.81 ^{b}

The PCA analysis also indicated that photosynthetic pigments responded strongly to light exposure (Figure 2.3C). The increase of light exposure in the HL environment resulted in a statistically significant increase in the xanthophyll, zeaxanthin (Table 2.2). This resulted in a larger xanthophyll pool size (V+A+Z) in HL samples. However, there was no significant difference in total carotenoid pools between LL and HL samples. Furthermore, increased light exposure lead to a significant decrease of chlorophyll a and b in HL samples (Table 2.2).

Table 2.2: Concentration of photosynthetic pigments (mg/g FW) from ground, whole berries of Sauvignon Blanc harvested from either a high light (HL) or low light (LL) microclimate. One -way ANOVA conducted per compound (row) between different samples (columns). Row-wise, different letters indicate statistical difference (Fisher's LSD, $p<0.05$). n=3

Compound	**Microclimates**	
	LL	**HL**
Carotenoids		
Noexanthin	785.48±93.21^{a}	273.41±30.31^{b}
Violaxanthin (V)	578.23±98.91^{a}	566.9±75.36 ^{a}
Antheraxanthin (A)	408.8±141.22^{a}	542.54±15.5^{a}
Zeaxanthin (Z)	1259.25±406.88^{a}	4815.69±637.18^{b}
V+A+Z	*2246.28±486.64* ^{a}	*5925.12±555.53*^{b}
Lutein epoxide (Lx)	196.98±187.99^{a}	ND
Lutein (L)	2118.21±519.5^{a}	1741.1±12.7^{a}
β-carotene (B)	12139.45±893.85^{a}	8760.31±446.43^{b}
Total carotenoids	*17486.41±1832.47*^{a}	*16699.94±966.69*^{a}

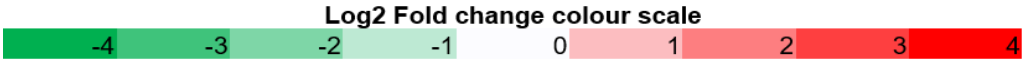
Total xanthophylls	*5346.95±950.47* [a]	*7939.63±523.26*[b]
B+L	*14257.66±1341.39* [a]	*10501.41±437.78*[b]
Lx:(Lx+L)	0.02 [a]	0 [a]
Chlorophylls		
Chlorophyll b (Chlb)	7428.73±699.67 [a]	2758.88±257.24[b]
Chlorophyll a (Chla)	21974.81±1766.65 [a]	11279.82±697.7[b]
Total Chlorophylls	*29403.54±2465.02* [a]	*14038.7±944.48*[b]
Chla:Chlb	2.96 [a]	4.1[b]
Ratio's		
β-carotene:Chlb	1.64 [a]	3.2[b]
lutein:Chla	0.09 [a]	0.15[b]
DEPS	0.73 [a]	0.9[b]
Total Carotenoids:total Chl	0.59 [a]	1.2[b]

Furthermore, the strong separation seen between HL and LL samples was driven by aroma-linked metabolites (Figure 2.3C). Chemical analysis of aroma-linked metabolites in homogenised whole berries (presented in Supplementary Table S2.1) showed that the majority of the aroma-linked metabolites that responded significantly to the light exposure were higher in the HL berries (Table 2.3). HL samples had significantly higher concentrations of, amongst others, linalool and total monoterpenes, 6-MHO, and IBMP. However light exposure also lead to a significant decrease of yeast preferred amino acids in HL samples (Table 2.3 and Supplementary Table S2.1).

Table 2.3: Concentrations and log_2-fold changes of selected metabolites (mg/g FW) from homogenised whole berries (skin, pulp and seed) of Sauvignon Blanc from either a high light (HL) or low light (LL) microclimate. Only the compounds that showed statistically significant responses to the light exposure treatment and had a Log_2-fold change >0.9 were selected. Totals for compound classes were calculated by taking the sum of all the compounds in the class (Supplementary Table S1.1), not only the compounds that responded significantly.

Compounds	LL	HL	Log2 fold change (HL/LL)
Amino Acids			
Aspartic acid	379.34±100.48	191.47±25.05	-0.99
Glutamic acid	41.86±11.21	9.01±0.3	-2.22
Total Yeast Preferred Amino Acids	*1550.88±337.97*	*810.77±191.34*	*-0.94*
Valine	10.63±1.12	26.85±1.54	1.34
Glycine	2.33±0	15,13±3,77	2.7
Proline	2.12±0	111,04±18,29	5.71
Methoxypyrazines			
IBMP	2.57±0.87	5.3±0.45	1.05
Major Volatiles			
Limonene	0.45±0.09	0.99±0.03	1.13
Linalool	0.61±0.41	6.94±2.21	3.5
Total Monoterpenes	*1.14±0.49*	*10.13±1.75*	*3.16*
2-Hexen-1-ol	5.14±3.46	14.93±1.11	1.54
Hexanoic acid	0.73±0.13	2.2±0.46	1.6
Hexyl formate	2.78±0.83	7.3±0.07	1.4
2-Heptanol	1.21±0.14	0.21±0.02	-2.5

6-MHO	3.34±1.39	8.18±0.59	1.29



The Log_2-fold change is indicated with a colour scale ranging from -4 to 4. A negative value (green) indicates that the compound concentration was higher in LL samples, and a positive value (red) indicates that the concentration of the compound was higher in HL samples.

From these results it was clear that not only groups of compounds, but also individual aroma compounds were affected when comparing the HL vs LL microclimates, with the majority of the statistically different compounds being elevated in the HL berries. Figure 2.4 provides a summary of the major chemical compounds responding to the HL microclimate in ripe SB berries at harvest. The model was compiled using the data represented in Supplementary table S2.1. The berries from the HL microclimate were considered to have a higher aromatic potential (HAP) than berries from the LL microclimate, having comparably lower concentrations of certain aroma linked metabolites (IBMP, hexanoic acid, branched chain and aromatic amino acids, monoterpenes, norisoprenoids and some C_6 compounds) and thus a lower aromatic potential (LAP). This observation was taken as an assumption in the next phase of experiments where grape processing steps were evaluated for their effectiveness in optimising extractability of aroma compounds into the juice and wine matrices from the HAP and LAP grapes.

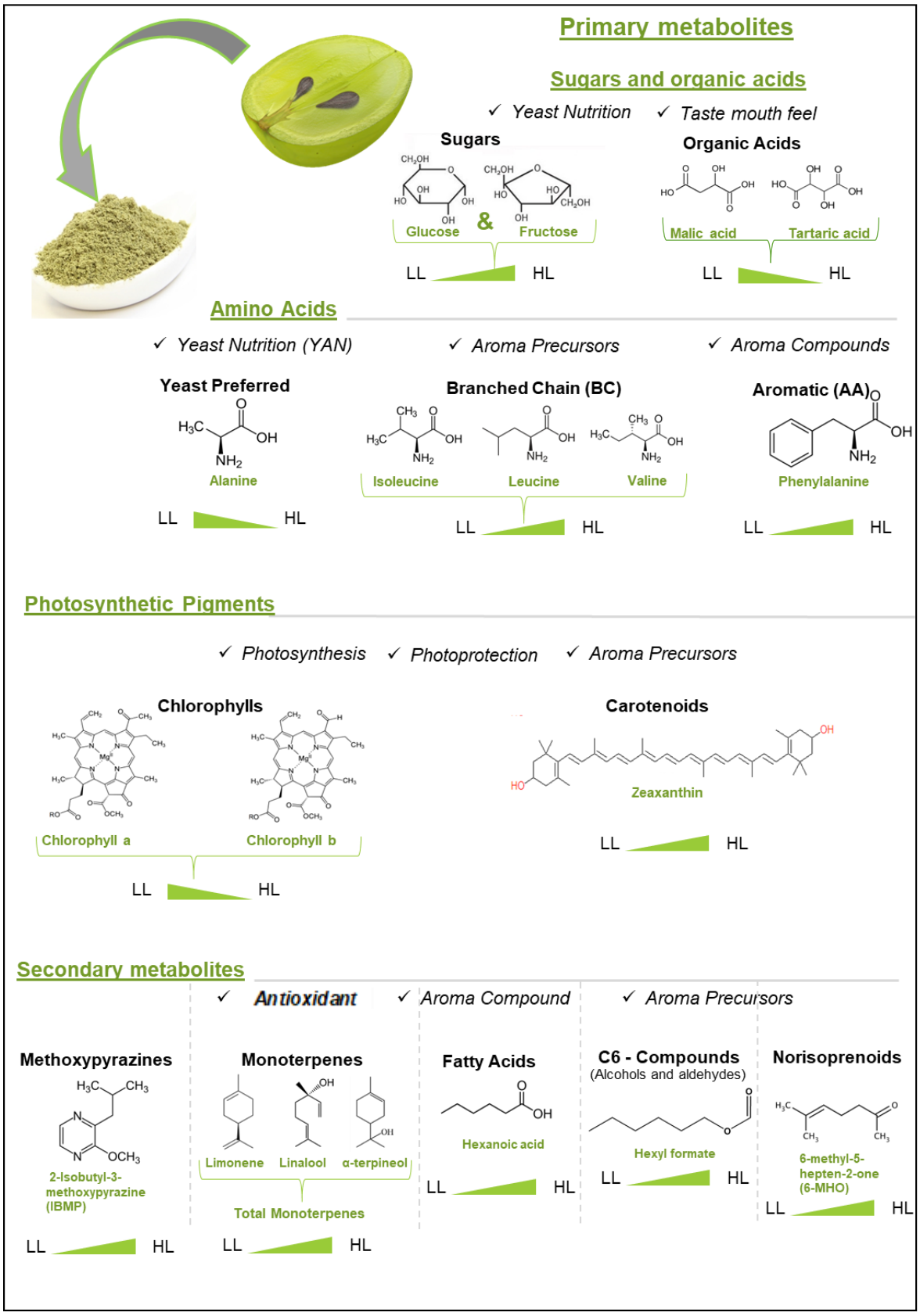


Figure 2.4: Model summarising the composition of berries from LL and HL microclimates. The wedge indicates in which microclimate the highest concentration of the compound/s was found.

2.3.2. Metabolic profiles of the juice samples and analysis of sediment metabolites

The metabolic profile of juice was determined by chemically analysing juice from different processing steps (free run, pressed and clarified juice) and of juice that received skin contact after pressing (all analytes are presented in Supplementary table S2.2 and Table 2.4.) Furthermore, the sediment fraction of the juice (residue after clarification, prior to fermentation) was also chemically analysed to determine whether the sediment retained any residual aroma-linked metabolites (presented in Supplementary table S2.2 and Table 2.4). The sediment data showed large standard deviations between biological repeats for some of the compounds, especially for the amino acid analysis, which is not reported on here, since the data was inconclusive.

Sugars and organic acids of the free run juice from LL and HL microclimate berries showed that the LL berries had lower sugars than the HL berries. As juice processing proceeded to the pressed and clarified stages, the differences in sugar levels in the LL versus HL samples became minimal and were no longer statistically significant (Table 2.4). Interestingly, sediment samples contained about two-fold more malic and succinic acids and about nine-fold more tartaric acids in both LL and HL samples when compared to juice samples. The sugars were also approximately two-fold more in the sediment samples (LL and HL) (Table 2.4). LL sediment samples contained significantly higher concentrations of malic acid whereas the HL sediment samples contained significantly higher concentrations of tartaric acid (except for the HL-Sc clarified samples). Moreover, skin contact lead to overall lower concentrations of malic and tartaric acid (in all samples), with clarified HL-skin contact and HL-skin contact sediment samples having the lowest organic acid concentration, although the decrease in acid concentration observed was not always significant.

Table 2.4: Concentration of major sugars and organic acids (mg/L) in juice from different processing stages: Free Run, Press and Clarified during standard wine making, or with the addition of skin contact. Juice was obtained from grape berries harvested from either a high light (HL) or low light (LL) microclimate. Values in brackets () are sediment sample values for the corresponding clarified juice fraction. Factorial ANOVA conducted per compound (row) between different samples (columns). Row-wise, different letters indicate statistical difference (Fisher's LSD, $p<0.5$). n=3

Compounds	Free run juice		Pressed juice		Pressed juice with skin contact		Clarified juice		Clarified juice with skin contact	
	LL	HL	LL	HL	LL	HL	LL	HL	LL	HL
Citric Acid	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD (0.39±0.13^{a})	<LOD (0.44±0.1 ^{a})	<LOD (0.48±0.09^{a})	<LOD (0.39±0.08^{a})
Tartaric acid	7.82±0.23^{ab}	8.27±0.72^{a}	6.97±0.34^{bc}	7.22±0.26^{ab}	5.28±0.16^{d}	5.25±0.81^{d}	5.92±0.99^{cd} (41.23±10.17^{a})	5.45±1.7^{d} (56.84±11.94^{b})	3.5±0.34^{e} (42.82±19.88^{ab})	3.81±0.29^{e} (34.85±2.69^{b})
Malic Acid	3.56±0.35^{a}	2.68±0.06^{ef}	3.26±0.17^{ab}	2.37±0.28^{de}	2.96±0.14^{bcf}	1.83±0.17^{g}	3.02±0.15^{bc} (6.6±1.52^{a})	2.21±0.14^{d} (4.96±1.55^{b})	2.74±0.18^{cf} (6.19±1.15^{ab})	1.8±0.14^{g} (3.17±0.65^{c})
Succinic acid	1.63±0.19^{def}	1.26±0.07^{b}	1.75±0.16^{ad}	1.45±0.1^{bcef}	1.97±0.08^{a}	1.56±0.16^{cdef}	1.65±0.15^{de} (3.71±0.81^{a})	1.39±0.1^{bcf} (3.68±1^{a})	1.9±0.11^{a} (3.69±0.76^{a})	1.32±0.24^{bc} (3.77±0.63^{a})
Total acids	*13.37±0.83^{a}*	*12.66±0.83 ^{ab}*	*12.37±0.63 ^{abc}*	*10.46±0.48^{bcd}*	*11.28±0.34^{de}*	*8.71±1.25^{f}*	*9.92±1 ^{cd} (51.93±12.37^{ab})*	*8.42±0.48 ^{ef} (65.77±13.76 ^{a})*	*9.63±1.68 ^{fg} (53.61±21.06 ^{ab})*	*7.1±0.92^{g} (41.12±3.47^{b})*
Glucose	98.23±2.22^{bc}	111.29±5.49^{a}	99.87±5.51^{bc}	105.42±5.64^{ab}	96.65±2.14^{cd}	102.24±4.06^{bc}	95.25±6.48^{cd} (131.91±40.24^{a})	96.08±5.56^{cd} (140.12±46.3^{a})	89.76±1.45^{d} (134.26±62.52^{a})	96.07±5.55^{cd} (145.23±84.07^{a})
Fructose	96.44±1.27^{cd}	111.7±5.48^{a}	98.47±6.29^{bcd}	105.28±5.81^{ab}	97.08±3.25^{cd}	104.12±3.02^{abc}	94.3±6.4^{d} (125.59±39.22^{a})	96.2±4.7^{cd} (133.42±39.12^{a})	91.07±1.2^{d} (127.81±51.41^{a})	98.3±5.45^{bcd} (138.86±32.54^{a})
Total Sugars	*195.32±9.82^{cde}*	*225.51±13.92 ^{a}*	*200.94±10.47^{bcd}*	*192.95±7.89 ^{ab}*	*210.71±11.39^{cde}*	*205.79±12.74^{bc}*	*187.56±13.88^{de} (257.5±79.31^{a})*	*180.83±4.87^{cde} (266.13±85.23^{a})*	*196.12±7.62^{e} (281.01±112.32^{a})*	*193.17±11.11^{cde} (265.11±106.11^{a})*

A PCA (Figure 2.5A) of aroma-linked metabolites (amino acids, methoxypyrazines and major volatiles) showed that, when only considering standard white winemaking, juice samples separated according to light exposure along the first- (PC1, 28% explained variance) and second component (PC2, 18.1% explained variance), with clearer separation in the free run and press samples, but with a closer grouping between the HL and LL samples when the clarification stage is reached. When considering skin contact samples, there was a clear separation between standard and skin contact samples in the pressed and clarified stages of both LL and HL, also further increasing the separation between LL and HL samples at both these processing stages. When considering the loadings and coefficient loadings plot (Figure 2.5 B & C), it was evident that HL samples were richer in hotrienol, IBMP, β-damascenone and hexyl formate, compared to the LL samples that had higher concentrations of yeast preferred amino acids driving the separation of LL and HL samples (Figure 2.5 A & C). However, the separation along PC1 and -2 only accounted for a small proportion of the explained variance (46%). Further analysis of the fourth and fifth principled components (Supplementary Figure S2.1 A & B) showed that juice samples separated according to skin contact along the fourth component (PC4, 11.2% explained variance) was driven by hexanal, aromatic amino acids and monoterpenes. Samples further separated somewhat along the fifth component (PC5; 6% explained variance) due to juice processing stage (Supplementary Figure S2.1B).

A PCA of the sediment (Figure 2.6) showed that skin contact was a main driver for sample separation (PC1, 28% explained variance) and had resulted in higher concentrations in LL samples of sugars and monoterpenes so that they grouped with HL- Sc samples. Light exposure was a main driver for the separation of standard samples (PC2, 21% explained variance). The loadings plot indicated that mostly volatiles and C_6 compounds drove the separation of the LL-Std samples from the HL-Std, HL-Sc and LL-Sc samples.

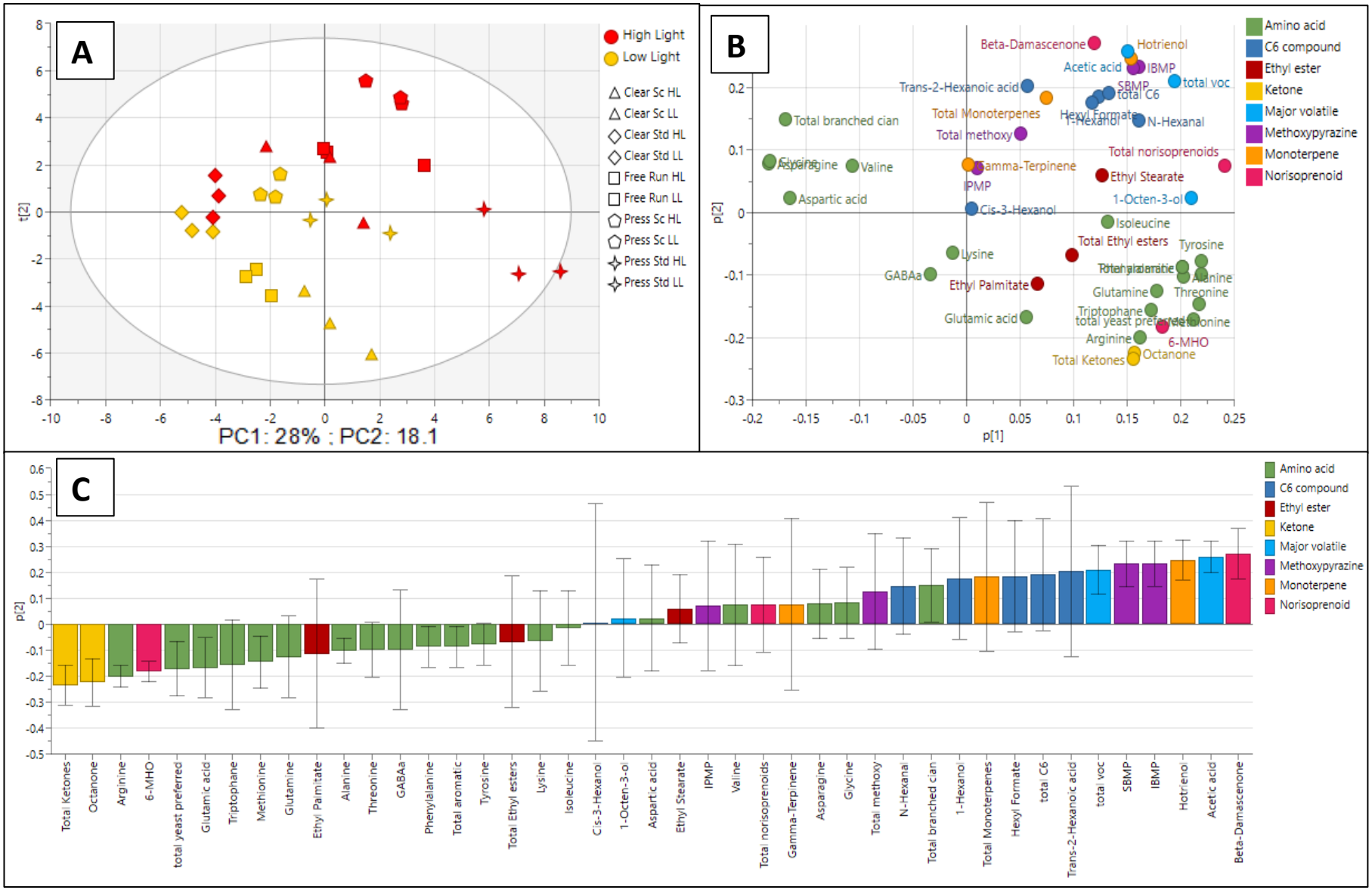


Figure 2.5: PCA score (A), loadings (B) and loadings coefficient (C) plots of aroma linked metabolites in juice from low light (LL) and high light (HL) microclimates. Juice is presented in Standard wine making conditions (Std), or receiving skin contact (Sc) within the different processing stages: Free Run , Press and Clear .

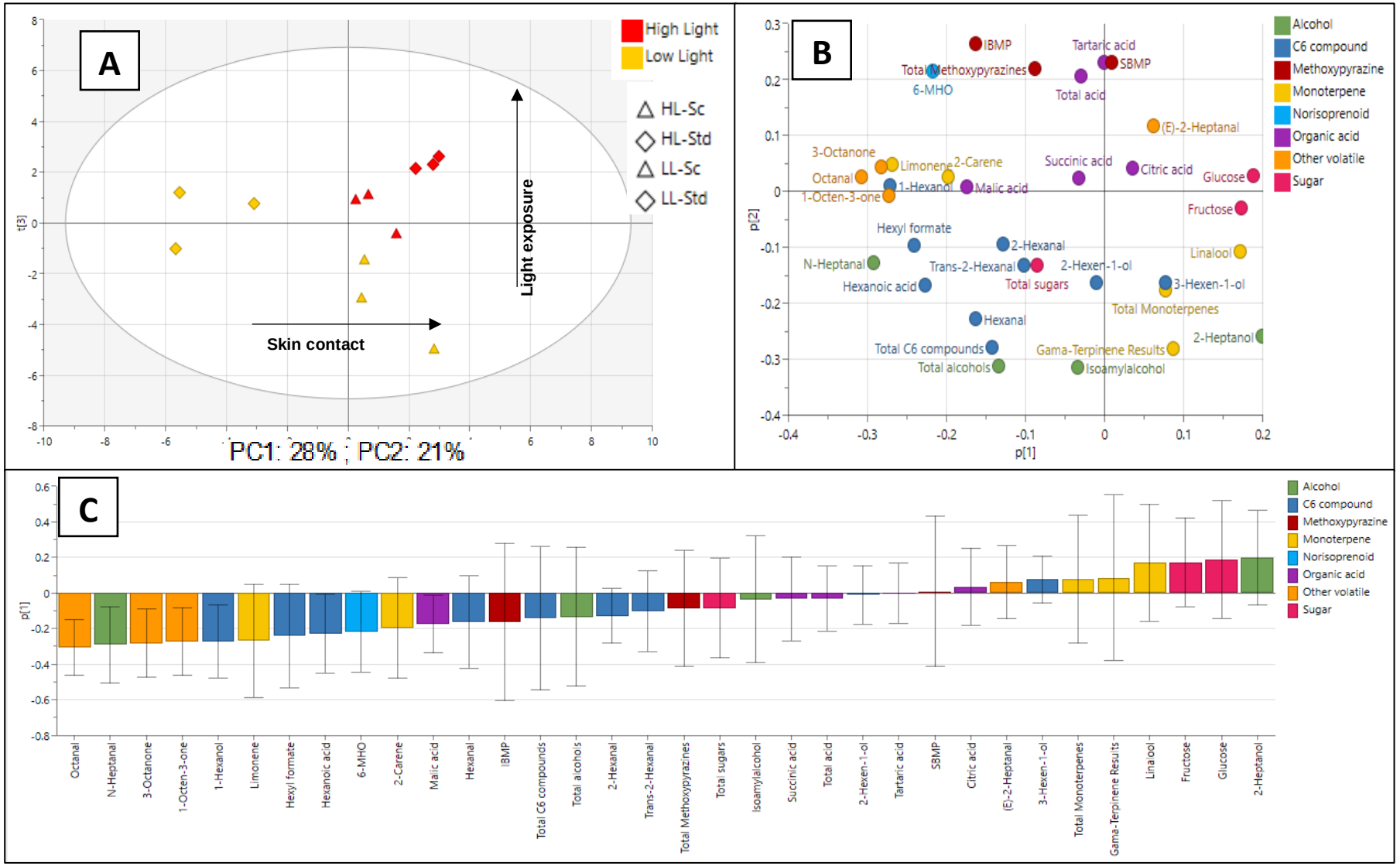


Figure 2.6: PCA score (A), loadings (B) and loadings coefficient (C) plots of aroma linked metabolites in juice sediment (residue left after clarification, when clarified juice has been racked off) from low light (LL) and high light (HL) microclimates from standard juice (Std) or juice with skin contact (Sc).

The results of the juice and sediment PCA were further investigated using a repeated measures ANOVA (Table 2.5). The repeated measures ANOVA was used to determine the significance of each compound's response to the main experimental factors (i.e. light exposure, juice processing and/or skin contact) (Table 2.5 A&B). The compounds are presented per compound group (amino acids, monoterpenes, methoxypyrazines, C_6 compounds, esters and norisoprenoids) and origin (grape-derived or processing-derived). Due to the nature of the repeated measures ANOVA analysis and the metabolite data, it was necessary to run two analyses: the first (A) investigating the response of each compound within standard wine making conditions to light exposure and juice processing (excluding skin contact samples). The second (B) investigated the response of each compound to light exposure, juice processing and skin contact (excluding standard samples).

The results confirmed that under standard processing conditions (Table 2.5A) grape-derived aroma-linked metabolites (for example IBMP, hotrienol and β-damascenone) displayed the strongest responses to light exposure with high F values of 50.0, 75.4 and 32.7 respectively. Furthermore, under standard processing conditions secondary grape-derived aroma metabolites (that are produced during processing like hexanol and hexyl formate, displayed a significant response to juice processing with F-values of 16.4 and 16.6, respectively. These results were mirrored in the skin contact samples (Table 2.5B), with IBMP, hotrienol and β-damascenone responding the strongest to light exposure with high F values of 324.6, 977.1 and 215.9 respectively, compared to the F values of the juice processing response (27.4, 48.2, 52.9) or skin contact (47.4, 81.7, 34.3). The C_6 compounds (specifically trans-2-hexanoic acid) responded the strongest (176 vs 38.94 for light exposure and 9.5 for juice processing) to skin contact. Most of the amino acids (including branched chain, aromatic and yeast preferred amino acids) were responsive to skin contact in combination with juice processing. A repeated measures ANOVA of the sediment data (Supplementary Table S2.3) indicated the compounds that were enriched in the sediment fractions due to the light exposure, skin contact or a combination of these two factors. It was clear that the skin contact treatment caused more compounds to be enriched in the sediment, particularly the linalool and 2-heptanol accumulated in this fraction.

Table 2.5: The repeated measures ANOVA results for: A - the interaction between juice processing and light exposure (Excluding skin contact samples), B - the interaction between juice processing, skin contact and light exposure (Excluding free run samples) and individual aroma linked metabolites are reported as F-values. Values are coloured from highest F- value (most significant) to the lowest F-value. **Green** indicates lower F-values (F>5), **yellow** indicates intermediate F- values (F>10) while **red** indicates high F-values values (F>20). All insignificant values (F ≤ 5) are coloured in grey. F values are inversely correlated to p-values – the larger the F value is, the smaller the corresponding p-value is and the higher the significance of the response. Maximum = p<0.001; intermediate =p<0.01; minimum = p<0.05; insignificant = p>0.05

A Aroma linked metabolite origin	Compound	Juice processing	Light exposure	Juice processing * Light Exposure
Grape-derived	**Amino acids**			
	Aspartic acid			
	Glutamic acid			
	Asparagine			
	Glutamine			6.7
	Glycine			
	Threonine			5.5
	Arginine			
	Alanine	5.2		
	GABAa			
	Tyrosine	5.7		
	Valine			
	Methionine			
	Tryptophan			
	Phenylalanine			
	Isoleucine		7.5	27.9
	Lysine			9.1
	Total yeast preferred amino acids			

B Aroma linked metabolite origin	Compound	Juice processing	Light exposure	Skin contact	Juice processing*Lignt exposure	Juice processing*Skin contact	Light exposure* Skin contact	Juice processing* Light exposure*Skin contact
Grape-derived	**Amino acids**							
	Aspartic acid					35.34		5.768
	Glutamic acid							
	Asparagine	16.3			21.12	83.67		28.99
	Glutamine	34.3			36.55	84.7		12.47
	Glycine	18			13.36	66.32		27.01
	Threonine	26.6			30.83	86.67		17.62
	Arginine	7.52					17	
	Alanine	5.3						
	GABAa	15.6		13.7				6.861
	Tyrosine	143	9.182	14.6	73.01	169.1	25.48	102.6
	Valine		10.51	5.04		24.31		7.398
	Methionine		10.71			41.75		10.28
	Tryptophan				5.583	22.96		12.02
	Phenylalanine	5.65						
	Isoleucine		8.081		21.63			
	Lysine				10.57			
	Total yeast preferred amino acids	15.6			12.97	28.32		

	Total branched chain amino acids					Total branched chain amino acids					14		
	Total aromatic amino acids					Total aromatic amino acids	5.65						
	Methoxypyrazines					**Methoxypyrazines**							
	IPMP		15.9	7.9		IPMP	6.65	78.1	42.9	35.62			
	IBMP	7.6	50.0			IBMP	27.4	324.6	47.2			25.15	
	SBMP	5.9	46.6			SBMP	17	301.2	51.6			20.84	6.958
	Total methoxypyrazines		*25.9*			*Total Methoxypyrazines*		*160.3*	*56.7*	*23.4*		*6.643*	
	Monoterpenes					**Monoterpenes**							
	Gamma-Terpinene					Gamma-Terpinene	6.3		126		9.249		
	Hotrienol	5.8	75.4			Hotrienol	48.2	977.1	81.7	6.47		57	
	Total Monoterpenes		*10.8*			*Total Monoterpenes*	*14.1*	*54.76*	*86.4*		*9.728*		
	Ethyl esters					**Ethyl esters**							
	Ethyl Palmitate					Ethyl Palmitate					8.941	7.749	
	Ethyl Stearate	6.5				Ethyl Stearate	5.51						
	Total ethyl esters					*Total ethyl esters*					*5.256*	*6.321*	
	Other volatiles					**Other volatiles**							
	1-Octen-3-ol	32.6				1-Octen-3-ol	32.1						
	Acetic acid	7.7	39.5			Acetic acid	11.8	77.7	5.38				
	Total Major Volatiles	*96.3*				*Total Major Volatiles*	*314*	*17.75*	*5.71*		*10.97*	*24.25*	
Processing-derived	**Norisoprenoids**				**Processing-derived**	**Norisoprenoids**							
	β-damascenone	11.7	32.7			β-damascenone	52.9	215.9	34.3	32.22		27.26	
	6-MHO	10.8				6-MHO	15.3						
	Total norisoprenoids	*16.7*				*Total norisoprenoids*	*19.9*	*5.846*					
	C_6 compounds					**C_6 compounds**							
	N-Hexanal	16.4	6.6			N-Hexanal	41.7	10.74	19.3	7.552			
	Cis-3-Hexanol					Cis-3-Hexanol		10.72	71.1				
	Hexyl Formate	16.6				Hexyl Formate	164	22.86	61.5		15.26	67.86	8.541
	Trans-2-Hexanoic acid					Trans-2-Hexanoic acid	9.5	38.94	176			59.66	
	1-Hexanol	17.3				1-Hexanol	116	21.04	42.7		11.62	44.9	
	Total C_6 compounds	*21.9*				*Total C_6 compounds*	*177*	*11.7*	*51.2*		*10.37*	*57.15*	

PCA and repeated measures ANOVA analyses were followed by clustering analysis to identify aroma-linked metabolites that share similar response patterns during juice processing. The clustering analysis dendrogram is presented in Supplementary Figure S2.2 and mean patterns of responses is presented in Figure 2.7. Samples grouped together based on light exposure and juice processing steps in three main groups, with skin contact mitigating the difference between samples due to light exposure (Supplementary Figure S2.2). Generally lower expressions were noted in LL and clarified samples.

Compounds that showed significant responses could be grouped in four distinctive groups according to similar response profiles (Figure 2.7) by using the clustering similarity matrix. The clustering analysis provided valuable insights on the response of different compounds, and the way different compounds respond in similar ways. Some aromatically important compounds were selected as a benchmark for the compound response group and further analysed. ANOVA plots were produced to visualise the statistical significance of the responses of different compounds to microclimate, juice processing and skin contact (presented in Figure 2.8 A-D).

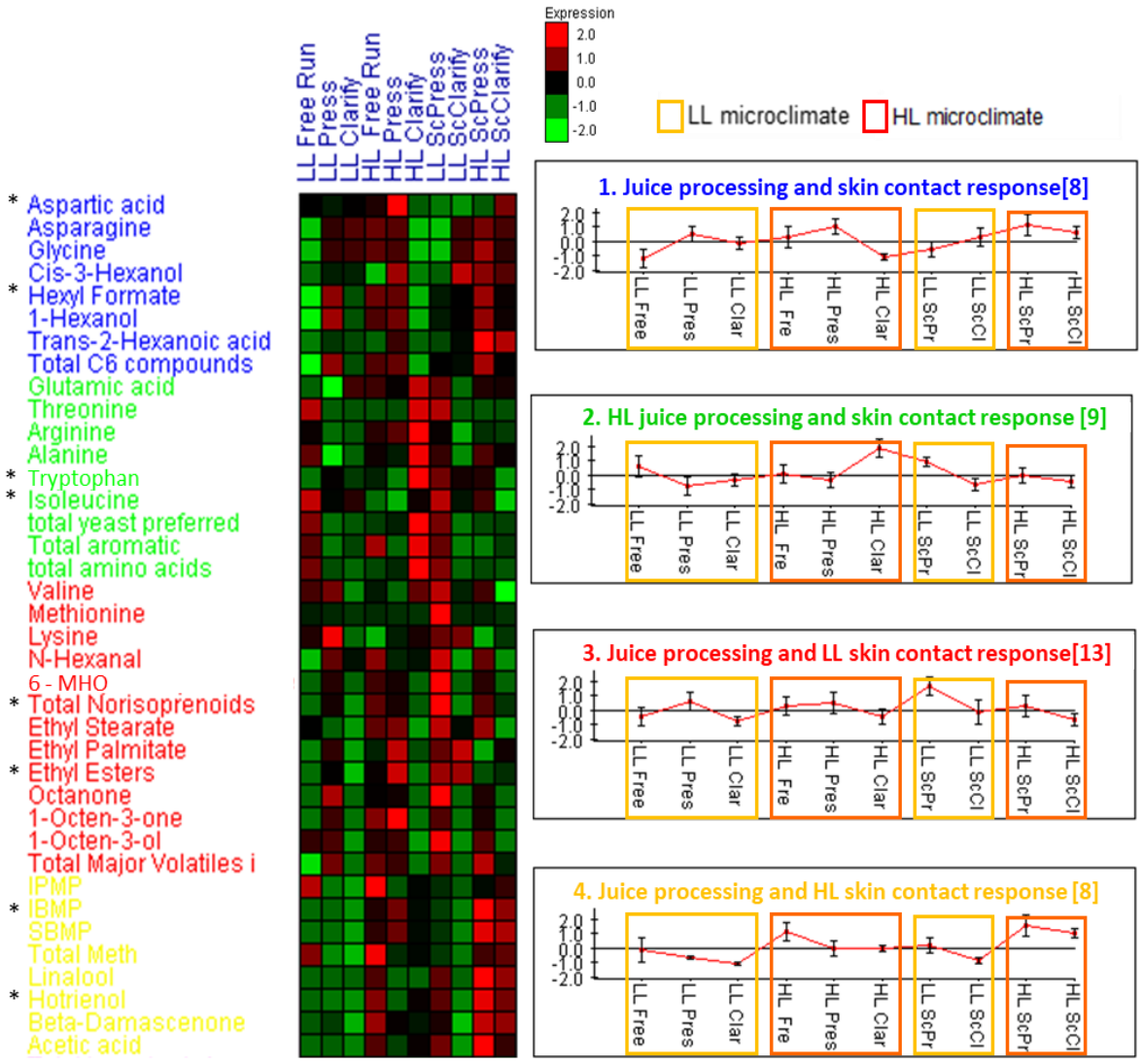


Figure 2.7: The clustering similarity matrix and mean patterns for compounds and compound groups measured in juice from different processing stages (Free run, pressed and clarify). Compounds or groups that exhibited similar response profiles to treatments were grouped together on the metabolite level. Red blocks indicate a high expression, and green blocks indicate a low expression of the specified compound within a sample. Asterix indicate examples from each group for the factorial ANOVA plots in Figure 2.8.

The first cluster contained 8 compounds, both standard LL and HL samples responded to juice processing, and HL-Sc increased slightly with skin contact (Figure 2.7). Compounds in this group included the grape derived aromatic C_6 compound, hexyl formate (associated with “apple” aromas and “green” flavours) and the yeast preferred amino acid, aspartic acid (Figure 2.8A). Aspartic acid increased in the press stage in HL standard samples, only to decrease after clarification; skin contact mitigated the loss in HL-Sc clarified samples, whereas LL samples did not show differences. Hexyl formate in LL-Std increased after pressing and then decreased after clarification, and HL samples decreased as juice processing proceeded. In both cases skin contact somewhat mitigated the processing effect, resulting in higher concentrations of hexyl formate in both LL and HL clarified samples. Analysis of the sediment (Supplementary Table S2.2) showed that a large proportion of

compounds in the sediment was C_6 compounds, predominantly hexyl formate. Furthermore, HL sediment and sediment Sc samples contained lower concentrations of hexyl formate (Supplementary Table S2.2).

The second cluster contained 9 compounds, and HL samples responded to juice processing and skin contact (Figure 2.7). Compounds in this group included the aromatic amino acid tryptophan and the yeast preferred amino acid arginine (Figure 2.8B). Tryptophan only responded to treatments in HL samples, with overall higher concentrations after clarification. Skin contact resulted in higher concentrations after pressing, but this effect was lost after clarification. Arginine had overall higher concentrations in HL samples after clarification. Skin contact resulted in lower concentrations of arginine in HL samples compared to standard samples.

The third cluster contained 13 compounds; samples responded to juice processing and LL samples to skin contact too (Figure 2.7). Compounds in this group included mostly aroma compounds like norisoprenoids and ethyl esters. Skin contact lead to higher concentrations of ethyl esters in LL juice samples. HL-clarified samples had significantly lower concentrations of norisoprenoids compared to free run juice, with skin contact having no effect (Figure 2.8C). The sediment contained high concentrations of the norisoprenoid 6-MHO and skin contact led to a reduction of 6-MHO (Supplementary table S2.2), similar to what was seen in the HL-Press samples.

The fourth cluster contained 8 compounds, and samples responded to juice processing and HL samples to skin contact (Figure 2.7). Compounds in this group included mostly grape-derived aroma compounds like the methoxypyrazine IBMP and monoterpenes like hotrienol. The concentrations of both hotrienol and IBMP lowered in samples as juice processing proceeded (Figure 2.8D), with significantly higher concentrations in HL samples which was further increased by skin contact. Low concentrations of monoterpenes were found in the sediment, with HL-Sc samples containing the highest concentration of monoterpenes especially linalool which was almost absent in the juice; hotrienol however was not detected (Supplementary Table S2.2). High concentrations of IBMP and SBMP were found in the sediment when skin contact occurred.

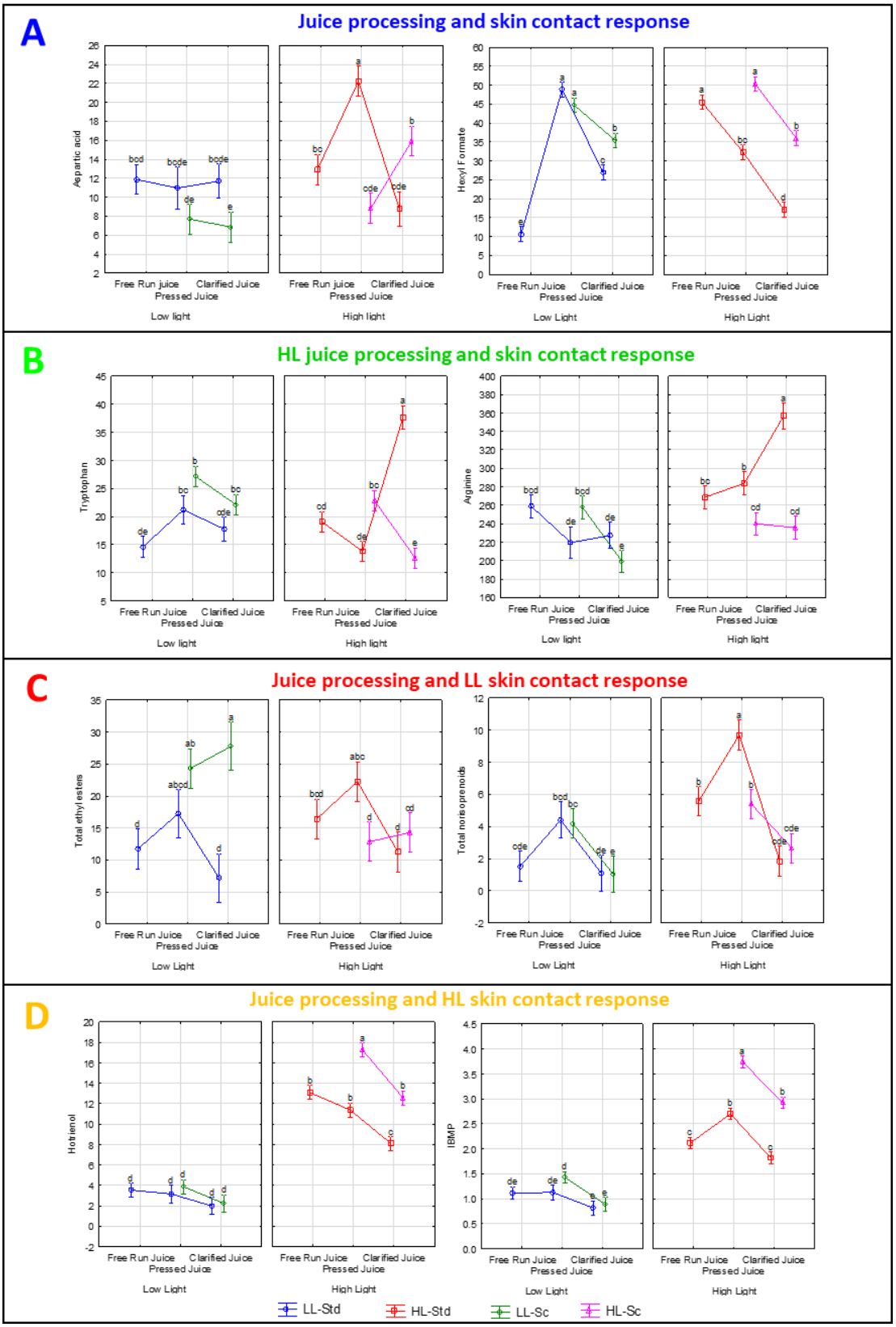


Figure 2.8: The ANOVA plots of different compound responses in LL- and HL juices from different juice processing stages (Free run, Pressed and Clarified) which responded to juice processing and skin contact. (A) aroma-linked compounds hexyl formate and aspartic acid; (B) aroma-linked compounds tryptophan and isoleucine; (C) aromatic ethyl esters and norisoprenoids; and (D) Grape-derived aromatic compounds hotrienol and IBMP. Different letters indicate statistical difference (Fischer's LSD $p<0.05$).

2.3.3. Analysis of wine metabolites

Alcoholic fermentation proceeded for an average of 24 days after inoculation. The sediment contact samples fermented faster than the no sediment samples with HL-Sed having the highest fermentation rate (supplementary figureS2.4). The fermentation rate was determined by dividing the % weight loss of the fermentation vessels and divided by the days of fermentation (Supplementary Figure S2.4). There were no statistically significant differences in the % alcohol produced (Table 2.6) between the different wine samples. HL samples generally had higher concentrations of fructose and LL- Std wine not only had the lowest concentration of alcohol, but also the lowest concentration of residual sugar whereas LL—Sc had the highest alcohol concentration. Both the light exposure and increased extraction through skin contact treatments led to significant increases in both glycerol and succinic acid concentrations in the LL and HL samples.

Table 2.6: Concentration of major sugars and organic acids (g/L) in wine made from berries from LL and HL microclimates. Results shown for standard wine making (control) and for modified wine making (addition of skin contact, sediment contact and skin + sediment contact. Factorial ANOVA conducted per compound (row) between different samples (columns). Row-wise, different letters indicate statistical difference (Fisher's LSD, $p<0.05$). n=3

	Standard wine making		**Wine with skin contact**		**Wine with sediment contact**		**Wine with skin + sediment contact**	
	LL	HL	LL	HL	LL	HL	LL	HL
Tartaric acid	3.23 ± 0.53^{a}	2.36 ± 0.06^{c}	3.17 ± 0.09^{ab}	2.17 ± 0.02^{c}	2.6 ± 0.23^{bc}	3.03 ± 0.58^{ab}	2.29 ± 0.18^{c}	3 ± 0.18^{ab}
Malic acid	2.15 ± 0.4^{a}	2.1 ± 0.05^{a}	1.94 ± 0.09^{a}	1.49 ± 0.05^{ab}	2.09 ± 0.4^{a}	2.11 ± 0.13^{a}	0.81 ± 1.15^{b}	1.72 ± 0.07^{a}
Succinic acid	1.67 ± 0.26^{d}	2.53 ± 0.05^{a}	1.68 ± 0.14^{cd}	2.26 ± 0.13^{ab}	1.9 ± 0.29^{bcd}	1.98 ± 0.07^{bcd}	1.83 ± 0.25^{cd}	2.04 ± 0.06^{bc}
Glucose	<1 g/L	<1 g/L	<1 g/L	<1 g/L	<1 g/L	<1 g/L	<1 g/L	<1 g/L
Fructose	1.1 ± 1.7^{cde}	9.74 ± 3.7^{ab}	6.0 ± 0.7^{bcd}	14 ± 8.3^{a}	3.4 ± 2.91^{e}	2.5 ± 1.3^{e}	0.9 ± 1.5^{de}	7.3 ± 2.7^{bc}
% Alcohol	12.56 ± 2^{b}	14 ± 0.26^{ab}	14.88 ± 0.77^{a}	14.29 ± 0.53^{ab}	13.9 ± 0.72^{ab}	13.35 ± 0.9^{ab}	14.68 ± 0.81^{a}	14.66 ± 0.31^{a}
Glycerol	13.09 ± 0.55^{d}	16.9 ± 0.64^{a}	14.66 ± 0.66^{bc}	18.39 ± 1.06^{a}	13.49 ± 0.85^{cd}	14.16 ± 0.36^{bcd}	15.95 ± 1.3^{b}	17 ± 0.1^{a}

Wine (after cold stabilisation, before bottling) was chemically analysed to determine the effect on skin contact, sediment contact or a combination of skin + sediment contact on the aromatic profile of final wines. Concentrations of all detected volatiles and statistical analysis are presented in Table S2.4 in the supplementary section of this chapter. Overall LL- Std and HL- Std wines were chemically distinct, retaining much of the vineyard- achieved aromatic potentials. HL-Std wine contained significantly higher concentrations of norisoprenoids and methoxypyrazines, compared to the other wines.

The PCA (Figure 2.9A) of aroma-linked metabolites (methoxypyrazines, volatile thiols, C_6 compounds, monoterpenes and major volatiles) showed that wine samples separated according to sediment contact along the first (PC1, 36% explained variance) and second component (PC2, 16.3% explained variance).

Within the no sediment contact group samples separate according to skin contact and the standard samples separated further according to light exposure. Interestingly LL-Sc grouped together with HL-Sc and HL-Std. No such clear distinctions were seen in the sediment contact group. The loadings plots (Figure 2.9 B &C) indicated that mostly monoterpenes (geranyl isobutyrate), esters (isoamyl acetate, butyl acetate) and some C_6 compounds contributed to the no-sediment contact sample separation. Conversely is was mainly alcohols and C_6 compounds that drove the separation of the skin contact samples. Further analysis of the third and fourth principal components (Supplementary Figure S2.3). showed that wine samples separated according to light exposure along the third component (PC3 10.8% explained variance) driven in the HL samples mostly by the grape derived aroma compounds: monoterpenes (linalool and hotrienol), norisoprenoids (β-damascenone) and methoxypyrazines (IBMP). Samples further separated along the fourth component (PC4 6.5% explained variance) due to skin contact (Supplementary Figure S2.3), without clear distinction between HL/LL samples.

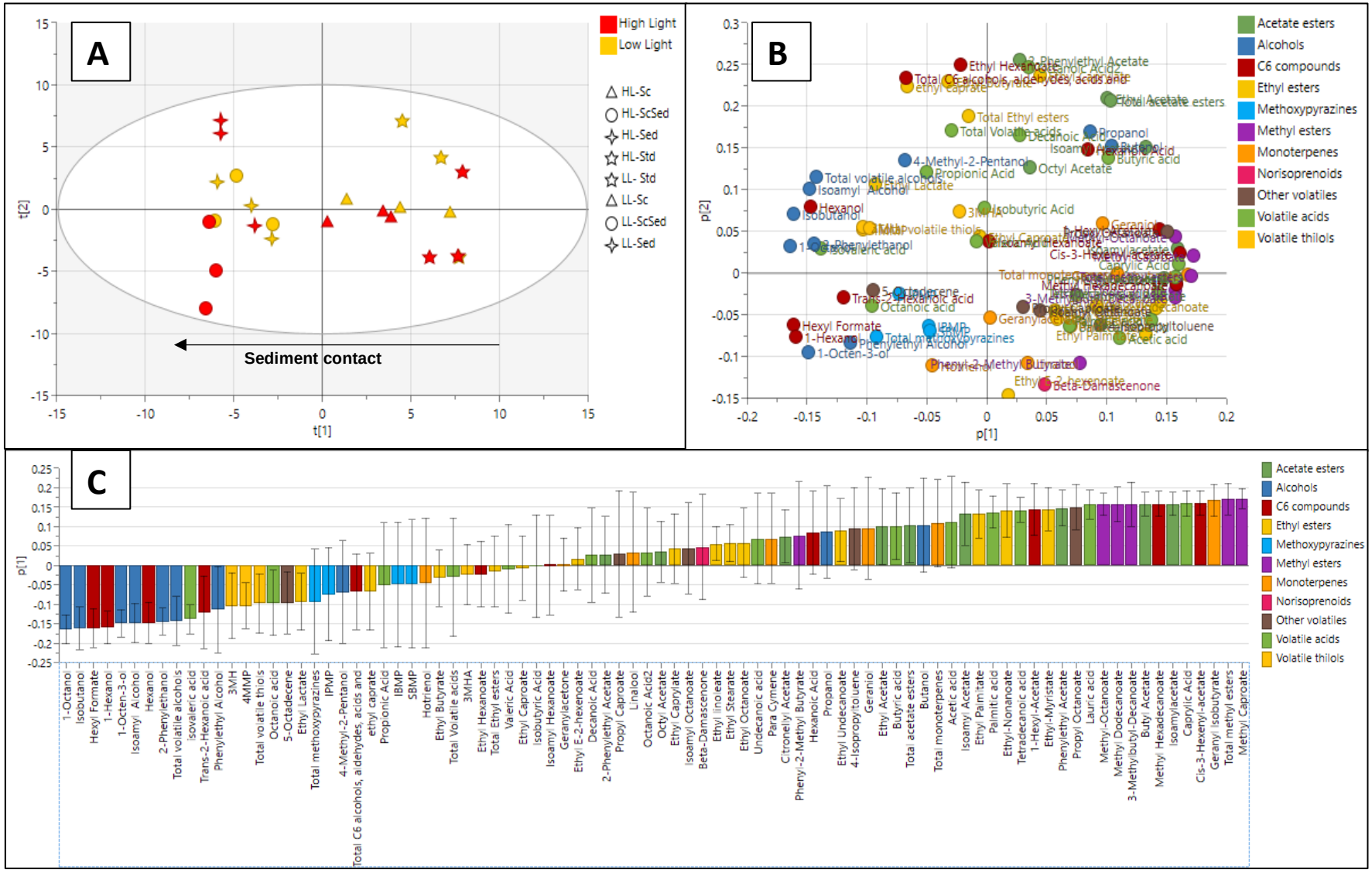


Figure 2.9: PCA score (A), loadings (B) and loadings coefficient (C) plots of aroma linked metabolites in standard (Std) LL and HL wine and in with different extraction treatments (skin contact – Sc, sediment contact – Sed and skin + sediment contact (ScSed). Different symbols indicate different wine samples. Score plot coloured according to light exposure: red = high light, yellow = low light).

Repeated measures ANOVA was used to rank the significance of the compound responses to the main experimental factors in the wine (i.e. light exposure, skin contact and sediment contact), individually and in combination (Table 2.7). The compounds are presented per compound group (monoterpenes, methoxypyrazines, C_6 compounds, norisoprenoids, acids, alcohols, various esters and volatile thiols) and origin (grape-derived or fermentation-derived). The results confirmed that sediment contact was the most significant driver for compound response (F-values for most of the compounds in this group was in the maximum range i.e. larger than 20). Most grape-derived aroma compounds (monoterpenes (43.4), hexanol (115.4), and to a lesser extent volatile thiols (5.8)) and fermentation-derived aroma compounds (ester (isoamyl acetate – 56.4), acids (lauric acid – 76.4) and alcohols (23.9) responded significantly to the sediment contact treatment. Some grape-derived aroma compounds, like IBMP (97.9), linalool (51.1), hotrienol (53.0) and β-damascenone (9.2), mostly reacted to light exposure; this was also seen in the PCA plots of the wines (Figure 2.10A). Interestingly, these were also the same compounds that also showed significant increases to light exposure in the juice samples.

Table 2.7. The repeated measures ANOVA results for the interaction between light exposure, skin contact and sediment contact and individual aroma linked metabolites in wines, reported as F-values. Values are coloured from highest (most significant). Green indicates lower F-values (F>5), yellow indicates intermediate F- values (F>10) while red indicates high F-values values (F>20). All insignificant values (F ≤ 5) are coloured in gray. F values correlate to p-values. Maximum = p<0.001; intermediate =p<0.01; minimum = p<0.05; insignificant = p>0.05

Compounds	Light exposure	Skin contact	Sediment contact	Light exposure* Skin contact	Light exposure* Sediment contact	Skin contact* Sediment contact	Light exposure* Skin contact* Sediment contact
GRAPE DERIVED AROMA COMPOUNDS							
Monoterpenes							
Para Cymene							
Linalool	51.1	9.9	11.0		16.7		6.0
Hotrienol	53.0				8.6		
Geranyl Isobutyrate			55.4				
Geraniol	29.6		53.7	13.4	34.3		24.4
Total monoterpenes			*43.4*	*5.1*	*33.7*		*8.2*
Norisoprenoids							
β-Damascenone	9.2				6.0		
C_6 - compounds							
Cis-3-Hexenyl-acetate		5.2	57.7				
1-Hexyl-Acetate			46.2				
Isoamyl Hexanoate	7.7			15.0	6.0		
Hexyl Formate			95.2				
1-Hexanol		11.5	115.4				5.1
Trans-2-Hexanoic acid			17.8		5.5		5.1
Methyl Hexadecanoate		6.9	58.1	6.2		16.4	4.5
Ethyl Hexanoate		5.1					7.0
Hexanol			31.2				
Hexanoic Acid			9.0				
Total C_6 compounds							
Volatile Thiols							
4MMP			7.4				
3MHA							
3MH			6.3				

Total Thiols			*5.8*				
Methoxypyrazine							
IPMP	6.5		11.8		6.6		
IBMP	97.9						
SBMP	147.4	5.1	5.3				
Total methoxypyrazines	*16.9*		*14.7*				
FERMENTATION DERIVED AROMA COMPOUNDS							
Acetate Esters							
2-Phenylethyl Acetate							5.3
Isoamyl acetate			56.4				
Octyl Acetate							
Phenylethyl Acetate			41.0				
Citronellyl Acetate			5.2	9.9			
Ethyl Acetate			13.3				
Total acetate esters			*14.6*				
Ethyl Esters							
Ethyl Butyrate		6.3					9.0
Ethyl Lactate			14.5				
ethyl caprate							5.8
Ethyl linoleate		18.0	7.3	22.1			
Ethyl Stearate		18.9	7.5	21.8			
Ethyl Palmitate			25.2	6.9		16.2	6.9
Ethyl Caproate							
Ethyl E-2-hexenoate		19.5				9.3	
Ethyl-Nonanoate			90.4	15.4	17.1		
Ethyl Undecanoate	6.0				5.4	11.1	
Ethyl-Myristate		10.6	47.8			30.5	6.1
Total Ethyl esters		*6.1*					
Methyl Esters							
Methyl Dodecanoate		10.3	52.2			13.1	
3-Methylbutyl-Decanoate		7.8	51.7			12.1	5.7
Methyl Caproate		5.4	156.7	5.1			
Phenyl-2-Methyl Butyrate	15.6		9.1			13.4	
Methyl-Octanoate	14.8	8.4	441.7			7.9	36.0
Total methyl esters		*7.9*	*135.9*			*6.9*	
Volatile Acids							
Undecanoic acid	41.6		29.3	65.9	54.6	16.9	21.6
Acetic acid	20.0		34.4	5.1		8.3	8.3
Octanoic acid			8.4				
Caprylic Acid			48.2	7.7			
Lauric acid		21.5	76.4			20.7	7.6
Tetradecanoic acid		13.4	39.3			28.1	
Palmitic acid			16.6			12.7	
Butyric acid		5.4	12.0				
isovaleric acid			22.3				
Alcohols							
Phenylethyl Alcohol	17.0	7.3	19.3				
1-Octen-3-ol		110.4	178.5		8.9		10.0
1-Octanol			192.5		13.1	14.1	
Propanol			7.4				
Isobutanol			51.0				
Butanol			17.8				
2-Phenylethanol	6.5	6.8	38.2				7.4
Isoamyl Alcohol			31.2				
Total volatile alcohols			*23.9*				
Other major volatiles							
Propyl Caproate	82.2		6.2	6.9			
4-Isopropyltoluene							
Propyl Octanoate	48.5	52.1	705.1	92.4	25.9	23.4	6.0
Isoamyl Octanoate							
5-Octadecene		8.3	38.9	6.7		5.6	

The PCA and repeated measures ANOVA analysis were followed by clustering analysis to identify aroma-linked metabolites that share similar response patterns between the standard and adapted wine processing treatments. The clustering analysis (presented in Supplementary Figure S2.5) generated three sample clusters, namely sediment contact, skin contact, or microclimate. Interestingly, the sediment contact samples formed sub-groups based on microclimate, whereas the no sediment contact samples separated based on skin contact. The skin contact pressed, and skin contact clarified samples clustered together with the free run samples. The mean patterns of responses is presented in Figure 2.10; compounds were grouped in three distinctive groups according to similar response profiles.

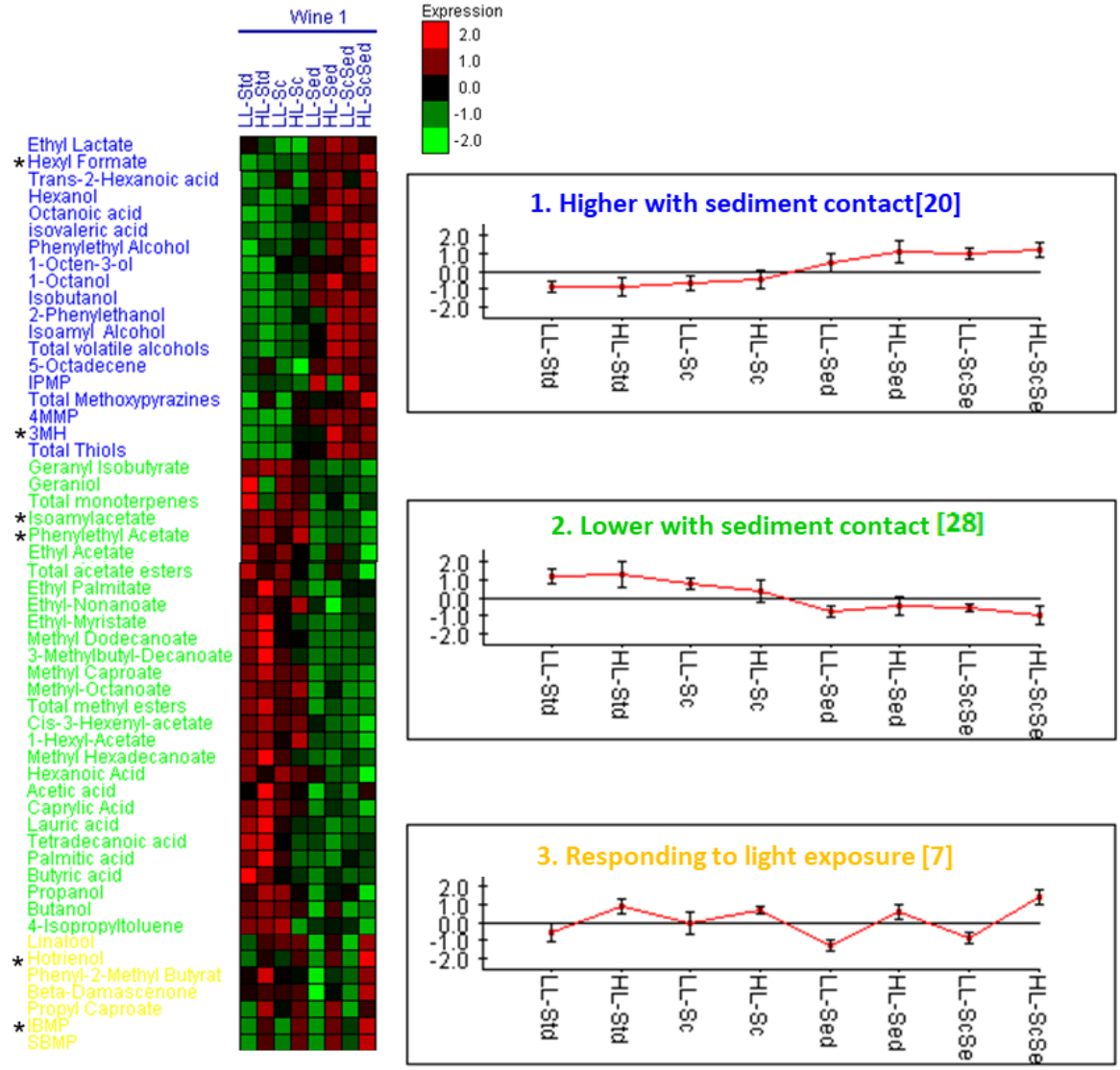


Figure 2.10: The cluster expression matrix and mean patterns for compounds and compound groups measured in standard wine and wine with various extraction treatments from two microclimates. Compounds and groups that exhibited similar response profiles to treatments were grouped together on the metabolite level. Abbreviations: LL, low light; HL, high light; Std, standard; Sc, skin contact; Sed, sediment contact; ScSed, skin + sediment contact. Red bocks indicate a high expression and green blocks indicate a low expression of the specific aroma compound within the wine sample. Asterix indicate examples from each group for the factorial ANOVA plots in Figure 2.11.

The first cluster contained 20 compounds which increased with sediment contact (Figure 2.10). Compounds in this group included the aromatic compounds hexyl formate (contributing “green” aromas) and 3MH (contributing SB varietal “tropical fruit” aromas) (Figure 2.11 A). Hexyl formate was also found in higher concentrations in the sediment (Supplementary Table S2.4). The ANOVA results indicate that the sediment contact treatment negated the light exposure and skin contact effects for the majority of compounds in this cluster (Figure 2.11A), with a few exceptions. For example, 3MH, of which significantly higher concentrations were found in HL sediment contact samples compared to LL sediment contact samples.

The second cluster was the biggest cluster, containing 28 compounds which mostly decreased with sediment contact (Figure 2.10). Compounds in this group included mostly esters like ethyl acetate and isoamyl acetate (Figure 2.11 B) – usually associated with “sweet fruity” and “floral” aromas in wine. As with the previous cluster, it would seem that the addition of sediment contact negated the light exposure and skin contact effects, irrespective of whether the sediment resulted in higher or lower concentrations of aroma compounds.

Most of the aroma compounds were found in the first two clusters (Figure 2.10), both of which responded to sediment contact and again reiterating the significant effect of sediment contact on the aromatic compounds in wine (mostly fermentation derived). However, some light exposure responses were still seen in grape-derived aroma compounds.

The third cluster contained 7 compounds which increased with light exposure (Figure 2.10). Compounds in this group included mostly grape-derived aroma compounds like linalool and IBMP (Figure 2.11C). Skin contact also led to the increase of these compounds in some samples (LL-Sc in linalool and HL-ScSed in both linalool and IBMP). The concentration ranges of aroma impact compounds are compared to the ranges found in literature and can be found in supplementary Table S2.5.

Furthermore, the odour activity values (OAV) values were calculated for samples that showed significant responses by dividing the detected concentration of that compound by known aroma thresholds found in literature (Supplementary Table S2.6) to determine the aromatic “strength” of the compounds detected, and the possibility of their contributions to the perceived sensory profile. It was found that most of the grape-derived and fermentation-derived aroma compounds were present at concentrations above their aroma thresholds, resulting in OAV values > 1 (Supplementary Table S2.6). Some of the aroma compounds found in specific samples had very large OAV values: isoamyl acetate (186), isoamyl alcohol (66) and β- damascenone (47.4).

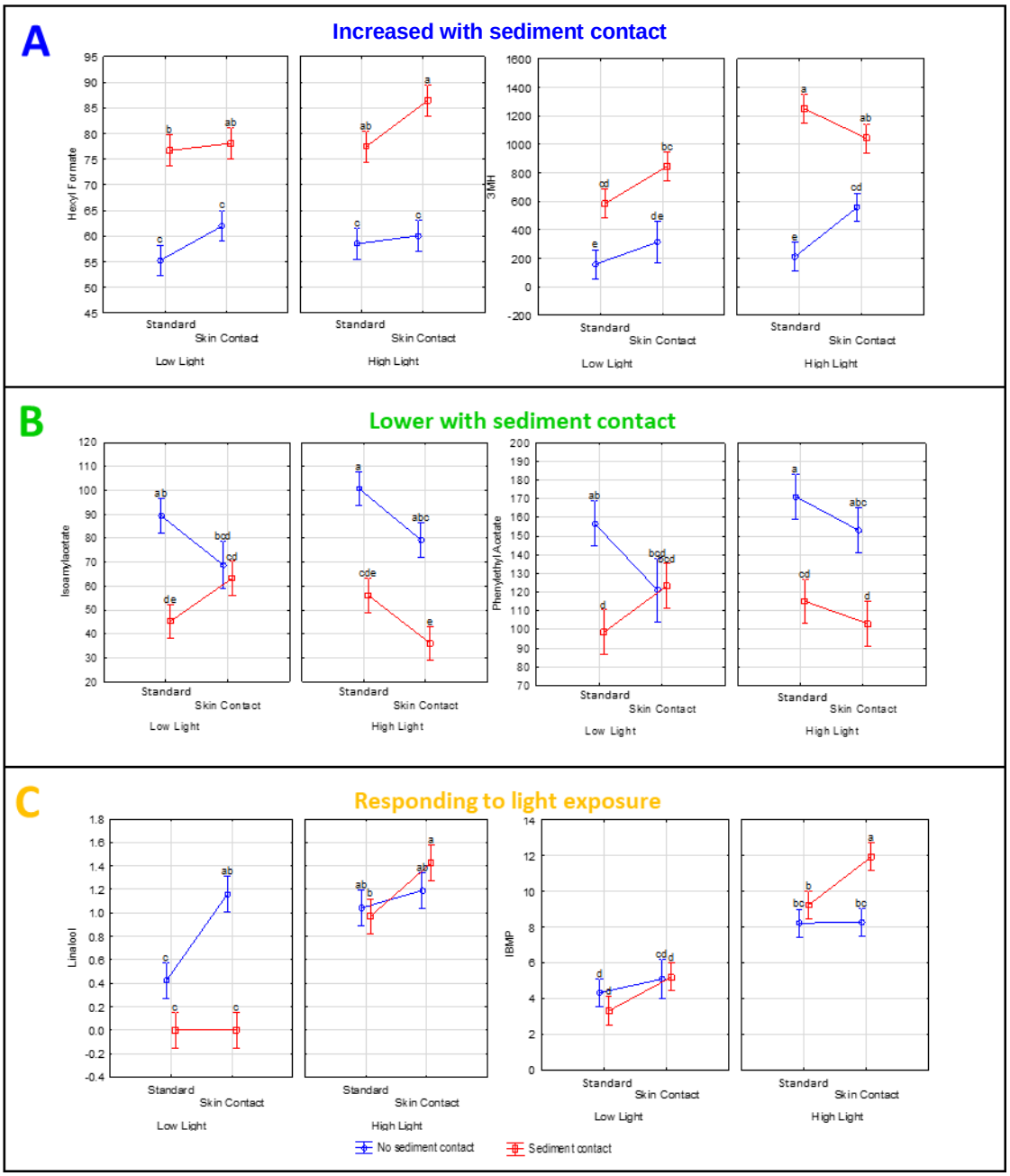


Figure 2. 11: The ANOVA plots of aroma compound in LL and HL wines with different extraction treatments (Sc, Sed or ScSed) which responded to light exposure and sediment contact. **(A)** Increased with sediment contact (hexyl formate and 3MH); **(B)** Decreased with sediment contact (isoamyl acetate and phenylethyl acetate); and **(C)** grape derived aroma compounds (linalool and IBMP)) responded to light exposure. Different letters indicate statistical difference (Fischer's LSD $p<0.05$).

2.3.4. Wine sensory analysis

Descriptive sensory analysis was performed on all wine samples after 6 months of bottle aging. Although wines were analyzed for taste, aroma and mouthfeel, focus will be placed on the aroma and taste; the phenolic profiles of the samples and sensory impacts on mouthfeel has been studied in a parallel study (Williams, 2019) and showed that sediment contact during fermentation had a significant impact on the bitterness and astringency of samples.

The panel performance was checked using PanelCheck software (Tomic *et al.*, 2009). The repeatability of the panel was checked as well as their consensus and discrimination ability. Two out of the 10 panelists were found not to be repeatable in their evaluations and their results were thus excluded. All the other judges were found to be repeatable and could discriminate between samples; the panel consensus was thus acceptable (results not shown). Most of the descriptors identified were typical of SB styles like “pineapple”, “peach” and “asparagus”. The data generated for all the descriptors identified are presented in Table 2.8.

The sensory profiles (descriptive analysis) of the various wines confirmed that eight wines with uniquely different descriptors were obtained from the experiments performed. When the data was analysed with PCA, there were two distinctive groupings identified in the PCA (Figure 2.12), driven by sediment extraction along the first principle component (PC1, 68.7% explained variance), and light exposure along the second principle component (PC2, 27.6% explained variance). All the sediment-contact samples formed a grouping, irrespective of light exposure or skin contact. Interestingly, in the no-sediment samples, LL-Sc grouped further from the LL-std sample. Overall, the further grouping of the LL-Sc to LL-std samples in both chemical and sensory data suggested that that the LL samples were more responsive to skin contact treatments after fermentation (Figure 2.9 and 2.12).

To visually compare the descriptive analysis of the wines, spider plots were prepared for all the wines (Figure 2.13). The wines made from HAP berries (Figure 2.13 E, F, G, H) were generally considered as tasting sweeter. HL- Std wine scored higher in sweetness, body and “banana” aroma than LL-Std wines(Figure 2.13 A & E). Whereas the LL-std wine tested higher for sour and astringency. Skin contact led to an increase of sweetness and banana aroma in LL-Sc wine (Figure 2.13 B). The LL-Sc wine had an very similar profile to the HL-Std wine (Figure 2.13 B & E). Sediment contact led to an increase in the perception of “savoury” and “cooked vegetable” characters in LL and HL wines (Figure 2.13 C, D, G, H), with a decrease in “peach” and “pineapple” aromas in HL-ScSed wines (Figure 2.13 H).

Table 2.8: Descriptive sensory analysis test scores (intensity) of standard SB wine and wine with different extraction treatments. Factorial ANOVA conducted per compound (row) between different samples (columns). Row-wise, different letters indicated statistical difference (Fisher's LSD, $p<0.05$). n=3

Taste and Aroma Descriptors	Standard Wine		Wine with skin contact		Wine with sediment contact		Wine with skin + sediment contact	
	LL	HL	LL	HL	LL	HL	LL	HL
Aroma descriptors								
Pineapple	3.18±0.07^{ab}	3.3±0.24^{a}	3.42±0.2^{a}	3.39±0.3^{a}	2.86±0.27^{b}	2.86±0.07^{b}	2.86±0.17^{b}	2.43±0.23^{c}
Peach	2.63±0.06^{a}	2.81±0.2^{a}	2.88±0.13^{a}	2.96±0.15^{a}	1.48±0.45^{b}	1.53±0.34^{b}	1.59±0.51^{b}	0.8±0.08^{c}
Banana	0.82±0.09^{ab}	1.07±0.11^{a}	1.04±0.17^{a}	0.82±0.37^{ab}	0.6±0.1^{bc}	0.41±0.13^{c}	0.53±0.13^{bc}	0.47±0.17^{c}
Passionfruit	2.06±0.45^{ab}	2.09±0.22^{ab}	2.44±0.09^{a}	2.35±0.39^{a}	1.41±0.35^{cd}	1.59±0.21^{bc}	1.41±0.38^{cd}	0.93±0.17^{d}
Grapefruit	2.36±0.13^{abc}	2.34±0.01^{abc}	2.66±0.26^{a}	2.48±0.4^{ab}	2.14±0.1^{bc}	2.27±0.32^{abc}	2.4±0.31^{ab}	1.94±0.16^{c}
Floral	1.17±0.06^{b}	1.8±0.3^{a}	1.26±0.2^{b}	1.31±0.53^{ab}	0.47±0.34^{c}	0.42±0.23^{c}	0.34±0.13^{c}	0.2±0.03^{c}
Baked apple	2.37±0.13^{b}	2.76±0.21^{a}	2.01±0.03^{cd}	2.27±0.32^{bc}	1.58±0.14^{ef}	1.78±0.17^{de}	1.23±0.17^{g}	1.32±0.13^{fg}
Gherkin/dill	0.23±0.05^{c}	0.1±0.11^{c}	0.43±0.09^{c}	0.32±0.11^{c}	0.83±0.39^{b}	0.78±0.21^{b}	0.86±0.07^{b}	1.51±0.04^{a}
Asparagus	0.09±0.01^{d}	0.05±0.02^{d}	0.11±0.05^{d}	0.05±0.03^{d}	0.52±0.17^{b}	0.35±0.11^{c}	0.49±0.12^{bc}	0.92±0.08^{a}
Cooked vegetables	0.2±0.13^{c}	0.15±0.11^{c}	0.24±0.05^{c}	0.14±0.11^{c}	1.52±0.61^{b}	1.26±0.41^{b}	1.64±0.64^{ab}	2.16±0.14^{a}
Savoury	0.31±0.26^{c}	0.2±0.15^{c}	0.44±0.04^{c}	0.21±0.06^{c}	1.16±0.53^{ab}	1.02±0.27^{b}	1.27±0.35^{ab}	1.62±0.3^{a}
Taste and mouthfeel descriptors								
Sweet	1.75±0.13^{d}	4.57±1.55^{ab}	2.77±0.78^{cd}	5±1.2^{a}	1.87±0.15^{cd}	2.29±0.17^{cd}	2.27±0.44^{cd}	3.32±1.14^{bc}
Sour	4.83±0.1^{a}	3.01±0.66^{de}	3.87±0.66^{c}	2.37±0.66^{e}	4.71±0.21^{ab}	4.34±0.11^{abc}	4.02±0.29^{bc}	3.56±0.45^{cd}
Bitter	1.74±0.11^{a}	1.31±0.35^{ab}	1.51±0.11^{ab}	1.15±0.51^{b}	1.5±0.14^{ab}	1.79±0.27^{a}	1.82±0.14^{a}	1.74±0.45^{a}
Astringent	1.17±0.08^{a}	0.81±0.17^{b}	1.16±0.05^{a}	0.79±0.3^{b}	1.25±0.23^{a}	1.16±0.06^{a}	1.12±0.13^{a}	1.24±0.23^{a}
Body	2.28±0.01^{b}	3.18±0.52^{a}	2.59±0.32^{b}	3.43±0.2^{a}	2.18±0.1^{b}	2.58±0.16^{b}	2.39±0.23^{b}	2.62±0.21^{b}

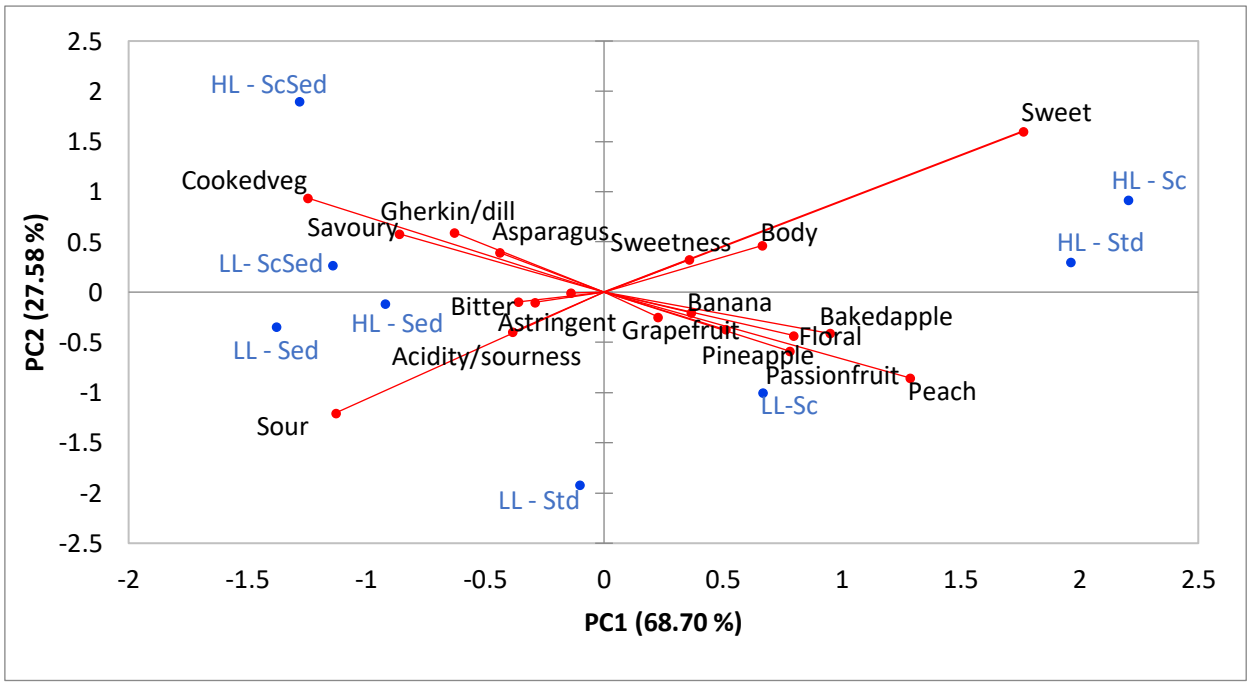


Figure 2.12: PCA bi plot of the descriptive sensory analysis indicating the correlation between different wine samples and descriptors. Proximity of samples to points representing aroma nuances indicates a positive correlation between the sample and the aroma nuance. Abbreviations: LL, low light; HL, high light; Std, standard; Sc, skin contact; Sed, sediment contact; ScSed, skin + sediment contact.

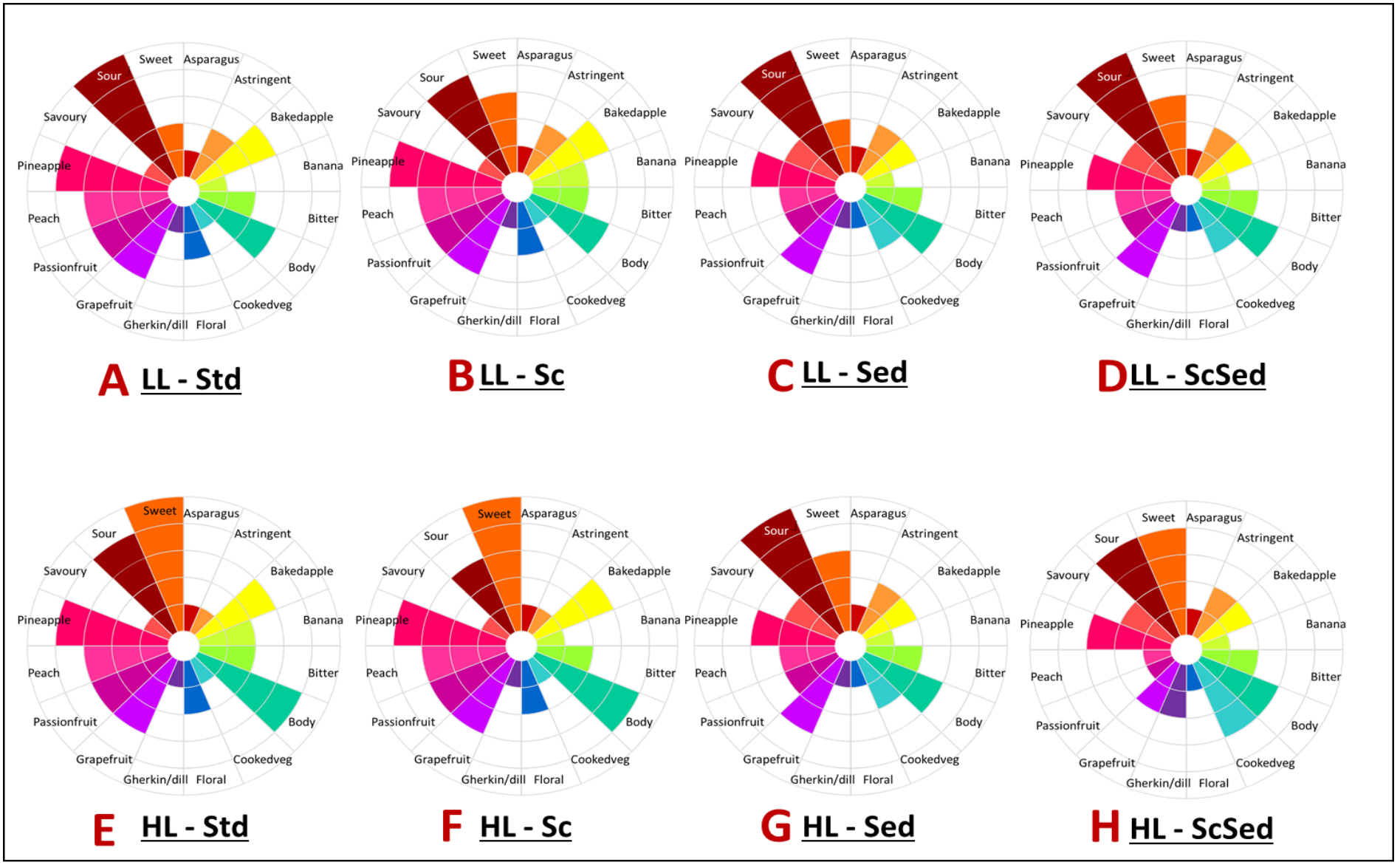


Figure 2.13: Spider-plots from the descriptive sensory analysis results representing the different aroma profiles of standard (Std), skin contact (Sc), sediment contact (Sed) and skin + sediment contact (ScSed) wines from LL and HL microclimates. Graphs calculated using DA sensory scores, n=3.

2.4. Discussion

Sauvignon Blanc berries grown under two microclimatic conditions, that differed in the light exposure in the bunch zone, were confirmed to be compositionally different, specifically with regards to aroma compounds and/or aroma-linked precursors (Figure 2.4). The berries from the HL microclimate accumulated slightly more sugars, less acids and overall less total amino acids, but were enriched in monoterpenes, norisoprenoids, methoxypyrazines and BCAA and AAA, compared to berries from the LL microclimate. The higher concentration of methoxypyrazines in HL samples is in contradiction to what has been found in previous studies (Šuklje *et al.*, 2014; Gregan *et al.* , 2016; Joubert *et al.*, 2016; Mosetti *et al.*, 2016) which found that increased light exposure usually lead to a decrease in methoxypyrazine concentration. This trend was observed throughout all the matrices, so the possibility of instrumental or labelling errors have been excluded. Moreover analysis of the photosynthetic pigments confirmed that the HL berries responded to the increased exposure by using the xanthophyll cycle to mitigate the increased light, resulting in HL berries with higher concentrations of xanthophylls, which are known aroma-compound precursors (Dunlevy *et al.*, 2009; Darriet, 2012), supporting similar findings by Joubert *et al.* (2016) and Young *et al.*, (2016). Our berry analysis confirmed that the berries from the HL microclimate had an overall higher aromatic potential (HAP), specifically with regards to grape-derived aroma compounds, compared to the lower aromatic potential (LAP) berries originating in the LL microclimate. The higher aromatic potential of the HL berries, however, are only an indication of the possibility that more aroma compounds and compound precursors are available for extraction. In the study it is assumed that when an increase of a compound concentration is seen, it is either due to increased extraction of said aroma compound, or the precursor of the aroma compound which is converted to the specified aroma compound during fermentation.

Subsequently, the impact of juice processing and winemaking steps were evaluated for specific volatile aroma compounds to explore whether these vineyard-achieved aromatic “potentials” can be more fully extracted to the resulting wines when non-standard white winemaking techniques were used. Conventional white winemaking procedures do not allow for prolonged extraction of metabolites from grape tissues such as the skin and/or sediment. A pre-fermentative skin contact and a fermentative sediment contact treatment, as well as a combination of the two, were introduced, to determine whether the extraction of aroma-linked metabolites would be increased into juices and wines, and how it might differ when working with berries with low and high aromatic potentials. The metabolic profiles, specifically the aromatic compounds were determined chemically from the different matrices and the impacts of the increased extraction treatments on the aroma and taste of wine were also determined.

The analysis of grape-derived aroma-linked metabolites in free run, pressed and clarified juices showed interesting and labile trends throughout juice processing (Figure 2.7 – 2.8) which corresponded to findings by Honeth (2018). Analysis of the juice matrix in the study by Honeth (2018) also revealed dynamic trends, especially in amino acids, but also in monoterpenes and norisoprenoids. The author suggested that this might be due to unaccounted for chemical and/or biochemical reactions during juice processing. The breaking of cell vacuoles during crushing and pressing could release various hydrolytic enzymes resulting in the enzymatic and oxidative release from glycosidically bound monoterpenes and norisoprenoids and the proteolytic degradation of proteins resulting in the release of amino acids. This is supported by the increase of many amino acids between the press and clarified stages (Figures 2.8A and B). The increase in amino acids after clarification is significant as the amino acids which increased are largely yeast preferred amino acids which prevent nitrogen deficiency during fermentation and consequently sluggish or stuck fermentations. The sediment contact samples had the highest fermentation rate, suggesting that the sediment matrix contained additional nutrients like lipids and possibly a portion of the yeast preferred amino acids that may have settled out upon clarification. Furthermore, in samples LL clarified samples (both standard and skin contact) contained significantly higher concentrations of BCAA, whereas HL-Sc clarified samples contained significantly higher concentrations of AAA. The metabolism of BCAA and AAA by yeast will result in the production of esters and higher alcohols which typically contribute to the sensory perception of wine (Rapp & Versini, 1995; Swiegers *et al.*, 2006; Hazelwood *et al.*, 2008). However, there were no significant differences in ester or alcohol concentrations between LL-Std and HL-Std wines. This might be due to the very high concentrations of yeast preferred amino acids present in the clarified juice prior to fermentation; due to the sequential metabolism of amino acids by yeast, the yeast would metabolise these first as they are preferred nitrogen sources over the BCAA and AAA (Crépin *et al.*, 2012).

The difference in aroma profile between HAP and LAP berries was still seen in the free run juice samples under standard winemaking conditions (LL-Std and HL-Std, Supplementary Table S2.2). The continued difference in aromatic profile between LAP and HAP samples through to the first stage of juice processing indicated that HAP berries resulted in juices with higher aromatic potentials. For example, significantly higher concentrations of monoterpenes and methoxypyrazines were observed in HL berry samples and in HL free run juice samples, when compared to the corresponding LL samples. Higher concentrations of hotrienol, IBMP and β-damascenone (important grape-derived aroma compounds) persisted in HAP samples throughout juice processing and fermentation, resulting in HL-Std wines with significantly higher concentrations of these compounds. The HL samples, however, did not ferment to dryness, although the fermentation weight was constant for a few days (indication that CO_2 release stopped) the residual fructose concentration was > 5 g/L. This could have had a significant impact on the sensory perception of HL wines. Of the other compounds present in higher concentrations, IBMP and β-damascenone had OAV values which are much larger

than 1 (Supplementary Table S2.6), the OAV of IBMP is 8.45 and for β-damascenone 47.41, showing that these compounds at the concentrations tested for will have a definite perceptible sensory effect on the aroma of the wine. In all the wine samples, it has been noted that the concentration of methoxypyrazines are higher when compared to the concentrations found in the juice. This is in contrast to what has been found in literature, especially in white wine (Hartmann *et al.*, 2002; Sala *et al.*, 2002, 2004; Darriet, 2012). No clear explanation as to the reason for the increase could be found. It is common for the concentration of methoxypyrazines to increase in red-wine production. This is usually due to the increase in extraction of methoxypyrazines from the pomace during the extended maceration period of red wine making. Furthermore, this study included the analysis of wine made through standard processing and modified processing with the purpose to increase extraction. The higher methoxypyrazine concentration in the modified winemaking samples indicated that these were effective in increasing the extraction of methoxypyrazines.

A general decrease in concentration of aroma-linked metabolites was observed as juice processing proceeded and particularly when considering the juice samples after clarification (Figure 2.8 A,C,D). This is in contrast to a study by Roland *et al.* (2011), who found that the concentration of volatile thiol precursors increased with pressing, although it is known that the cysteinylated and glutathionylated volatile thiol precursors are linked to only a fraction of the final thiol concentrations in wine (Coetzee *et al.* 2012). The findings are in agreement to the findings of Patel *et al.* (2010), which showed that pressing resulted in significantly lower concentrations of volatile thiols in final wine. It was proposed by Patel *et al*. (2010) that this loss was due to non-enzymatic oxidation of volatile thiols. The reduction of volatile thiols in wine that received no sediment contact is probably due to the precipitation of thiol precursors into the sediment after clarification. This is supported by the significant higher concentrations of volatile thiols in the sediment contact wine.

In terms of grape-derived aroma compounds, the distinction between LAP and HAP samples were maintained from berry to LL-Std and HL-Std wine, respectively (Tables S2.1, S2.2 and S2.3). The aroma impact compound (methoxypyrazines and volatile thiols) concentrations of all samples fell within ranges observed elsewhere in literature (Supplementary Table S2.5). After alcoholic fermentation, many of the fermentative aroma compounds (esters, acids, alcohols) and volatile thiols showed no significant differences between LL-Std and HL-Std wines (chemically). Although the differences in chemical composition of LL-Std and HL-Std wines were not remarkable, there were clear differences in the sensory analysis of LL-Std and HL-Std wines: LL-Std wines had a definite “sour” and “fruity” style, where the HL-Std and HL-skin had similar “sweet” and “fruity” profiles with high scores for body, similarly to previous studies with grapes from the same vineyard (Šuklje *et al.*, 2014; Honeth, 2018). Chemical analysis showed that the HL samples contained significantly higher concentrations of fructose and glycerol. Studies have shown that sugar is a larger contributor to wine mouthfeel than glycerol, and can be the reason for the increased perception of body in HL-Std

samples (Nurgel *et al., 2005; Gawel et al., 2008)*. Glycerol is also a stress metabolite (Bauer & Pretorius, 2000), indicating that the HL no sediment contact fermentations experienced some stress, possibly due to the lower yeast preferred amino acid concentration in the HL berries. Only the HL wines that did not receive sediment contact tested high for “sweet” descriptors. The sweetness perceptions in the sediment contact wines could have been somewhat masked by the increased perception of bitterness, and the lower concentration of residual fructose in these samples.

This notable distinction between LL-Std and HL-Std wines (but also the other samples) was pronounced at a sensory level, with minor differences recorded on a chemical level. This is likely linked to the following, amongst others: 1) It is possible that the aroma compounds were subject to aroma compound x aroma compound interactions and that these interactions may cause an additive effect which is not accounted for in chemical profiling. 2) There are likely additional aroma compounds present in the wines that were not part of our analyses, which also contributes to and promoted the distinction between LL-Std and HL-Std wines at the sensory level. 3) Finally, the wine samples used for chemical analysis were taken directly after fermentation, whereas sensory analysis was conducted after 6 months of aging. It would have been interesting to also have an analytical dataset of the wines at the time the sensory evaluation occurred. It would therefore seem that the vineyard achieved aromatic potentials were maintained, specifically in terms of grape-derived aroma compounds, resulting in wines with two distinct sensory profiles.

The aroma compounds that persisted in the HL-Std wine shortly after fermentation were grape-derived aroma compounds. When examining the distribution of aroma-linked metabolites in the grape berry, most of the grape-derived aroma-linked metabolites were found in the skin of the grape berry (Conde *et al.*, 2007; Fontes *et al.*, 2011; Jackson, 2014). Many grape-derived terpenoid compounds are mainly stored as glucoside derivatives that are water-soluble in the skin of grape berries, or as amino acid conjugates in grape cell walls (Lund & Bohlmann, 2006). Cell walls and vacuoles are broken during crushing, bringing the non-volatile glycosides into contact with water and enzymes required for cleaving of the conjugated non-volatile compounds, making them volatile (Lund & Bohlmann, 2006). Chemical analysis of clarified juice showed that skin contact resulted in higher concentrations of C_6 compounds (leading to higher total volatile concentrations in HL-Sc samples), the rest of the aroma-linked metabolites were either unresponsive (some amino acids, norisoprenoids, methoxypyrazines) or showed a decrease due to skin contact (monoterpenes and other amino acids), in contrast to what has previously been found in literature.

Studies by Marais (1998) and Selli *et al (*2006) found that skin contact resulted in significantly higher concentrations of volatile monoterpenes, alcohols and methoxypyrazines in juice and musts. The addition of skin contact has also been shown to increase the concentrations of aromatic compounds in wines (Cabaroglu *et al.*, 1997; Marais, 1998; Lanaridis *et al.*, 2002; Darias-Martín *et al.*, 2004;

Selli, Canbas, Cabaroglu, Erten, Lepoutre, *et al.*, 2006b; Nicolau *et al.*, 2008). Wine samples with skin contact however showed significantly higher concentrations of monoterpenes and thiols, and can be explained by the biochemical liberation of terpene precursors and thiol precursors from the skin of the grape berry during skin contact in juice processing (Lund & Bohlmann, 2006).

Different aroma compounds responded to skin contact in wine samples compared to juice samples, it is possible that the prolonged skin contact resulted more in the extraction of aroma compound precursors, which was liberated during fermentation. This correlates to a study by Swiegers *et al*. (2009) who found that compounds like thiols and monoterpenes responsible for more fruity attributes in wines appeared to be dependent on the strain of yeast used during fermentation. The yeast used in this study, Cross Evolution (Lallemand), is characterised by its ability to produce wines with increased body and aromas of “fresh fruit” and “floral characters” and likely contributed to the increase in “fruity” aromas in the wine by liberating the volatiles from their precursor complexes. Interestingly, skin contact was effective in the extraction of compounds responsible for “green” aromas (C_6 compounds) in juice, similar to what was found in a study by Swiegers *et al.* (2009). These compounds are secondary grape-derived aroma compounds, meaning that they are produced through chemical reactions during juice processing (Dunlevy *et al.*, 2009; Darriet, 2012), and that with prolonged skin contact there was longer contact time between enzymes and substrates. On the other hand, the sensory profile of LL-Sc wines were similar to HL-Std and HL-Sc wines (Figure 2.13 A,B and E), receiving high scores for both “fruity” (“grapefruit”, “passion fruit”, “pineapple”, “peach”) and “sour” attributes – which differs significantly from the aroma profile of LL-Std wines. While there were significant concentrations of methoxypyrazines and C_6-compounds (linked to “green” attributes) present the fold differences between their OAV values are less pronounced than the OAV values for the thiols, monoterpenes and esters, it is possible that their effect on the sensory profile was masked by high concentrations and relative sensory strength of volatile thiols, monoterpenes and esters (linked to fruity attributes). This is supported by a study by van Wyngaard (2013) which found that methoxypyrazines reduce the tropical character of the thiols, and that thiols in turn reduce the “green” (“asparagus”) character of methoxypyrazines, resulting in a mutual suppression. Furthermore, skin contact wine samples of both LL and HL conditions had the highest concentration of esters (Figure 2.11B) and it has been found that some esters can potentially enhance the “fruity” aroma perceptions of thiols (Campo *et al.*, 2005; King *et al.*, 2011), which could be another reason for the increased fruity perceptions in skin contact wines. Much research has been done on the positive (and sometimes negative) effect of skin contact on the aroma profile of wine (Cabaroglu *et al.*, 1997; Marais, 1998; Lanaridis *et al.*, 2002; Darias-Martín *et al.*, 2004; Selli *et al.*, 2006b; Nicolau *et al.*, 2008). Skin contact is usually avoided in the production of white wines like Sauvignon Blanc, primarily due to the increased extraction of phenolic compounds associated with this treatment, which, although positive in red wine production, can be detrimental to the sensory perception of white wine due to increased extraction of polyphenols, resulting in unwanted taste and mouthfeel

characteristics (Marais, 1998; Darias-Martín et al., 2004; Gómez-Míguez et al., 2007; Hernanz et al., 2007; Gawel et al., 2014). Furthermore, prolonged contact time with the skin my introduce other natural occurring microorganisms which would be in competition with the inoculated yeast, resulting in yeast stress and the production of off flavours. Williams (2019) found that the total level of polyphenols decreased considerably from free run to clarified juice and that skin contact increased the total polyphenol concentrations in final wines, but did not have an adverse effect on the taste and mouthfeel of the wines.

The reduction in aroma-linked metabolites (amino acids, monoterpenes and C_6 compounds) found after clarification, suggested that the berry particulates that settle out as sediment during clarification were rich in the number aroma-linked metabolites. Analysis of the sediment revealed high concentrations of sugars, tartaric acid, and aroma-linked metabolites such as monoterpenes and C_6 compounds (Supplementary Table S2.2). It was found that fermentative sediment contact led to higher fermentation rates and indeed also higher concentrations of the C_6 compounds, alcohols, methoxypyrazines, volatile thiols and monoterpenes, but resulted in diminished concentrations of many esters.

Sediment contact likely permitted vital yeast nutrients like sugars, fatty acids, amino acids and sterols to remain available during fermentation (Jackson, 2014), thereby contributing to the increase in the rate of fermentation. Furthermore, the fatty acids and sterols found in the sediments are known precursors to the aromatic C_6 compounds and higher alcohols (Dunlevy *et al.*, 2009; Darriet, 2012). The reduction in ester concentration in the presence of sediment during fermentation was also found by Houtman *et al.* (1980a) who reasoned that this was due to other factors in the system which is not clearly defined. Prolonged sediment contact time probably lead to more precursors being available for the conversion into C_6 compounds and alcohols, thereby strongly impacting the aromatic potentials and sensory descriptions of the wines. Furthermore, Nicolini *et al.* (2011) found that increased juice turbidity led to increased fermentation rates and as juice turbidity increased, the concentrations of C_6 compounds and alcohols also increased while esters and fatty acids decreased, which ultimately resulted in complex wine aromas.

The sensory descriptive analysis also confirmed that completely different aroma profiles were achieved in the wines made with sediment contact, compared to the no-sediment contact wines with no distinction between LAP and HAP samples (Figure 2.13). All the sediment contact wines scored higher for aroma and taste descriptors like “gherkin/dill”, “cooked veg”, “bitterness” and “savoury” in spite of having the highest concentrations of volatile thiols. Taking into account the high concentrations organic acids in the sediment, it is interesting that sediment samples did not score higher for “sour” attributes, indicating that the organic acids were not further extracted during

fermentation. As already determined, sediment contact had a negative effect on monoterpene and ester concentrations (associated “fruity” and “floral” aromas) and higher concentrations of volatile thiols, alcohols, C_6 compounds and methoxypyrazines (associated with “herbaceous”, “green” and “vegetative characters”). The resulting “vegetative” (specifically “cooked vegetable”) aroma profiles of sediment contact wines could be due to the suppression of thiols by methoxypyrazines (van Wyngaard, 2013), also, some esters have been linked to the enhancement of volatile thiol aromas (Campo *et al.*, 2005; King *et al.*, 2011), and the lower ester concentrations in the sediment contact wine samples could have further contributed the masking effect of the thiol-associates aromas in the sensory profile of the wine. Furthermore, reductive aroma compounds have been linked to cooked vegetables have been known to mask thiol driven aromas. Reductive aromas (like “cooked vegetable” – which was very prominent in the sediment contact wines – LL-Sed, LL-ScSed, HL-Sed, HL-ScSed) have been associated with too little oxygen during wine making (Coetzee & Du Toit, 2015). As stated earlier, a reductive approach was used during wine making, to limit oxidation, this coupled with the increased fermentation rate (the yeast quickly expelling any oxygen as they produce CO_2) could have resulted in too little oxygen during fermentation. Reductive aroma compounds are known to form after a period in the bottle (Coetzee & Du Toit, 2015), and could explain why the 6-month old sediment contact wine (LL-Sed, LL-ScSed, HL-Sed, HL-ScSed) wine exhibited pronounced “cooked vegetable” aromas in the sensory analysis. Hydrogen sulphide (H_2S) and methyl mercaptan (MeSH) has mostly been attributed to post bottling reduction. It has been established that the sediment fraction did contain a pool of “un-utilised” aroma compounds and precursors, and it is possible that precursors for H_2S and MeSH also resided in the sediment, resulting in their increased extraction and formation after bottling in the samples that received sediment contact. However the formation of reductive aroma compounds is still poorly understood (Coetzee & Du Toit, 2015).

Furthermore, sediment contact also interacted with light exposure so that all HL wines that tested high for residual sugars, but only the combination of HL and no sediment treatment resulted in subsequent descriptors of “sweetness” and “body” during descriptive sensory analysis. The “sweetness“ perceptions seem somewhat masked by the increased perception of “bitterness“ produced by the sediment contact samples. In a parallel study, Williams (2019) analysed the effect of polyphenols on the taste of the same wine and found that the increase of catechin content in the sediment contact wines led to an increase in “bitter“ and “astringent“ sensory perceptions.

Extraction treatments like skin contact and sediment contact (when individually applied) has been shown to result in increased extraction of certain aroma linked metabolites in juice and wine. The combination of skin contact and sediment contact during fermentation did not have an additive response, but rather resulted in unique chemical and sensory profiles in the wines. It was found the wine ScSed samples contained high concentrations of C_6 compounds, IBMP 3MH and linalool, which

was mirrored by the sensory descriptors; these wines had the lowest scores for “fruity“ attributes and the highest scores for “cooked vegetable“. The prevalence of the “cooked vegetable“ aroma in the presence of high concentrations of volatile thiols may be due to three reasons: (1) a masking effect of methoxypyrazines on volatile thiols (van Wyngaard, 2013) or (2) high concentrations of volatile thiols (as seen in this study with OAV values about 10 fold higher than that of IBMP) have been found to cause off odours like sweatiness or cat urine (Swiegers *et al.*, 2006). Furthermore, analysis of the phenolic fraction of the wine revealed that ScSed wines had the highest concentration of coutaric and caftaric acids (Williams, 2019). These findings indicate that the ScSed samples were subject to interesting and complex interaction effects on both chemical and sensory levels which were not easy to predict.

2.5. Conclusion

It was confirmed that light exposure of bunches will determine which compounds/substrates are formed and/or metabolised in the grapevine berries. It is possible to manipulate these processes by modulating the light microclimate. Furthermore, due to the general increase of aroma compounds and precursors in berries from a HL environment, it can be said that HL microclimates produced berries with a higher aromatic potential (HAP). The vineyard achieved aromatic potential in terms of grape derived aroma compounds persisted throughout juice processing and in standard wine, however there was no significant differences observed between fermentation derived aroma compounds. Distinct sensory profiles between LL-Std and HL-Std wines confirmed the importance of grape derived aroma compounds to the wine style produced but that this can be altered by controlled extraction treatments like skin contact. Skin Contact resulted in LL-Sc samples having similar sensory profiles than HL-Std samples, indicating that although the LL microclimate resulted in berries with LAP, a simple pre-fermentative skin contact enabled the potential to be more fully utilised, elevating the aromatic perception of the wine. Furthermore, our study also found that the “fate” of the aromatic compounds and precursors of grape berries can be modulated throughout juice processing and wine making. The addition of skin contact, sediment contact and skin + sediment contact steps resulted in the increased concentrations of alcohols, IBMP, hotrienol and 3MH and, to a lesser extent, a decrease in predominantly esters, which significantly altered the aromatic potential and sensory perception of the final wine. These changes in aromatic potential (especially the changes facilitated by sediment contact and skin + sediment contact steps) were reflected in the sensory perception of the wines. Not all changes necessarily contributed to the desired styles of SB as both desirable and undesirable aroma characteristics were increased in some of the scenarios. Our results show that if SB is manipulated in the vineyard, it is important to define the aromatic potential of the berries to implement juice and wine processing steps to enhance/minimise the desired compounds.

2.6. References

Ancín, C., Ayestarán, B., Corroza, M., Garrido, J. & González, A., 1996. Influence of prefermentation clarification on the higher alcohol contents of wines. Food Chem. 55(3), 241–249.

Cabaroglu, T., Canbas, A., Baumes, R., Bayonove, C., Lepoutre, J.P. & Günata, Z., 1997. Aroma composition of a white wine of Vitis vinifera L. cv. Emir as affected by skin contact. J. Food Sci. 62(4), 680–683.

Campo, E., Ferreira, V., Escudero, A. & Cacho, J., 2005. Prediction of the wine sensory properties related to grape variety from dynamic-headspace gas chromatography-olfactometry data. J. Agric. Food Chem. 53(14), 5682–5690.

Coetzee, C. & du Toit, W.J., 2012. A comprehensive review on Sauvignon Blanc aroma with a focus on certain positive volatile thiols. Food Res. Int. 45(1), 287–298.

Conde, C., Silva, P., Fontes, N., Dias, A.C.P., Tavares, R.M., Sousa, M.J., Agasse, A., Delrot, S. & Gerós, H., 2007. Biochemical changes throughout grape berry development and fruit and wine quality. Food 1 1–22.

Coombe, B.G., 1987. Distribution of Solutes within the Developing Grape Berry in Relation to Its Morphology. Am. J. Enol. Vitic. 38(2), 120–127.

Crépin, L., Nidelet, T., Sanchez, I., Dequin, S. & Camarasa, C., 2012. Sequential use of nitrogen compounds by saccharomyces cerevisiae during wine fermentation: A model based on kinetic and regulation characteristics of nitrogen permeases. Appl. Environ. Microbiol. 78(22), 8102–8111.

Culleré, L., Escudero, A., Cacho, J. & Ferreira, V., 2004. Gas Chromatography-Olfactometry and Chemical Quantitative Study of the Aroma of Six Premium Quality Spanish Aged Red Wines. J. Agric. Food Chem. 52(6), 1653–1660.

Darias-Martín, J., Díaz-González, D. & Díaz-Romero, C., 2004. Influence of two pressing processes on the quality of must in white wine production. J. Food Eng. 63(3), 335–340.

Darriet, P., 2012. Aroma and aroma precursors in grape berry. Biochem. Grape Berry 111–136.

Drappier, J., Thibon, C., Rabot, A. & Geny-Denis, L., 2019. Relationship between wine composition and temperature: Impact on Bordeaux wine typicity in the context of global warming—Review. Crit. Rev. Food Sci. Nutr. 59(1), 14–30.

Dunlevy, J.D., Kalua, C.M., Keyzers, R.A. & Boss, P.K., 2009. The production of flavour & aroma compounds in grape berries. In: Grapevine Mol. Physiol. Biotechnol. Second Ed. 293–340.

Eyéghé-Bickong, H.A., Alexandersson, E.O., Gouws, L.M., Young, P.R. & Vivier, M.A., 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.

Ferrando, M., Güell, C. & López, F., 1998. Industrial wine making: Comparison of must clarification treatments. J. Agric. Food Chem. 46(4), 1523–1528.

Ferreira, V., Ortín, N., Escudero, A., López, R. & Cacho, J., 2002. Chemical characterization of the aroma of Grenache rosé wines: Aroma extract dilution analysis, quantitative determination, and sensory reconstitution studies. J. Agric. Food Chem. 50(14), 4048–4054.

Fontes, N., Gerós, H. & Delrot, S., 2011. Grape berry vacuole: A complex and heterogeneous membrane system specialized in the accumulation of solutes. Am. J. Enol. Vitic. 62(3), 270–278.

Des Gachons, C.P., Van Leeuwen, C., Tominaga, T., Soyer, J.P., Gaudillère, J.P. & Dubourdieu, D., 2005. Influence of water and nitrogen deficit on fruit ripening and aroma potential of Vitis vinifera L cv Sauvignon Blanc in field conditions. J. Sci. Food Agric. 85(1), 73–85.

Gawel, R. & Waters, E.J., 2008. The effect of glycerol on the perceived viscosity of dry white table wine. J. Wine Res. 19(2), 109–114.

Gawel, R., Day, M., Van Sluyter, S.C., Holt, H., Waters, E.J. & Smith, P.A., 2014. White wine taste and mouthfeel as affected by juice extraction and processing. J. Agric. Food Chem. 62(41), 10008–10014.

Gewu, W., 1997. Quantitation and sensory studies of character impact odorants of different white wine varieties. J. Agric. Food Chem. 45(8), 3027–3032.

Gómez-Míguez, M.J., González-Miret, M.L., Hernanz, D., Fernández, M.Á., Vicario, I.M. & Heredia, F.J., 2007. Effects of prefermentative skin contact conditions on colour and phenolic content of white wines. J. Food Eng. 78(1), 238–245.

Gregan, S.M. & Jordan, B., 2016. Methoxypyrazine accumulation and O-Methyltransferase gene expression in Sauvignon Blanc grapes: The role of leaf removal, light exposure, and berry development. J. Agric. Food Chem. 64(11), 2200–2208.

Gregan, S.M., Wargent, J.J., Liu, L., Shinkle, J., Hofmann, R., Winefield, C., Trought, M. & Jordan, B., 2012. Effects of solar ultraviolet radiation and canopy manipulation on the biochemical composition of Sauvignon Blanc grapes. Aust. J. Grape Wine Res. 18(2), 227–238.

Hazelwood, L.A., Daran, J.M., Van Maris, A.J.A., Pronk, J.T. & Dickinson, J.R., 2008. The Ehrlich pathway for fusel alcohol production: A century of research on Saccharomyces cerevisiae metabolism (Applied and Environmental Microbiology (2008) 74, 8, (2259-2266)). Appl. Environ. Microbiol. 74(12), 3920.

Hernanz, D., Recamales, Á.F., González-Miret, M.L., Gómez-Míguez, M.J., Vicario, I.M. & Heredia, F.J., 2007. Phenolic composition of white wines with a prefermentative maceration at experimental and industrial scale. J. Food Eng. 80(1), 327–335.

Honeth, C., 2018. The impact of UV-light on grapevine berry and wine metabolites. (PhD), Stellenbosch University, Stellenbosch, South Africa.

Houtman, A.C. & Du Plessis, C.S., 1981. The effect of juice clarity and several conditions promoting yeast growth on fermentation rate, the production of aroma components and wine quality. South African J. Enol. Vitic. 2(2), 71–81.

Houtman, A.C., Marais, J. & Du Plessis, C.S., 1980a. The possibilities of applying present-day knowledge of wine aroma components: Influence of several juice factors on fermentation rate and ester production during fermentation. South African J. Enol. Vitic. 1(1), 27–33.

Houtman, A.C., Marais, J. & Du Plessis, C.S., 1980b. Factors affecting the reproducibility of fermentation of grapes jiuce and of the aroma composition of wines. I. Grape maturity, sugar, inoculum concentration, aeration, juice turbidity and ergosterol. Vitis 54(19), 37–54.

Jackson, R.S., 2014. Wine science: principles, practice, perception. Fourth Edition. Academic Press.

Joubert, C., Young, P.R., Eyeghe-Bickong, H.A. & Vivier, M.A., 2016. Field-grown grapevine berries use carotenoids and the associated xanthophyll cycles to acclimate to UV exposure differentially in high and low light (shade) conditions. Front. Plant Sci. 7(June), 786.

Joubert, C., Young, P.R., Eyéghé-bickong, H.A. & Castellarin, S.D., 2016. Field-grown grapevine berries use carotenoids and the associated xanthophyll cycles to acclimate to UV exposure differentially in high and low light (shade) conditions. Front. Plant Sci. 7(June), 1–17.

King, E.S., Osidacz, P., Curtin, C., Bastian, S.E.P. & Francis, I.L., 2011. Assessing desirable levels of sensory properties in Sauvignon Blanc wines - consumer preferences and contribution of key aroma compounds. Aust. J. Grape Wine Res. 17(2), 169–180.

Kobayashi, H., Takase, H., Suzuki, Y., Tanzawa, F., Takata, R., Fujita, K., Kohno, M., Mochizuki, M., Suzuki, S. & Konno, T., 2011. Environmental stress enhances biosynthesis of flavor precursors, S-3-(hexan-1-ol)-glutathione and S-3-(hexan-1-ol)-L-cysteine, in grapevine through glutathione S-transferase activation. J. Exp. Bot. 62(3), 1325–1336.

Lanaridis, P., Salaha, M.J., Tzourou, I., Tsoutsouras, E. & Karagiannis, S., 2002. Volatile compounds in grapes and wines from two Muscat varieties cultivated in Greek Islands. J. Int. des Sci. la Vigne du Vin 36(1), 39–47.

Lashbrooke, J.G., Young, P.R., Strever, A.E., Stander, C. & Vivier, M.A., 2010. The development of a method for the extraction of carotenoids and chlorophylls from grapevine leaves and berries for HPLC profiling. Aust. J. Grape Wine Res. 16(2), 349–360.

Lund, S.T. & Bohlmann, J., 2006. The molecular basis for wine grape quality--a volatile subject. Science 311(5762), 804–5.

Maggu, M., Winz, R., Kilmartin, P.A., Trought, M.C.T. & Nicolau, L., 2007. Effect of skin contact and pressure on the composition of Sauvignon Blanc must. J. Agric. Food Chem. 55(25), 10281–10288.

Malherbe, S., 2010. Investigation of the impact of commercial malolactic fermentation starter cultures on red wine aroma compounds, sensory properties and consumer preference. Stellenbosch : University of Stellenbosch.

Marais, J., 1998. Effect of grape temperature, oxidation and skin contact on Sauvignon blanc juice and wine composition and wine quality. South African J. Enol. Vitic. Stellenbosch 19(1), 10–16.

Marais, J., Hunter, J.J. & Haasbroek, P.D., 1999. Effect of canopy microclimate , season and region on Sauvignon blanc grape composition and wine quality. South African J. Oenology Vitic. 20(1), 1–30.

Martin, D., Grose, C., Fedrizzi, B., Stuart, L., Albright, A. & McLachlan, A., 2016. Grape cluster microclimate influences the aroma composition of Sauvignon blanc wine. Food Chem. 210 640–647.

Mollendorff, A. von & von Mollendorff, A., 2013. The impact of wine yeast strains on the aromatic profiles of Sauvignon blanc wines derived from characterized viticultural treatments.

Mosetti, D., Herrera, J.C., Sabbatini, P., Green, A., Alberti, G., Peterlunger, E., Lisjak, K. & Castellarin, S.D., 2016. Impact of leaf removal after berry set on fruit composition and bunch rot in “Sauvignon blanc”. Vitis - J. Grapevine Res. 55(2), 57–64.

Nicolau, L., Trought, M.C.T., Maggu, M. & Kilmartin, P., 2008. Effect of skin contact and pressure on the composition of Sauvignon Blanc must. Aust. New Zeal. Grapegrow. Winemak. 2008(533a-Annual technical Issue), 73–77.

Nicolini, G., Moser, S., Román, T., Mazzi, E. & Larcher, R., 2011. Effect of juice turbidity on fermentative volatile compounds in white wines. Vitis - J. Grapevine Res. 50(3), 131–135.

Nurgel, C. & Pickering, G., 2005. Contribution of glycerol, ethanol and sugar to the perception of viscosity and density elicited by model white wines. J. Texture Stud. 36(3), 303–323.

Patel, P., Herbst-Johnstone, M., Lee, S.A., Gardner, R.C., Weaver, R., Nicolau, L. & Kilmartin, P.A., 2010. Influence of juice pressing conditions on polyphenols, antioxidants, and varietal aroma of sauvignon blanc microferments. J. Agric. Food Chem. 58(12), 7280–7288.

Pineau, B., Barbe, J.C., Leeuwen, C. Van & Dubourdieu, D., 2009. Examples of perceptive interactions involved in specific “red-” and “black-berry” aromas in red wines. J. Agric. Food Chem. 57(9), 3702–3708.

du Plessis, K., Young, P.R., Eyéghé-Bickong, H.A. & Vivier, M.A., 2017. The transcriptional responses and metabolic consequences of acclimation to elevated light exposure in grapevine berries. Front. Plant Sci. 8(July).

Rapp, A. & Versini, G., 1995. Influence of nitrogen compounds in grapes on aroma compounds of wines. Dev. Food Sci. 37(C), 1659–1694.

Reynolds, A.G., Pool, R.M. & Mattick, L.R., 1986. Influence of cluster exposure on fruit composition and wine quality of Seyval blanc grapes. VITIS - J. Grapevine Res. 25(2), 85.

Ribéreau-Gayon, P., Boidron, J.N. & Terrier, A., 1975. Aroma of muscat grape varieties. J. Agric. Food Chem.

23(6), 1042–1047.

Roland, A., Schneider, R., Charrier, F., Cavelier, F., Rossignol, M. & Razungles, A., 2011. Distribution of varietal thiol precursors in the skin and the pulp of Melon B. and Sauvignon Blanc grapes. Food Chem. 125(1), 139–144.

Ryona, I., Pan, B.S., Intrigliolo, D.S., Lakso, A.N. & Sacks, G.L., 2008. Effects of cluster light exposure on 3-isobutyl-2-methoxypyrazine accumulation and degradation patterns in red wine grapes (Vitis vinifera L. Cv. Cabernet Franc). J. Agric. Food Chem. 56(22), 10838–10846.

Sadras, V.O., Moran, M.A. & Bonada, M., 2013. Effects of elevated temperature in grapevine. I Berry sensory traits. Aust. J. Grape Wine Res. 19(1), 95–106.

Seifert, R.M., Buttery, R.G., Guadagni, D.G., Black, D.R. & Harris, J.G., 1972. Synthesis and Odor Properties of Some Additional Compounds Related to 2-Isobutyl-3-methoxy pyrazine. J. Agric. Food Chem. 20(1), 135–137.

Selli, S., Canbas, A., Cabaroglu, T., Erten, H., Lepoutre, J. & Gunata, Z., 2006a. Effect of skin contact on the free and bound aroma compounds of the white wine of Vitis vinifera L. cv Narince,. Food Control 17(1), 75–82.

Selli, S., Canbas, A., Cabaroglu, T., Erten, H. & Günata, Z., 2006. Aroma components of cv. Muscat of Bornova wines and influence of skin contact treatment. Food Chem. 94(3), 319–326.

Selli, S., Canbas, A., Cabaroglu, T., Erten, H., Lepoutre, J.P. & Gunata, Z., 2006b. Effect of skin contact on the free and bound aroma compounds of the white wine of Vitis vinifera L. cv Narince. Food Control 17(1), 75–82.

Sivilotti, P., Herrera, J.C., Lisjak, K., Baša Česnik, H., Sabbatini, P., Peterlunger, E. & Castellarin, S.D., 2016. Impact of Leaf Removal, Applied before and after Flowering, on Anthocyanin, Tannin, and Methoxypyrazine Concentrations in Merlot' (Vitis vinifera L.) Grapes and Wines. J. Agric. Food Chem. 64(22), 4487–4496.

Song, J., Smart, R., Wang, H., Dambergs, B., Sparrow, A. & Qian, M.C., 2015. Effect of grape bunch sunlight exposure and UV radiation on phenolics and volatile composition of Vitis vinifera L. cv. Pinot noir wine. Food Chem. 173 424–431.

Šuklje, K., Antalick, G., Coetzee, Z., Schmidtke, L.M., Baša Česnik, H., Brandt, J., du Toit, W.J., 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. 20(2), 223–233.

Šuklje, K., Antalick, G., Buica, A., Langlois, J., Coetzee, Z.A., Gouot, J., Schmidtke, L.M. & Deloire, A., 2016. Clonal differences and impact of defoliation on Sauvignon blanc (Vitis vinifera L.) wines: A chemical and sensory investigation. J. Sci. Food Agric. 96(3), 915–926.

Swiegers, J.H., Willmott, R., Hill-Ling, A., Capone, D.L., Pardon, K.H., Elsey, G.M., Howell, K.S., de Barros Lopes, M.A., Sefton, M.A., Lilly, M. & Pretorius, I.S., 2006. Modulation of volatile thiol and ester aromas by modified wine yeast. Dev. Food Sci. 43(C), 113–116.

Swiegers, J.H., Kievit, R.L., Siebert, T., Lattey, K.A., Bramley, B.R., Francis, I.L., King, E.S. & Pretorius, I.S., 2009. The influence of yeast on the aroma of Sauvignon Blanc wine. Food Microbiol. 26(2), 204–211.

Tomic, O., Luciano, G., Nilsen, A., Hyldig, G., Lorensen, K. & Næs, T., 2009. Analysing sensory panel performance in a proficiency test using the PanelCheck software. Eur. Food Res. Technol. 230(3), 497–511.

Williams, D.L., 2019. Linking the phenolic potential of grape berries with polyphenol distribution during wine making : A Sauvignon Blanc case study. (MSc), Stellenbosch University, Stellenbosch, South Africa.

van Wyngaard, E., 2013. Volatiles playing an important role in South African Sauvignon blanc wines. (MSc), Stellenbosch University, Stellenbosch, South Africa.

Yang, Y.X., Li, X.L., Wang, L., Han, S.Y., Zhang, Y.R., Pratheeshkumar, P., Wang, X., Lu, J., Yin, Y.Q., Sun, L.J., Budhraja, A., Hitron, A.J. & Ding, S.Z., 2013. Anti-apoptotic proteins and catalase-dependent apoptosis resistance in nickel chloride-transformed human lung epithelial cells. Int. J. Oncol. 43(3), 936–946.

Young, P., Eyeghe-Bickong, H.A., du Plessis, K., Alexandersson, E., Jacobson, D.A., Coetzee, Z.A., Deloire, A. & Vivier, M.A., 2015. Grapevine plasticity in response to an altered microclimate: Sauvignon Blanc modulates specific metabolites in response to increased berry exposure. Plant Physiol. 170(March), 1235–1254.

Young, P., Eyeghe-Bickong, H.A., du Plessis, K., Alexandersson, E., Jacobson, D.A., Coetzee, Z.A., Deloire, A. & Vivier, M.A., 2016. Grapevine plasticity in response to an altered microclimate: Sauvignon Blanc modulates specific metabolites in response to increased berry exposure. Plant Physiol. 170(March), 1235–1254.

2.7. Supplementary tables/figures

Table S2.1: Concentration of aroma linked metabolites (mg/gFW) of ripe grape berries, in response to a high light (HL) microclimate compared to a low light (LL) microclimate. . The results are an average of n=3 samples. One-way ANOVA conducted per compound (row) between different samples (columns). Row-wise, different letters indicate statistical difference (Fisher's LSD, $p<0.05$).

Compounds	Low Light	High Light
Amino Acids		
Aspartic acid	379.34±100.48[a]	191.47±25.05[b]
Glutamic acid	41.86±11.21 [a]	9.01±0.3 [b]
Arginine	145.19±21.14 [a]	161.6±15.85 [a]
Alanine	650.54±111.65 [a]	394.43±61.42 [b]
Serine	9.28±3.83 [a]	12.04±0.65 [a]
Asparagine	<LOD	<LOD
Glutamine	326.31±87.34 [a]	274.18±35.01 [a]
Total Yeast Preferred Amino Acids	*1550.88±337.97 [a]*	*810.77±191.34 [b]*
Isoleucine	49.05±9.93 [a]	32.38±23.67 [a]
Leucine	40.88±0 [a]	248.29±152.27 [a]
Valine	10.63±1.12 [a]	26.85±1.54 [b]
Total Branched Chain Amino Acids	*48.14±4.45 [a]*	*210.35±92.95 [a]*
Phenylalanine	13.47±19.05 [a]	72.72±27.74 [a]
Tyrosine	254.78±9.41 [a]	265.31±51.83 [a]
Tryptophan	<LOD	<LOD
Total Aromatic Amino Acids	*268.25±9.64 [a]*	*289.5±39.24 [a]*
Cysteine	1338.02±310.64 [a]	723.69±46.43 [b]
Histidine	344.82±126 [a]	200.21±57.67 [a]
Glycine	<LOD	15.13±3.77 [b]
Threonine	24.39±6.38 [a]	31.39±7.39 [a]
GABAa	237.79±8.8 [a]	326.28±72.42 [a]
GABAb	<LOD	<LOD
Methionine	8.41±0 [a]	43.21±3.76 [a]
Lysine	101.73±16.54 [a]	132.88±95.15 [a]
Hydroxy proline	223.28±64.86 [a]	259.03±85.7 [a]
Proline	<LOD	111.04±18.29 [b]
Total amino acids	*4020.64±813.98 [a]*	*3041.82±559.64 [a]*
Methoxypyrazines		
SBMP	2.16±0.81 [a]	2.09±0.04 [a]
IBMP	2.57±0.87 [a]	5.3±0.45 [b]
Total methoxypyrazines	*5,67±0,13 [a]*	*8,1±1,19 [b]*
Monoterpenes		
Limonene	0.45±0.09 [a]	0.99±0.03 [b]
Cineol	<LOD	<LOD
α-Pinene	0.29±0 [a]	1.37±0.38 [a]
Hortrienol	<LOD	<LOD
Eucalyptol	<LOD	<LOD
2-Carene	0.15±0 [a]	0.84±0.05 [a]
p-Cymene	<LOD	<LOD
y-Terpinene	<LOD	<LOD
Linalool	0.61±0.41 [a]	6.94±2.21 [b]
Total Monoterpenes	*1.14±0.49 [a]*	*10.13±1.75 [b]*
C_6-Compounds		
Hexanal	575.95±115.02 [a]	599.01±174.58 [a]
2-Hexanal	7.55±0.56 [a]	8.19±0.83 [a]
Trans-2-Hexanal	579.64±107.55 [a]	497.79±98.24 [a]
1-Hexanol	2.56±0.9 [a]	1.55±0.1 [a]
3-Hexen-1-ol	1.22±0.09 [a]	3.88±1.87 [a]

(E)-2-Hexanal	<LOD	<LOD
2-Hexen-1-ol	5.14±3.46 [a]	14.93±1.11 [b]
(E)-2-Hexenal	<LOD	<LOD
Hexanoic acid	0.73±0.13 [a]	2.2±0.46 [b]
Hexyl formate	2.78±0.83 [a]	7.3±0.07 [b]
Total C_6 compounds	*1172.06±105.95* [a]	*1125.35±274.51* [a]
Norisoprenoids		
6-MHO	3.34±1.39 [a]	8.18±0.59 [b]
α-Ionone	<LOD	<LOD
Total Norisoprenoids	*3.34±1.39* [a]	*8.18±0.59* [b]
Other Volatiles		
3-Octanone	0.81±0.08 [a]	1.55±0.22 [b]
Octanal	2.89±0.34 [a]	2.38±0.23 [a]
1-Octen-3-one	2.61±0.91 [a]	1.45±0.09 [a]
(E)-2-Heptanal	2.03±0.5 [a]	3.21±0.27 [a]
2-Heptanol	1.21±0.14 [a]	0.21±0.02 [b]
N-Heptanal	7.6±0.8 [a]	7.58±0.78 [a]
Ethyl-Caproate	<LOD	<LOD
1-Hepten-3-one	<LOD	<LOD
Heptenal	<LOD	<LOD
2-Methyl-4-pentanol	<LOD	<LOD
2-Octen-1-ol	<LOD	<LOD
2-Methyl-4-pentanol	<LOD	<LOD
3-Octanoate	<LOD	<LOD

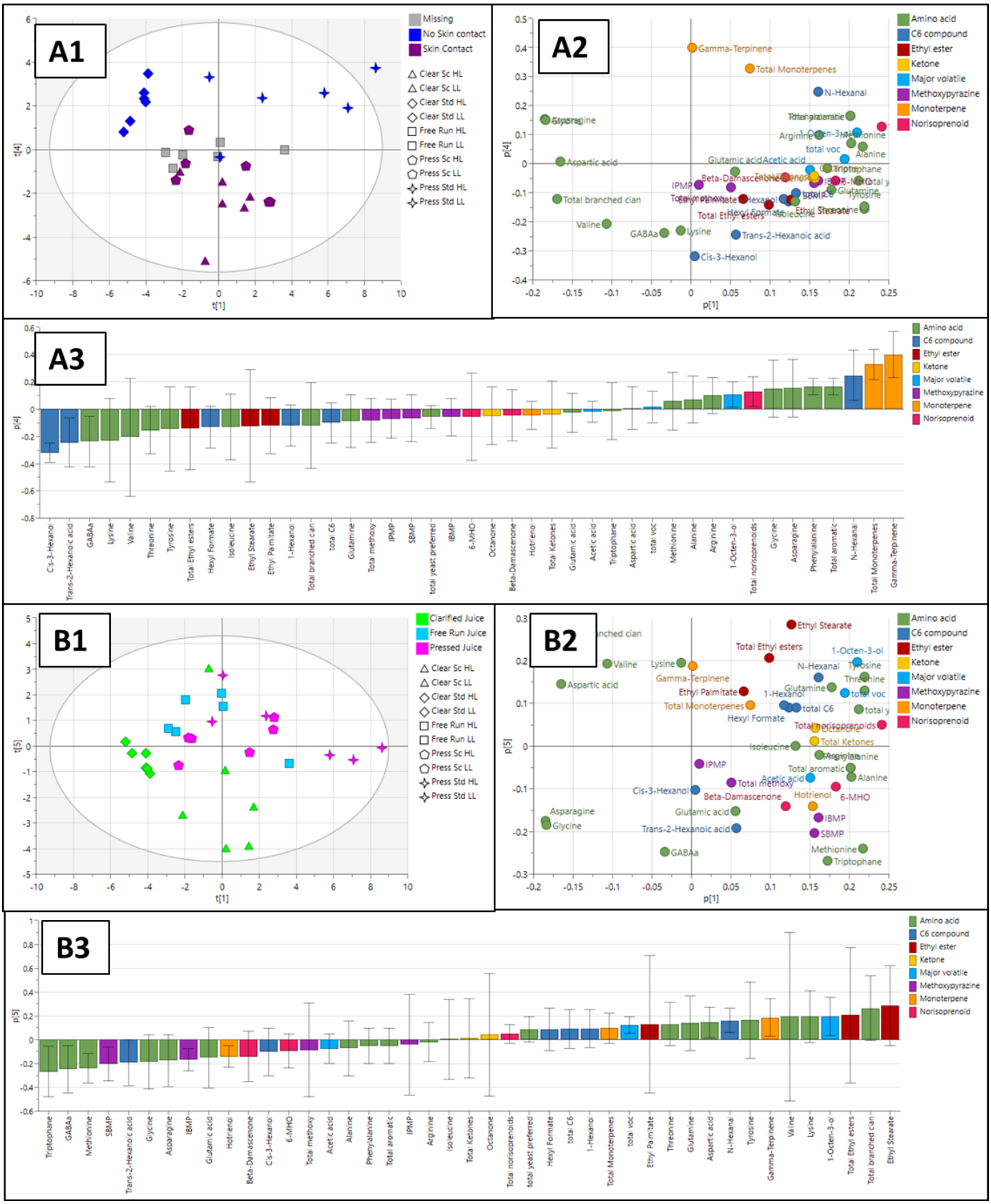


Figure S2.1: PCA score (1) , loadings (2) and coefficient (3) plots of aroma linked metabolites in juice from Low light (LL) and high light (HL) microclimates. Juice is presented in Standard wine making conditions (Std), or receiving skin contact (Sc) within the different processing stages: Free Run, Press and Clarified. Plots A are coloured according to skin contact and plots B according to juice processing step.

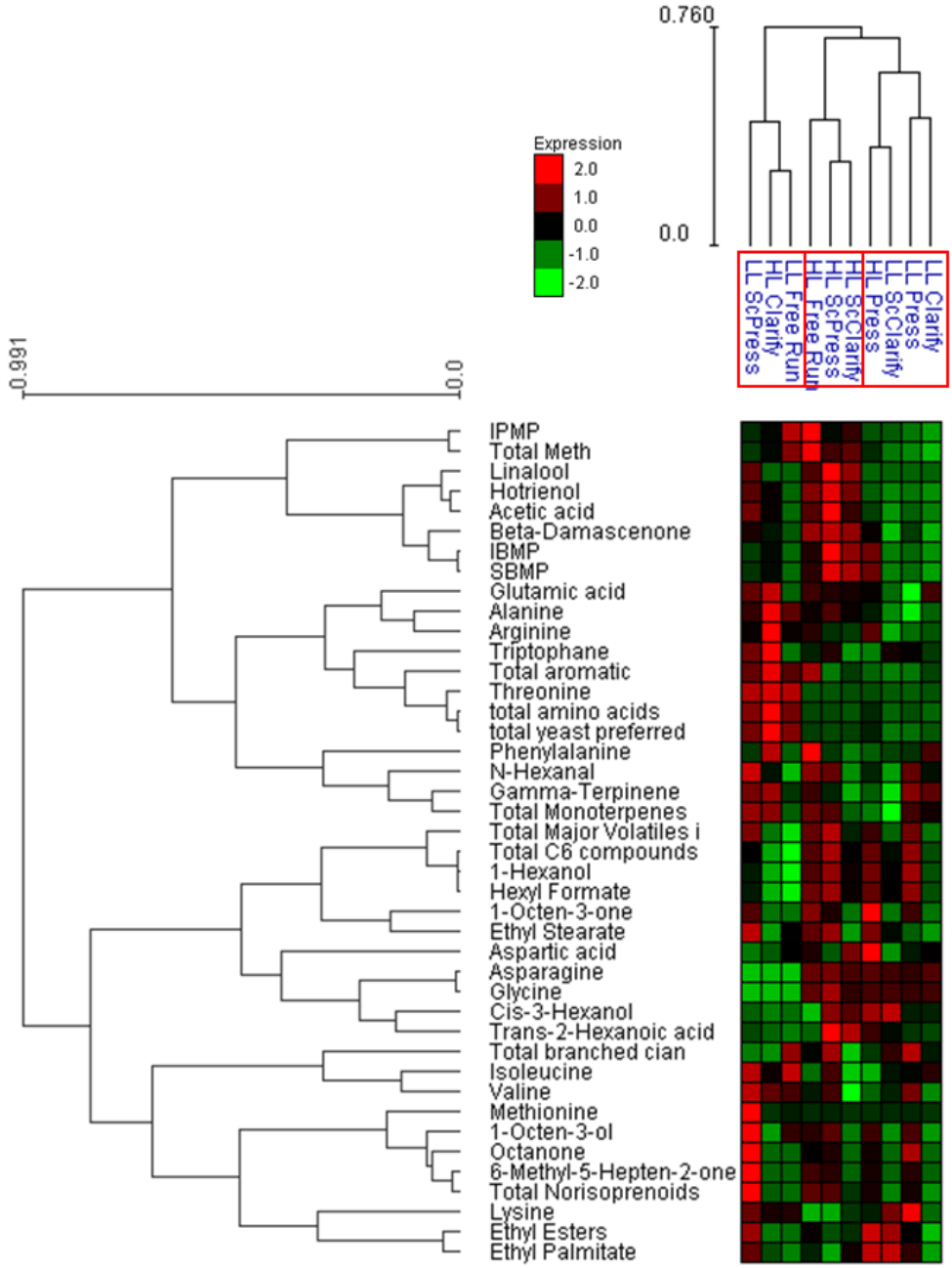


Figure S2.2: Hierarchical cluster analysis of selected aroma impact metabolites from juice during different processing stages. Selection based on significance of metabolite response.

Table S2.2: Concentration of aroma linked metabolites (mg/L) in juice from different processing stages: Free Run, Press and Clarified during standard wine making or with the addition of skin contact. Juice was obtained from grape berries harvested from either a high light (HL) or low light (LL) microclimate. The results are an average of n=3 samples. Factorial ANOVA conducted per compound (row) between different samples (columns). Row-wise, different letters indicate statistical difference (Fisher's LSD, $p<0.05$). Values in brackets () are sediment sample values for the corresponding clarified juice fraction

Compounds	Free run juice		Pressed juice		Pressed juice with skin contact		Clarified juice		Clarified juice with skin contact	
	LL	HL	LL	HL	LL	HL	LL	HL	LL	HL
Amino acids										
Aspartic acid	11.86±4.18^{bcd}	12.91±1.6^{bc}	10.98±0.04^{bcde}	22.22±1.5^{a}	7.7±1.9^{de}	8.83±0.43^{cde}	11.92±0.67^{bcde}	8.78±2.58^{cde}	6.86±0.33^{e}	15.92±7.42^{b}
Glutamic acid	69.64±7.29^{cd}	77.1±8.83^{abc}	60.43±0.05^{e}	74.49±3.64^{bcd}	78.86±6.47^{ab}	75.72±1.68^{bc}	77.3±2.01^{abc}	83.4±0.81^{a}	69.16±3.48^{d}	75.23±1.9^{bcd}
Asparagine	3.94±0.25^{e}	47.69±6.69^{a}	39.66±0.67^{d}	45.26±2.47^{b}	4.16±0.14^{e}	49.66±3.43^{a}	44.42±0.89^{bc}	5.06±0.2^{e}	41.93±0.97^{cd}	42.96±2.64^{bcd}
Glutamine	201.93±29.01^{b}	24.08±2.34^{cd}	24.71±0.89^{c}	<LOD	189.17±9.36^{b}	<LOD	17.83±0.72^{cd}	256.06±4.52^{a}	20.65±0.4^{c}	17.38±2.37^{cd}
Arginine	258.89±8.17^{cd}	268.63±56.3^{b}	219.58±5.91^{ef}	283.82±4.19^{bc}	257.85±14.21^{cd}	239.95±13.32^{de}	227.62±2.07^{ef}	356.86±19.6^{a}	199.18±2.13^{f}	235.73±35.72^{de}
Alanine	107.11±11.9^{ab}	98.84±9.72^{abc}	73.03±0.71^{e}	94.56±1.09^{bcd}	102.76±5.98^{bc}	105.3±13.32^{b}	87.33±5.9^{cde}	122.25±11.31^{a}	83.14±8.52^{de}	98.49±12.04^{bc}
Total Yeast Preferred AA	*649.44±58.02^{cd}*	*443.02±109.02^{b}*	*388.73±7.53^{ef}*	*475.08±6.96^{bc}*	*636.34±9.46^{cd}*	*429.79±28.09^{d}*	*413.09±6.05^{a}*	*827.36±16.66^{de}*	*378.99±10.62^{d}*	*438.4±54.3^{f}*
Valine	28.73±2.67^{b}	25.61±2.82^{bc}	30.3±0.08^{ab}	23.17±2.12^{de}	32.64±0.01^{a}	28.57±1.14^{b}	21.9±1.76^{e}	<LOD	25.33±3.74^{cd}	18.01±0.83^{f}
Isoleucine	8.58±0.54^{a}	5.02±0.85^{bc}	6.25±0.07^{abc}	4.21±0.11^{c}	8.47±1.59^{a}	7.1±0.15^{ab}	6.76±5.09^{ab}	<LOD	6.08±0.57^{abc}	3.94±0.07^{c}
Total Branched Chain AA	*35.17±6.38^{ab}*	*29.38±3.57^{bcd}*	*36.55±0.01^{abc}*	*27.38±2.22^{d}*	*24.79±20.43^{a}*	*35.67±0.99^{bc}*	*28.66±6.43^{d}*	*24.06±20.84^{bc}*	*31.41±4.31^{cd}*	*21.95±0.76^{e}*
Phenylalanine	1.68±0.34^{c}	5.95±4.25^{bc}	2.19±0.02^{bc}	1.71±0.16^{c}	2.13±0.19^{bc}	2.13±0.07^{bc}	3.58±3.03^{ab}	4.84±2.61^{a}	2±0.51^{c}	1.31±0.09^{c}
Tyrosine	5.97±0.49^{a}	4.88±0.3^{a}	<LOD	<LOD	5.85±1.32^{a}	<LOD	<LOD	6.69±1.1^{a}	<LOD	3.3±0.17^{e}
Total Aromatic AA	*7.65±0.83^{bc}*	*9.61±5.86^{cd}*	*2.19±0.02^{d}*	*2.71±1.91^{d}*	*7.98±1.51^{b}*	*2.13±0.07^{d}*	*3.58±3.03^{d}*	*11.53±1.51^{a}*	*2±0.51^{d}*	*3.78±1.59^{d}*
Methionine	2.8±0.32^{b}	2.22±0.32^{b}	2.37±0.04^{b}	2.04±0.02^{b}	20.33±20.12^{a}	2.55±0.18^{b}	1.81±0.04^{b}	47.06±4.32^{b}	2.19±0.03^{b}	1.92±0.15^{b}
Tryptophan	14.61±0.96^{d}	19.08±5.37^{bc}	21.2±0.89^{bc}	13.83±2.42^{d}	27.16±4.66^{b}	22.83±1.81^{bc}	17.8±1.99^{cd}	37.63±2.35^{a}	22.1±5.99^{c}	12.63±3.49^{d}
Lysine	29.23±31.93^{abcd}	1.66±1.88^{cd}	56.74±0.18^{a}	20.99±22.56^{abcd}	38.62±27.28^{abc}	2.4±2.02^{d}	11.25±19.55^{d}	28.03±17.83^{abcd}	40.52±25.06^{ab}	17.83±17.55^{bcd}
Threonine	42.98±3.33^{b}	3.21±1.09^{c}	1.64±0.04^{c}	4.04±0.11^{c}	42.71±0.68^{b}	2.88±0.84^{c}	3.08±0.22^{c}	48.41±5.38^{a}	1.78±0.37^{c}	3.61±0.89^{c}
Glycine	2.01±0.36^{e}	22.02±2.28^{b}	19.33±0.21^{cd}	20.42±0.67^{c}	1.76±0.47^{e}	27.04±2.02^{a}	19.99±0.33^{bc}	1.73±0.41^{e}	20.33±0.22^{cd}	18.82±1.04^{d}
Total Amino Acids	787.83±100.98^{b}	577.88±123.37^{c}	568.42±9.19^{de}	611.74±22.57^{cd}	803.85±22.01^{b}	574.95±32.04^{de}	539.23±17.75^{e}	1030.87±10.75^{a}	541.26±23.76^{de}	561.9±61.88^{de}
Methoxypyrazines										
IPMP	16.09±11.38^{ab}	19.66±6.62^{a}	2.95±0.33^{d}	4.57±1.5^{cd}	6.26±0.67^{cd}	7.79±0.99^{cd}	1.29±0.22^{d} (ND)	7.91±0.74^{cd} (ND)	4.2±1.6^{cd} (ND)	10.5±3^{bc} (ND)
SBMP	1.12±0.02^{def}	2.12±0.17^{c}	1.13±0.05^{de}	2.7±0.16^{b}	1.43±0.11^{d}	3.74±0.4^{a}	0.8±0.04^{f} (3.74±0.9^{ab})	1.82±0.16^{c} (4.46±0.74^{a})	0.95±0.1^{ef} (3.42±0.31^{b})	2.92±0.32^{b} (3.88±0.3^{ab})
IBMP	1.27±0.07^{f}	2.39±0.08^{c}	1.27±0.11^{f}	2.85±0.11^{d}	1.69±0.14^{e}	3.72±0.43^{a}	0.89±0.05^{g} (4.3±1.19^{a})	1.99±0.14^{e} (4.57±0.52^{a})	1.11±0.18^{fg} (2.79±0.75^{b})	3.33±0.35^{b} (3.15±0.71^{b})
Total Methoxypyrazines	*18.49±11.03^{ab}*	*24.17±6.25^{a}*	*5.36±1.03^{de}*	*10.12±1.91^{cde}*	*9.39±1.68^{cde}*	*14.75±2.44^{bc}*	*2.98±0.78^{e}* (8.04±1.96^{a})	*11.72±2.05^{bcd}* (9.02±1.08^{a})	*6.26±2.89^{de}* (5.18±2.69^{b})	*16.75±4.39^{abc}* (7.03±0.81^{ab})
Monoterpenes										
γ-Terpinene	38.85±2.45^{c}	48.37±1.39^{b}	55.08±2.62^{a}	53.67±6.16^{ab}	35.36±5.02^{c}	40.24±2.82^{c}	51.48±0.82^{ab} (0.6±0.2^{a})	57.09±2.64^{a} (<LOD)	24.17±0.68^{d} (0.61±1.21^{a})	28.32±4.96^{d} (1.78±1.39^{a})
Linalool	<LOD	0.95±0.11^{c}	<LOD	1.04±0.09^{c}	<LOD	1.94±0.06^{a}	<LOD (1.14±0.16^{b})	<LOD (2.51±3.17^{b})	<LOD (1.23±0.55^{b})	1.32±0.11^{b} (8.56±2.49^{a})
Hotrienol	3.55±0.21^{ef}	13.11±2.68^{b}	2.87±0.57^{efg}	11.38±1.02^{c}	3.87±0.32^{e}	17.26±0.88^{a}	1.82±0.34^{g} <LOD	8.1±1.44^{d} <LOD	2.12±0.17^{fg} <LOD	12.57±0.98^{bc} <LOD
Limonene	\	\	\	\	\	\	(1.38±1.36^{a})	(0.41±0.43^{ab})	(0.2±0.11^{b})	(0.27±0.38^{b})
Total Monoterpenes	*42.4±2.33^{e}*	*62.11±1.85^{abc}*	*57.95±2.7^{cd}*	*66.09±6.61^{a}*	*39.23±4.91^{e}*	*59.45±2.23^{bc}*	*53.3±0.97^{d}*	*65.19±3.97^{ab}*	*26.29±0.63^{f}*	*42.21±4.97^{e}*

							(3.42±1.24^{b})	(3.08±3.58^{b})	(2.2±1.43^{b})	(9.23±5.11 ^{a})
C_6 - Compounds										
N-Hexanal	13.25±0.73^{d}	29.3±0.43^{ab}	27.41±5.05^{b}	32.62±1.58^{a}	20.75±4.11^{c}	27.45±2.8^{b}	21.73±1.02^{c} (412.12±24.25 ^{a})	21.67±1.53^{c} (219.14±54.41 ^{a})	14.64±0.53^{d} (292.9±210.28 ^{a})	15.44±0.91^{d} (336.08±117.28 ^{a})
Cis-3-Hexanol	3.44±0.58^{cde}	1.9±0.43^{e}	4.99±1.91^{c}	3.7±0.8^{cd}	8.66±1.1^{ab}	8.21±0.29^{ab}	4.93±1.33^{c}	3.07±1.04^{de}	9.28±0.48^{a}	7.2±0.38^{b}
Hexyl Formate	10.67±1.64^{e}	45.48±3.19^{a}	48.85±8.14^{a}	32.33±3.02^{bc}	44.67±2.38^{a}	50.31±2.02^{a}	26.91±1.87^{c} (145.9±51.85 ^{a})	17.02±1.88^{d} (62.51±29.77^{b})	34.85±1.04^{b} (68.92±26.36^{b})	35.95±3.08^{b} (70.67±13.39^{b})
1-Hexanol	37.51±4.5^{f}	92.86±5.16^{b}	107.01±13.34^{a}	75.39±5.5^{cd}	99.21±4.3^{ab}	104.94±2.5^{a}	65.73±4.42^{d} (5.34±1.48 ^{a})	49.65±2.87^{e} (2.16±1.04^{b})	80±3.37^{c} (2.27±1.1^{b})	82.24±8.79^{c} (2.28±0.82^{b})
Trans-2-Hexanoic acid	1.71±0.13^{g}	2.1±0.36^{fg}	2.84±0.34^{e}	2.54±0.26^{ef}	4.55±0.76^{c}	8.03±0.56^{a}	2.55±0.14^{ef} (4.79±3.79 ^{a})	2.1±0.41^{fg} (0.63±0.27^{b})	3.53±0.16^{d} (1.58±0.57^{b})	6.43±0.58^{b} (2.53±0.35 ^{ab})
Trans-2-Hexanal	\	\	\	\	\	\	(149.08±42 ^{a})	(119.97±24.85 ^{a})	(102.04±45.64 ^{a})	(133.8±25.2 ^{a})
2-Hexen-1-ol	\	\	\	\	\	\	(117.87±12.79 ^{ab})	(62.03±24.16^{b})	(123.05±34.79 ^{a})	(128.18±24.11 ^{a})
Total C_6 compounds	*66.57±6.55^{g}*	*171.64±8.82^{c}*	*191.1±18.62^{ab}*	*146.58±8.85^{d}*	*177.83±6.04^{bc}*	*198.94±7.14^{a}*	*121.85±6.82^{e}* (841.77±54.94 ^{a})	*93.51±4.6^{f}* (470.39±129.45^{b})	*142.29±4.62^{d}* (601.68±240.03 ^{ab})	*147.26±12.76^{d}* (680.99±121.76 ^{ab})
Norisoprenoids										
β-Damascenone	1.52±0.12^{e}	2.67±0.28^{a}	1.66±0.26^{de}	2.07±0.09^{b}	1.97±0.04^{bd}	2.86±0.17^{a}	1.1±0.17^{c} <LOD	1.84±0.39^{bde} <LOD	1.03±0.08^{c} <LOD	2.65±0.34^{a} <LOD
6-MHO	<LOD	2.91±1.61^{b}	2.45±0.41^{bcd}	7.63±4.21^{a}	2.22±0.61^{bcde}	2.53±0.34^{bc}	<LOD (8.45±1.49 ^{a})	<LOD (7.79±2.42 ^{ab})	<LOD (4.97±1.91^{c})	<LOD (5.51±0.77^{bc})
Total Norisoprenoids	*1.52±0.12^{d}*	*5.58±1.88^{b}*	*4.11±0.64^{bc}*	*9.71±4.14^{a}*	*4.19±0.64^{bd}*	*5.4±0.28^{b}*	*1.1±0.17^{d} (8.45±1.49 ^{a})*	*1.84±0.39^{cd} (7.79±2.42 ^{ab})*	*1.03±0.08^{d} (4.97±1.91^{c})*	*2.65±0.34^{cd} (5.51±0.77^{bc})*
Esters										
Ethyl Stearate	4.97±1.1^{abc}	6.44±1.38^{ab}	3.55±0.98^{bc}	7.87±3.16^{a}	6.87±2.89^{ab}	7.29±1.98^{a}	2.65±0.65^{c}	2.62±0.73^{c}	4.87±3.01^{abc}	2.63±0.35^{c}
Ethyl Palmitate	6.76±3.89^{cd}	9.99±3.46^{bcd}	12.67±1.36^{abc}	14.4±2.35^{ab}	17.45±4.35^{a}	5.59±3.32^{d}	5.82±1.77^{d}	8.62±1.57^{bcd}	17.61±7.82^{a}	11.68±1.22^{abcd}
Total Ethyl Esters	*11.73±4.93^{c}*	*16.43±4.3^{abc}*	*16.22±2.14^{abc}*	*22.27±1.31^{ab}*	*24.32±6.74^{a}*	*12.88±5.26^{c}*	*8.47±2.37^{c}*	*11.24±2.3^{c}*	*22.48±10.82^{ab}*	*14.31±1.57^{bc}*
Other volatiles										
3-Octanone	<LOD	3.24±2.14^{bcd}	8.27±2.05^{ab}	11.6±6.62^{a}	3.6±1.02^{cd}	4.05±0.71^{bc}	<LOD (18.79±11.23 ^{a})	<LOD (10.58±5.15 ^{ab})	<LOD (8.25±5.89 ^{ab})	<LOD (5.94±1.14^{b})
1-Octen-3-one	<LOD	2.66±1.52^{ab}	1.9±0.24^{b}	4.09±2.38^{a}	2.13±0.86^{b}	<LOD	<LOD (5.72±1.43 ^{a})	<LOD (2.5±0.94^{b})	<LOD (2.68±0.96^{b})	<LOD (2.76±0.7^{b})
1-Octen-3-ol	10.62±2.15^{b}	10.15±3.63^{b}	11.59±1.67^{b}	19.24±8.34^{a}	10.95±2.49^{b}	12.31±1.63^{b}	1.79±0.29^{c}	1.59±0.25 ^{c}	2.17±0.19 ^{c}	3.09±0.21 ^{c}
Acetic acid	27.52±1.01^{ef}	42.84±6.69^{bc}	28.22±0.83^{ef}	45.37±7.77^{b}	30.94±2.72^{de}	58.81±4.19^{a}	25.4±3.47^{ef}	36.79±2.57^{cd}	23.63±1.12^{f}	40.49±3.37^{bc}
Octanal	\	\	\	\	\	\	(4.24±0.99 ^{a})	(2.31±0.41^{b})	(2.01±0.32^{b})	(1.84±0.33^{b})
(E)-2-Heptanal	\	\	\	\	\	\	(1.73±0.31 ^{ab})	(1.91±0.49 ^{a})	(1.17±0.61^{b})	(2.45±0.55 ^{a})
Isoamyl Alcohol	\	\	\	\	\	\	(0.23±0.16 ^{a})	(0.03±0.06^{b})	(0.2±0.29 ^{a})	(0.19±0.16 ^{ab})
2-Heptanol	\	\	\	\	\	\	(0.15±0.14 ^{b})	(0.06±0.08^{b})	(1.88±0.38 ^{a})	(2.24±0.48 ^{a})
N-Heptanal	\	\	\	\	\	\	(6.26±1.91 ^{a})	(3.28±0.94^{b})	(3.18±1.27^{b})	(3.3±0.51^{b})
Total alcohols	\	\	\	\	\	\	(6.65±1.96 ^{a})	(3.37±1.04^{b})	(5.26±1.91 ^{a})	(5.73±0.93 ^{a})
Total Major Volatiles in Juice	*160.37±13.16^{f}*	*313.76±21.94^{bc}*	*319.38±20.06^{b}*	*324.93±11.4^{b}*	*293.19±12.44^{c}*	*352.35±4.69^{a}*	*211.53±9.62^{e}*	*210.16±10.83^{e}*	*217.89±14.16^{e}*	*250±20.05^{d}*

Compounds measures in sediment	Light	Skin contact	Light*Skin contact
Monoterpenes			
Limonene		6.5	
y-Terpinene		3.3	
2-Carene	8.6		
Linalool	30.0	17.9	15.4
Total Monoterpenes	*3.7*	*3.6*	*7.2*
C6 aldehydes, esters and alcohols			
Hexanal	3.1		
2-Hexanal			
Trans-2-Hexanal			
Hexyl formate	8.8	4.1	7.6
1-Hexanol	6.5	6.4	7.4
3-Hexen-1-ol	8.2	14.7	
2-Hexen-1-ol		3.7	
Hexanoic acid	4.4		11.0
Total C6 compounds			*5.5*
Alcohols			
Isoamylalcohol	8.0	3.9	
2-Heptanol		330.6	
N-Heptanal	8.6	5.1	7.4
Total alcohols	*4.7*		*5.3*
Aldehydes and Ketones			
6-MHO		14.2	
Octanal	10.2	16.3	5.8
(E)-2-Heptanal	8.7		5.0
3-Octanone	4.6	5.1	
1-Octen-3-one	7.4	6.8	9.4
Methoxypyrazines			
IBMP		16.7	
SBMP	4.4		
Total Methoxypyrazines	*4.6*	*13.5*	

Table S2.3. The repeated measures ANOVA results for sediment. Showing the interaction between light exposure and skin contact and individual aroma linked metabolites which are reported as F-values. Values are coloured from highest (most significant). Green indicates lower F-values (F>5), yellow indicates intermediate F-values (F>10) while red indicates high F-values values (F>20). All insignificant values (F ≤ 5) are coloured in grey. F values correlate to p-values. Maximum = p<0.001; intermediate =p<0.01; minimum = p<0.05; insignificant = p>0.05

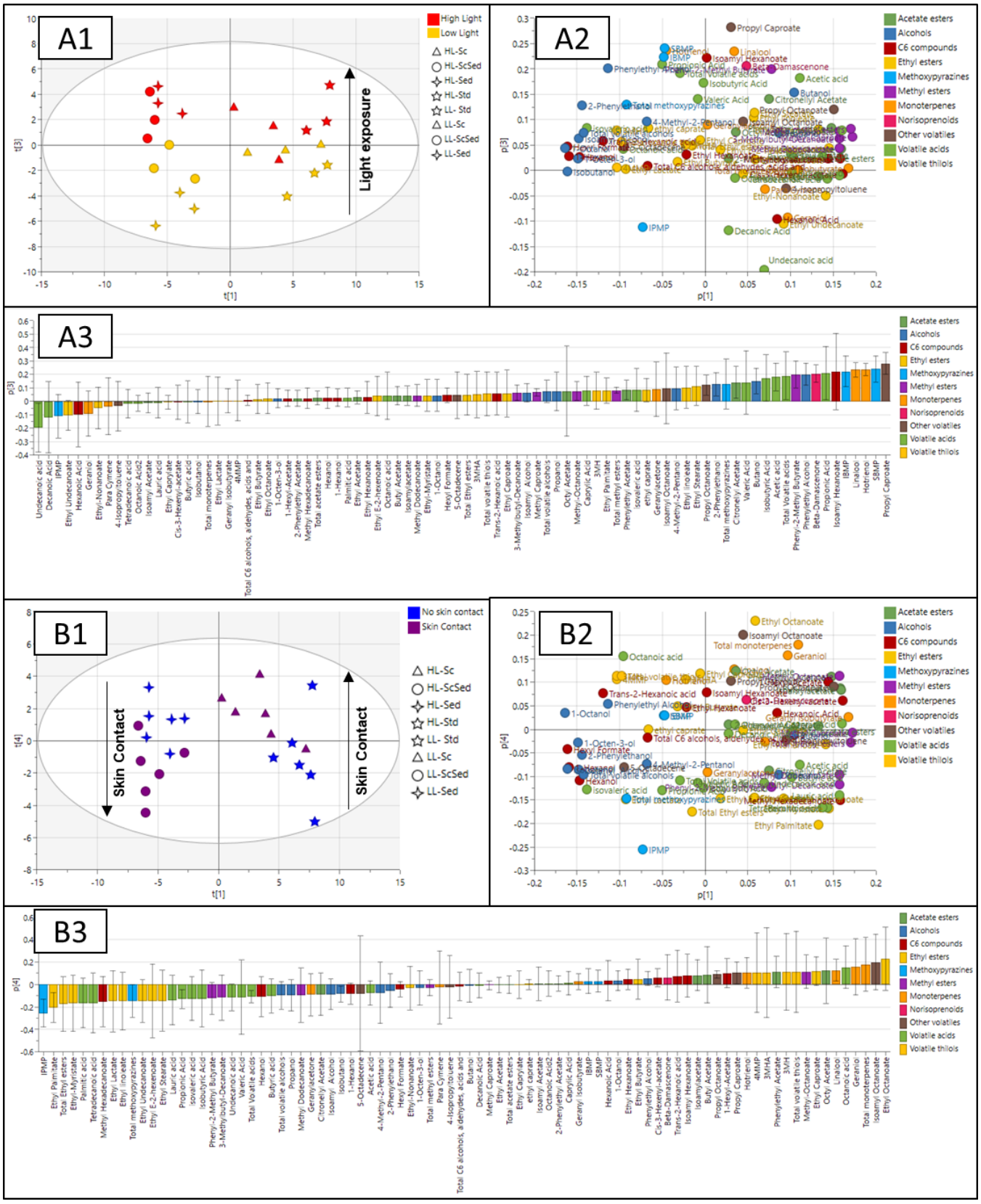


Figure S2.3: PCA score (A), loadings (B) plots of aroma linked metabolites in standard (Std) LL and HL wine and with different extraction treatments (skin contect – Sc, sediment contact – Sed and skin + sediment contact (ScSed). Different symbols indicate different wine samples. Score plot coloured according to A – light exposure and B – skin contact

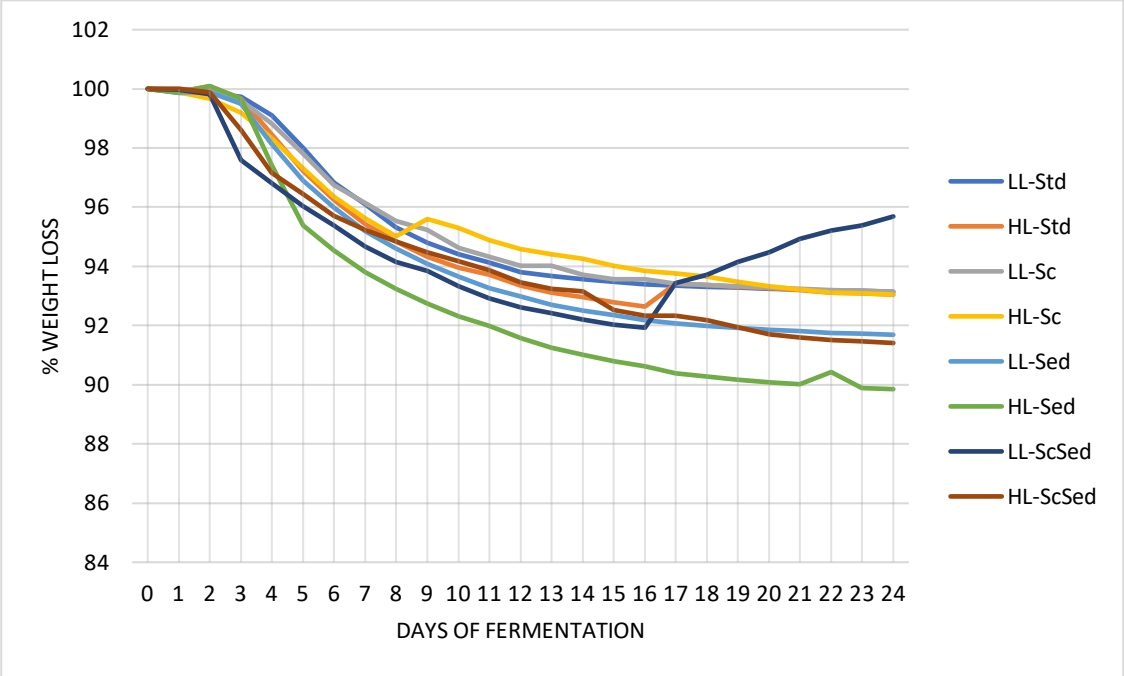


Figure S2.4: Line graph of the rate of fermentation as % weight loss over time (days after inoculation.

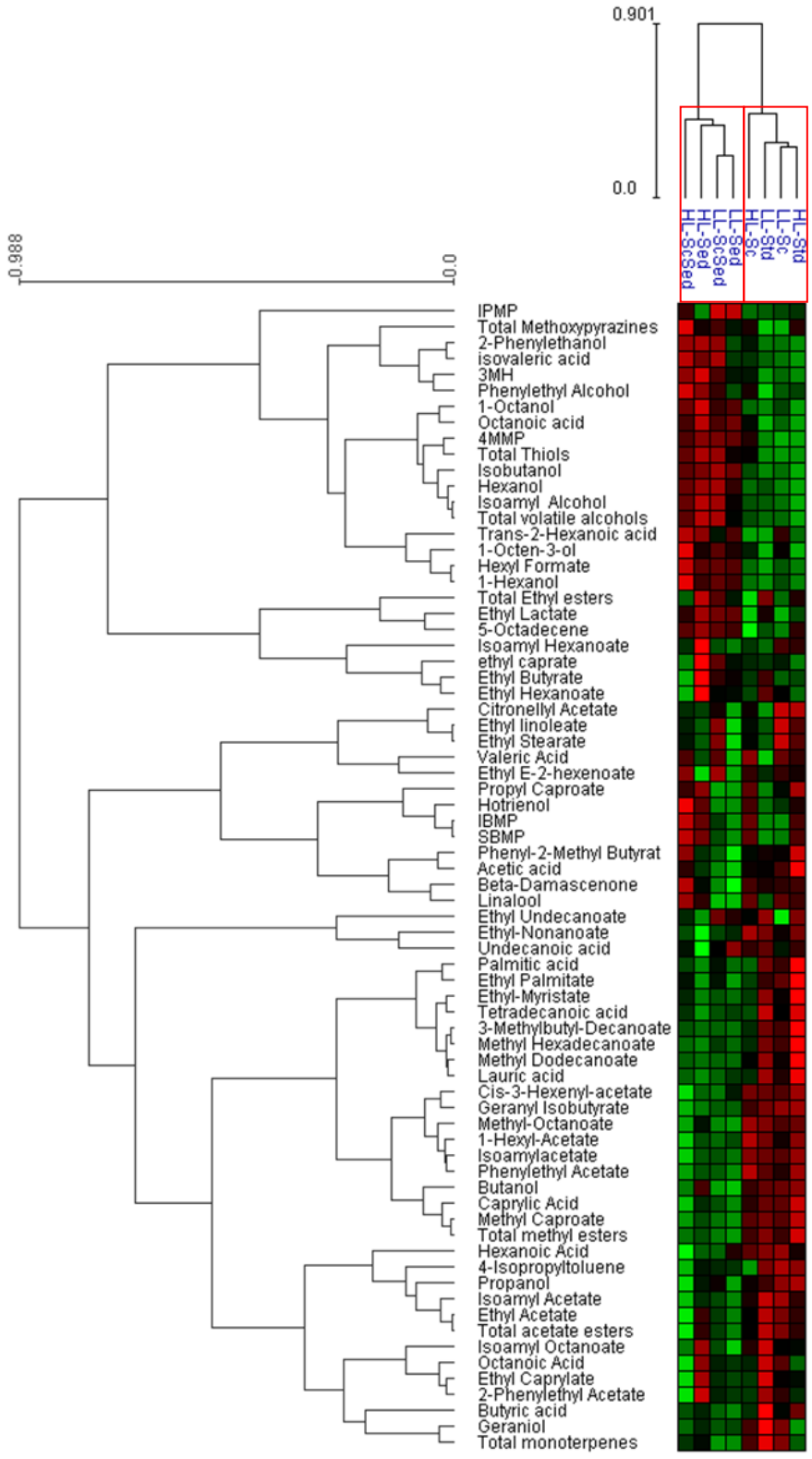


Figure S2.5: Hierarchical cluster analysis of selected aroma impact metabolites in standard wine and wine with various extraction treatments (Sc, Sed and ScSed) from two microclimates. Selection based on significance of metabolite response. Abbreviations: LL, low light; HL, high light; Std, standard; Sc, skin contact; Sed, sediment contact; ScSed, skin + sediment contact. Red blocks indicate first order groupings (first separation of compounds).

Table S2.4: Concentration of aroma linked metabolites in different fresh wine samples in response to skin contact, sediment contact or skin and sediment contact. n=3. Unit = ug.L unless otherwise specified. The results are an average of n=3 samples. Factorial ANOVA conducted per compound (row) between different samples (columns). Row-wise, different letters indicate statistical difference (Fisher's LSD, $p<0.05$).

Grape derived aroma compounds	Standard wine		Wine with skin contact		Wine with sediment contact		Wine with skin + sediment contact	
	LL-Std	**HL-Std**	**LL-Sc**	**HL-Sc**	**LL-Sed**	**HL-Sed**	**LL-ScSed**	**HL-ScSed**
Monoterpenes								
Para Cymene	13.12±4.47^{a}	12.63±1.37^{a}	12.93±2.45^{a}	7.07±2.73^{a}	10.01±4.41^{a}	11.21±6.91^{a}	10.11±2.45^{a}	8.04±0.58^{a}
Linalool	0.42±0.73^{b}	1.04±0.05^{a}	1.16±0^{a}	1.19±0.07^{a}	<LOD	0.97±0.05^{a}	<LOD	1.43±0.05^{a}
Hotrienol	8.51±4.95^{cd}	15.61±3.41^{b}	11.44±6.32^{bc}	17.5±6.43^{b}	6.8±1.78^{d}	18.76±1.68^{b}	7.14±1.34^{cd}	26.07±2.15^{a}
Geranyl Isobutyrate	6.9±1.51^{a}	7.69±2.56^{a}	7.39±2.1^{ab}	5.84±1.07^{a}	2.84±1.94^{bc}	2.32±0.19^{bc}	2.9±1.47^{bc}	1.29±0.59^{c}
Geraniol	55.55±5.34^{a}	<LOD	37.91±22.11^{bc}	30.35±6.23^{b}	11±5.12^{de}	15.73±3.23^{cd}	8.82±5.03^{de}	6.39±0.93^{ef}
Geranylacetone	2.05±0.05^{a}	2.19±0.74^{a}	1.13±0.37^{a}	1.38±0.31^{a}	1.45±0.45^{a}	2.25±1.29^{a}	1.71±0.55^{a}	2.03±0.46^{a}
Total monoterpenes	*86.55±15.83^{a}*	*39.16±2.86^{cd}*	*71.38±19.89^{bc}*	*63.34±3.55^{b}*	*32.11±11.85^{d}*	*51.24±8.29^{bc}*	*30.68±5.29^{d}*	*45.23±0.48^{cd}*
Norisoprenoids								
β-Damascenone	4.88±1.49^{ab}	5.1±0.77^{c}	4.98±0.01^{ab}	5.13±0.92^{bc}	3.2±0.61^{ab}	4.66±0.45^{ab}	3.87±0.33^{ab}	5.79±0.43^{a}
Total Norisoprenoids	*4.88±1.49^{ab}*	*5.1±0.77^{c}*	*4.98±0.01^{ab}*	*5.13±0.92^{bc}*	*3.2±0.61^{ab}*	*4.66±0.45^{ab}*	*3.87±0.33^{ab}*	*5.79±0.43^{a}*
Volatile thiols								
4MMP (ng/L)	303.76±31.4^{d}	142.46±117.69^{d}	111.8±11.67^{d}	1235.03±90.55^{c}	1580.83±161.19^{ab}	1778.68±311.75^{a}	1695.41±215.51^{ab}	1482.25±147.36^{b}
3MHA (ng/L)	107.35±28.58^{e}	170.84±48.72^{e}	235.31±19.87^{de}	249.58±37.84^{cd}	163.66±54.47^{cd}	228.41±75.82^{a}	196.69±25.82^{bc}	114.9±53.19^{ab}
3MH (ng/L)	158.88±64.77^{b}	210.67±42.58^{ab}	313.55±38.52^{a}	556.76±169.89^{a}	555.5±196.81^{ab}	1251.21±177.19^{a}	845.23±134.2^{ab}	1042.37±148.95^{b}
Total varietal thiols (ng/L)	*379.99±292.18^{b}*	*349.32±283.54^{b}*	*330.33±364.31^{b}*	*1224.82±1074.42^{a}*	*1277.78±1230.21^{a}*	*2172.2±1669.26^{a}*	*2053±1297.3^{a}*	*1759.68±1329.91^{a}*
Methoxypyrazines								
IPMP (ng/L)	4.85±3.27^{b}	5.94±3.01^{b}	5.43±1.48^{b}	4.39±2.05^{b}	12.32±3.6^{a}	3.73±3.46^{b}	12.83±3.17^{a}	8.74±3.63^{ab}
IBMP (ng/L)	4.07±0.67^{c}	8.45±0.48^{b}	3.93±1.64^{c}	9.11±2.41^{ab}	3.79±0.92^{c}	9.07±0.35^{ab}	5.74±0.98^{c}	11±1.62^{a}
SBMP (ng/L)	3.59±0.34^{c}	7.96±0.36^{b}	4.02±1.17^{c}	8.3±1.85^{b}	3.64±0.81^{c}	8.72±0.21^{ab}	4.94±1.03^{c}	10.17±1.1^{a}
Total Methoxypyrazinres (ng/L)	*12.51±3.84^{d}*	*22.34±2.61^{b}*	*13.38±1.33^{cd}*	*21.8±5.03^{b}*	*19.75±5.29^{bc}*	*21.52±3.32^{b}*	*23.5±5.05^{ab}*	*29.91±3.45^{a}*
C_6 Compounds								
Cis-3-Hexenyl-acetate	10.37±1.31^{ab}	11.51±0.98^{a}	9.89±3.38^{ab}	10.63±0.25^{a}	7.62±2.89^{bc}	6.01±0.73^{cd}	5.61±1.51^{cd}	3.49±0.76^{d}
1-Hexyl-Acetate	136.32±3.72^{a}	140.82±15.71^{a}	116.22±3.2^{ab}	149.59±32.58^{a}	96.61±20.71^{bc}	96.12±4.18^{bc}	90.24±15.51^{bc}	69.73±18.29^{c}
Isoamyl Hexanoate	6.55±0.8^{b}	8.01±0.67^{b}	8.23±0.9^{b}	7.03±0.95^{b}	6.35±1.78^{b}	10.28±0.87^{a}	6.8±0.99^{b}	7.11±0.77^{b}
Hexyl Formate	55.23±3.78^{c}	58.47±3.43^{c}	61.94±3.84^{c}	60.07±11.62^{c}	76.69±3.28^{b}	77.48±2.56^{ab}	78.09±5.07^{ab}	86.46±2.39^{a}
1-Hexanol	34.05±2.43^{c}	35.38±2.29^{c}	41.32±7^{c}	37.17±7.55^{c}	56.13±2.75^{b}	55.57±2.7^{b}	59.48±10.61^{b}	73.75±4.95^{a}
Trans-2-Hexanoic acid	4.06±1.02^{d}	4.84±1.63^{cd}	6.94±1.65^{abcd}	4.09±1.98^{d}	7.27±0.83^{abc}	8.15±2.62^{ab}	5.98±0.05^{bcd}	8.65±0.88^{a}
Methyl Hexadecanoate	3.49±0.33^{b}	4.5±0.52^{a}	3.08±1.16^{bc}	2.28±0.78^{cd}	1.81±0.33^{d}	1.59±0.03^{d}	2.17±0.18^{cd}	1.8±0.21^{d}

Ethyl Hexanoate	937.82±337.43 ab	639.71±159.8 bc	795.17±3.8 abc	694.92±51.57 bc	782.63±90.3 bc	1198.99±328.65 a	792.72±107.33 bc	525.39±248.55 c
Hexanoic Acid	375.21±119.16 a	297.63±71.03 ab	386.47±0.71 a	346.03±56.46 a	305.7±270.18 ab	213.89±67.71 ab	209.53±61.43 ab	105.71±68.81 b
Total C_6 compounds	*2212.81±582.66 ab*	*1715.01±308.58 b*	*2000.1±37.92 ab*	*1918.87±143.61 b*	*2194.25±315.83 ab*	*2688.58±501.33 a*	*2283.42±401.73 ab*	*1823.8±531.87 b*
Fermentation derived aroma compounds	**Standard wine**		**Wine with skin contact**		**Wine with sediment contact**		**Wine with skin + sediment contact**	
	LL-Std	**HL-Std**	**LL-Sc**	**HL-Sc**	**LL-Sed**	**HL-Sed**	**LL-ScSed**	**HL-ScSed**
Esters								
Phenylethyl Acetate	151.9±22.83 a	166.8±9.97 a	140.19±27.01 ab	172.15±34.02 a	101.89±18.08 bc	114.7±2.75 bc	112.4±19.09 bc	97.61±14.82 c
Isoamyl Acetate	81,8±2,76 ab	85,77±3,6 a	100,7±1,54 bcd	71,75±3,55 abc	55,94±4,31 de	57,3±1,36 cde	61,72±2,39 cd	26,93±0,79 e
Butyl Acetate	1.57±0.2 a	1.87±0.05 a	1.75±0.02 a	2.09±0.92 a	<LOD	<LOD	<LOD	<LOD
Octyl Acetate	11.25±0.66 ab	11.29±0.89 ab	10.74±1.99 ab	11.37±1.94 ab	10.65±3.63 ab	14.25±0.98 a	10.5±3.62 ab	7.23±3.12 b
Citronellyl Acetate	1.07±0.14 c	1.7±0.36 a	1.74±0.56 ab	1.38±0.19 abc	1.03±0.08 c	1.23±0.06 bc	1.4±0.27 abc	1.27±0.31 abc
Ethyl Acetate	63695.73±13298.6 a	53833.5±11019.18 abc	59180.74±1388.57 ab	50412.04±6652.23 abc	41075.9±3931.36 cd	54552.05±11213.25 abc	46033.86±5567.13 bcd	33368.53±9810.49 d
2-Phenylethyl Acetate	2769.97±811.71 a	2158.56±413.18 ab	2430.41±10.8 ab	2114.8±157.63 ab	2114.14±194.52 ab	2903.21±593.07 a	2187.59±298.76 ab	1607.29±493.09 b
Total acetate esters	*72326.94±15802.88 a*	*60188.84±12442.8 abc*	*67057.93±1433.44 ab*	*56478.22±8011.02 abc*	*45171.37±4378.16 cd*	*60474.31±12848.38 abc*	*50913.4±6527.83 bc d*	*36272.61±11104.04 d*
Ethyl Butyrate	317.04±106.36 ab	240.97±49.25 bc	228.25±69.1 bc	250.1±26.8 bc	286.88±34.35 bc	429.62±137.54 a	307.36±29.24 abc	180.92±84.26 c
Ethyl Caprylate	1219.41±476.15 a	846.76±172.45 abc	873.99±1.6 abc	770.07±139.32 bc	752.11±27.28 bc	1134.68±308.23 ab	792.37±104.69 bc	512.32±158.8 c
Ethyl Lactate	7752.18±299.31 abc	7395.12±568.87 abc	7021.47±253.43 bc	6928.82±157.17 c	8029.8±501.58 ab	8264.21±610.97 a	8089.86±374.04 a	7834.16±886.82 ab
ethyl caprate	422.16±124.33 b	374.25±109.94 b	440.91±37.86 b	431.93±32.23 b	448.93±93.55 b	653.63±138.72 a	523.22±102.85 ab	368.23±120.12 b
Ethyl linoleate	3.57±0.41 de	4.63±0.35 abc	5.39±0.07 a	4.04±0.36 bcd	2.79±0.35 e	3.53±0.94 de	4.96±0.35 ab	3.92±0.92 cd
Ethyl Stearate	70.58±9.12 cd	100.78±11.92 ab	120.22±3.77 a	85.28±4.18 bc	49.89±10.98 d	71.1±25.89 cd	107.76±9.51 ab	82.71±23.96 bc
Ethyl Palmitate	1046.09±168.55 b	1465.22±110.98 a	1132.63±515.57 ab	798.99±137.96 bc	604.71±126.19 c	610.21±110.96 c	892.1±49.11 bc	896.62±151.91 bc
Ethyl Caproate	97.25±41.36 a	100.82±40.72 a	92.24±6.63 a	102.3±66.28 a	122.43±35.27 a	121.45±19.08 a	79.25±54.11 a	94.98±53.37 a
Ethyl E-2-hexenoate	2.4±0.21 bcd	2.65±0.34 abc	2.76±0.84 abc	2.77±0.42 ab	1.89±0.4 cd	1.72±0.03 d	3.19±0.73 a	3.09±0.39 ab
Ethyl-Nonanoate	14.77±0.71 ab	14.78±3.27 ab	11.13±1.59 bc	16.37±2.82 a	9.4±1.02 c	2.55±1.65 d	8.43±0.99 c	8.12±1.37 c
Ethyl Octanoate	19.82±33 a	3.73±0.38 a	2.22±3.13 a	25.48±23.3 a	2.43±0.44 a	3.12±0.28 a	2.71±0.33 a	1.92±0.24 a
Ethyl Undecanoate	2.68±0.39 a	2.58±0.61 a	0.98±1.39 c	2.01±0.31 abc	2.19±0.47 ab	1.35±0.04 bc	2.51±0.55 a	1.85±0.2 abc
Ethyl-Myristate	12.89±2.03 b	17.3±3.01 a	7.82±6.32 c	5.33±2.28 cd	3.46±0.95 cd	2.58±0.49 d	4.31±0.66 cd	6.14±2.32 cd
Total Ethyl esters	*10980.83±756.74 a*	*10569.58±993.76 ab*	*9940.02±315.52 ab*	*9423.49±29.44 b*	*10316.91±636.96 ab*	*11299.77±897.09 a*	*10818.02±441.41 a*	*9994.99±1174.96 ab*
Methyl Dodecanoate	28.23±11.79 ab	37.84±7.65 a	15.93±9.63 bc	14.35±9.01 c	4.9±1.14 c	4.31±0.39 c	5.38±1.24 c	5.96±1.43 c
3-Methylbutyl-Decanoate	9.59±2.35 b	13.65±1.42 a	8.33±6 bc	5.28±0.73 cd	3.35±0.98 d	3.49±0.05 d	3.75±0.61 d	4.16±0.96 d
Methyl Caproate	43.61±1.14 ab	50.75±7.11 a	40.28±14.27 b	40.55±2.89 b	16.79±2.72 c	24±2.27 c	19.9±1.9 c	16.26±2.56 c
Phenyl-2-Methyl Butyrate	16.67±3.2 bc	21.93±1.52 a	16.35±3.97 bc	15.89±1.24 bc	9.62±2.58 d	14.85±0.65 c	13.46±0.66 cd	20.13±4.91 ab

Methyl-Octanoate	36.3±1.36 ^{ab}	34.68±2.03 ^{b}	32.33±5.33 ^{b}	38.5±1.05 ^{a}	14.52±2.19 ^{d}	25.24±1.87 ^{c}	16.01±1 ^{d}	13.94±2.18 ^{d}
Total methyl esters	*134.41±19.51 ^{ab}*	*158.85±15.93 ^{a}*	*113.22±39.2 ^{b}*	*114.57±12.77 ^{b}*	*49.19±9.22 ^{c}*	*71.88±4.45 ^{c}*	*58.5±4.32 ^{c}*	*60.46±7.37 ^{c}*
Volatile acids								
Undecanoic acid	4.05±0.24 ^{ab}	3.62±0.47 ^{bc}	2.85±0.23 ^{c}	3.59±0.75 ^{bc}	4.47±0.44 ^{a}	<LOD	3.09±0.52 ^{c}	2.91±0.15 ^{c}
Acetic acid	941.68±130.79 ^{b}	1291.03±105.63 ^{a}	1031.74±213.13 ^{b}	953.66±106.99 ^{b}	647.06±30.26 ^{d}	857.58±33.36 ^{bc}	737.14±66.33 ^{cd}	999.47±119.6 ^{b}
Octanoic acid	1330.51±970.88 ^{b}	1403.92±792.58 ^{b}	1661.94±408.48 ^{ab}	2041.72±1725.69 ^{ab}	2534.13±348.94 ^{ab}	2846.29±11.15 ^{a}	2394.64±291.18 ^{ab}	2364.71±205.15 ^{ab}
Caprylic Acid	1487.31±166.53 ^{ab}	1642.34±76.26 ^{a}	1471.79±254.79 ^{abc}	1389.17±100.3 ^{bcd}	1107.39±195.54 ^{ef}	1255.01±7.95 ^{cde}	1198.21±10.9 ^{def}	1018.76±97.57 ^{f}
Lauric acid	325.73±41.32 ^{a}	399.4±54.25 ^{a}	213.9±127.5 ^{b}	146.19±64.28 ^{bc}	136.55±17.53 ^{bc}	60.48±12.83 ^{c}	96.26±11.23 ^{c}	97.41±38 ^{c}
Tetradecanoic acid	74.24±7.89 ^{a}	77.77±9.21 ^{a}	46.53±25.24 ^{b}	35.3±10.7 ^{bc}	36.95±5.36 ^{bc}	24.42±4.78 ^{c}	34.79±4.18 ^{bc}	39.43±9.38 ^{bc}
Palmitic acid	41.76±6.82 ^{ab}	50.68±0.9 ^{a}	37.9±19.13 ^{abc}	27.93±3.48 ^{c}	27.43±7.16 ^{c}	25.36±2.95 ^{c}	33.24±1.7 ^{bc}	29.92±4.3 ^{c}
Propionic Acid	20072.11±4702.95 ^{a}	24944.77±6479.01 ^{a}	19217.46±63.13 ^{a}	22843.22±1336.2 ^{a}	20545.01±4073.9 ^{a}	26881.67±4222.64 ^{a}	22449±1962.66 ^{a}	25107.28±4155.34 ^{a}
Isobutyric Acid	808.95±156.23 ^{a}	1516.28±1444.7 ^{a}	924.46±38.13 ^{a}	1419.61±893.52 ^{a}	840.78±50.94 ^{a}	1317.89±662.95 ^{a}	1209.64±252.89 ^{a}	1046.5±151.85 ^{a}
Butyric acid	1683.74±576.11 ^{a}	1323.74±212.2 ^{ab}	1086.53±35.91 ^{bc}	997.89±197.8 ^{bc}	822.94±76.66 ^{c}	1024.99±224.08 ^{bc}	891.21±94.68 ^{bc}	928.3±153.49 ^{bc}
isovaleric acid	801.07±130.16 ^{c}	717.45±82.95 ^{c}	774.3±12.63 ^{bc}	871.92±195.59 ^{c}	867.44±117.18 ^{bc}	1120.53±14.9 ^{ab}	1203.46±321.5 ^{a}	1228.59±180.17 ^{a}
Valeric Acid	158.48±21.65 ^{a}	218.53±66.71 ^{a}	230.33±56.89 ^{a}	239.2±20.2 ^{a}	161.26±55.45 ^{a}	187.37±45.35 ^{a}	227.23±75.76 ^{a}	224.42±50.92 ^{a}
Octanoic Acid	5115.14±1741.8 ^{a}	3289.52±1142.46 ^{bc}	4429.56±53.12 ^{abc}	3680.12±288.85 ^{abc}	3570.32±340.95 ^{abc}	4749.04±1037.2 ^{ab}	3700.11±633.38 ^{abc}	2551.19±1016.17 ^{c}
Decanoic Acid	1809.4±500.13 ^{a}	1385.71±217.9 ^{a}	1669.63±48.47 ^{a}	1415.78±105.79 ^{a}	1759.32±1052.95 ^{a}	1430.59±198.36 ^{a}	1299.48±146.87 ^{a}	1090.11±324.93 ^{a}
Total Volatile acids	*34654.15±6080.29 ^{a}*	*38264.74±9123.38 ^{a}*	*32798.94±1166.27 ^{a}*	*36065.28±3566.52 ^{a}*	*33061.05±4974.3 ^{a}*	*41781.21±5742.8 ^{a}*	*35477.51±3015.8 ^{a}*	*36729±6189.25 ^{a}*
Volatile alcohols								
Phenylethyl Alcohol	86.22±11.18 ^{d}	99.62±4.43 ^{cd}	97.46±2.88 ^{cd}	108.23±16.95 ^{bc}	98.57±7.36 ^{cd}	119.36±4.88 ^{ab}	111.25±6.9 ^{abc}	126.1±8.35 ^{a}
Propanol	33990.49±5435.05 ^{abc}	37304.73±8258.88 ^{a}	36441.85±5672.81 ^{ab}	32497.71±2653.2 ^{abc}	27153.28±3615.78 ^{bc}	31418.79±5549.63 ^{abc}	32517.05±5997.62 ^{abc}	25404.5±5330.16 ^{c}
Isobutanol	16109.36±3097.97 ^{b}	14277.28±2734.27 ^{b}	17374.34±661.65 ^{b}	19124±3386.37 ^{b}	28363.66±6053.14 ^{a}	29122.41±3195.86 ^{a}	31095.28±6385.78 ^{a}	27692.33±5205.25 ^{a}
Butanol	1353.24±223.76 ^{a}	1405.3±253.86 ^{a}	1362.02±68.74 ^{ab}	1295.76±151.8 ^{ab}	967.17±96.55 ^{c}	1315.49±102.67 ^{ab}	1007.82±100.22 ^{c}	1056.42±150.82 ^{bc}
2-Phenylethanol	8096.36±2104.47 ^{b}	7230.09±1404.73 ^{b}	8424.66±288.65 ^{b}	12101.95±4890.65 ^{b}	9949.15±2373.04 ^{b}	19588.17±1368.86 ^{a}	18573.52±5510.69 ^{a}	18982.79±2904.31 ^{a}
4-Methyl-2-Pentanol	5905.69±1428.66 ^{a}	5902.68±1796.25 ^{a}	7314.74±1140.07 ^{a}	7019.33±745.66 ^{a}	6830.91±1770.25 ^{a}	7905.13±1171.33 ^{a}	7056.22±1336.13 ^{a}	7013.74±2159.58 ^{a}
Isoamyl Alcohol	98712.18±25721.98 ^{bc}	78175.01±16407.71 ^{c}	94034.02±4534.78 ^{bc}	101299.81±21545.26 ^{bc}	124216.09±19555.27 ^{ab}	168397.08±19085.55 ^{a}	162612.24±42372.76 ^{a}	147722.23±34114.59 ^{a}
Total volatile alcohols	*164255.35±37623.13 ^{bc}*	*144396.11±30812.35 ^{c}*	*165052.83±12371.54 ^{bc}*	*173449.75±28131.89 ^{bc}*	*197584.94±29712.71 ^{abc}*	*257874.36±29598.99 ^{a}*	*252979.36±56321.27 ^{a}*	*228005.56±48399.25 ^{ab}*

Table S2.5: Comparison of concentration ranges of aroma impact metabolites found in this study in all samples (LL and HL combined) compared to the ranges found in literature.

Aroma compound	Matrix	Concentration range	Concentration range in literature	How does it compare to literature	Reference
Methoxypyrazines	Wine	3.59 - 12.89 ng/L	3.57 -38.2 ng/L	It falls within the lower part of the range previously determined in a study based on the same block, as well as a study based in New Zealand	(Lacey *et al.*, 1991; Marais *et al.*, 1999; Šuklje *et al.*, 2014; Martin *et al.*, 2016; Šuklje *et al.*, 2016)
Volatile thiols	Wine	107.35 - 1251.21 ng/L	97.9.0 - 969.7 ng/L	The concentrations found in the wine samples of this study exceeds the range what has previously been found in literature	(Šuklje *et al.*, 2014)

Table S2.6: Odour activity values (OAV) of volatiles responding to different treatments in wine samples. OAV calculated by dividing concentration by known aroma threshold. Threshold units are ug/L unless otherwise specified. Only OAV>1 will be taken as having aromatic importance and discussed. OAV<1 is not significant (grey)

Compound	Threshold* (µg/L)	Standard wine		Wine with skin contact		Wine with sediment. contact		Wine with Skin & Sed. contact	
		LL Std	HL Std	LL Sc	HL Sc	LL Sed	HL Sed	LL ScSed	HL ScSed
Ethyl Acetate	12264[1]	5.2	4.4	4.8	4.1	3.4	4.5	2.7	3.8
2-Phenylethyl Acetate	250[2]	11.1	8.6	9.7	8.5	8.5	11.6	6.4	8.8
Isoamyl Acetate	301[1]	186.9	130.6	173.6	122.4	60.6	94.5	38.6	83.8
Ethyl Hexanoate	14[4]	67.0	45.7	56.8	49.6	55.9	85.6	37.5	56.6
Ethyl-Myristate	1000[5]	11.0	10.6	9.9	9.4	10.3	11.3	10.0	10.8
Ethyl Butyrate	20[4]	61.0	42.3	43.7	38.5	37.6	56.7	25.6	39.6
Octanoic acid	500[4]	3.0	3.3	2.9	2.8	2.2	2.5	2.0	2.4
Isovaleric acid	33.4[1]	4.7	6.5	6.9	7.2	4.8	5.6	6.7	6.8
Propanol	5000[3]	3.2	2.9	3.5	3.8	5.7	5.8	5.5	6.2
Butanol	15000[3]	0.5	0.5	0.6	0.8	0.7	1.3	1.3	1.2
Isoamyl Alcohol	3000[1]	54.8	48.1	55.0	57.8	65.9	86.0	76.0	84.3
B-Damascenone	0.05[1]	32.2	47.4	38.4	43.8	30.6	46.0	41.8	30.3
3MHA	4[6]	26.8	42.7	62.4	58.8	40.9	57.1	28.7	49.2
3MH	60[6]	2.7	3.5	9.3	5.2	9.3	20.9	17.4	14.1
IPMP (ng/L)	2[7]	2.4	3.0	2.7	2.2	6.2	1.9	4.4	6.4
IBMP (ng/L)	1[7]	4.1	8.5	3.9	9.1	3.8	9.1	11.0	5.7
SBMP (ng/L)	1[7]	3.6	8.0	4.0	8.3	3.6	8.7	10.2	4.9

*Threshold references: 1. Ferreira et al. (2002), 2. Gewu (1997), 3. Li et al. (2008), 4. Culleré, et al. (2004), 5. Moreno et al. (2005), 6. Coetzee et al. (2012), 7. Seifert et al. (1972).

OAV values are indicated with a colour scale: **Green** to **Red** (1 – 50) and saturate after 50.

Chapter 3
General discussion and conclusions

Sauvignon blanc is a highly aromatic wine, with well-studied aroma profiles (ranging from “green” to “fruity”) and known aroma impact metabolites (methoxypyrazines and volatile thiols, for example) which impart the different aroma profiles (Coetzee & du Toit, 2012; van Wyngaard, 2013; Šuklje *et al.*, 2014; Martin *et al.*, 2016). Winemaking is an extensive process that starts with the cultivation of the grape berry and concludes with the bottling of the wine. Most studies focus on specific components of the winemaking pathway, for example, either on the viticultural aspects or oenological aspect and the effects of these on berries or wine, respectively, but rarely in an integrated fashion (Lacey & Allen, 1991; Naor *et al.*, 2002; Dubourdieu *et al.*, 2006; Swiegers *et al.*, 2009; Olejar *et al.*, 2015; Costa *et al.*, 2016; Martin *et al.*, 2016; Gregan *et al.*, 2017). Berry studies focused for example on the effects of light exposure (Gregan *et al.*, 2017), UV radiation (Gregan *et al.*, 2012; Šuklje *et al.*, 2014), on berry composition; whereas studies on wine focused on either vineyard factors (without subsequent analysis of the berry composition) or wine making factors, such as parameters for the wine processing steps or choices of yeast and the composition of wine (Dubourdieu *et al.*, 2006; Maggu, Winz, *et al.*, 2007; Swiegers *et al.*, 2009; Patel *et al.*, 2010; Green *et al.*, 2011; Olejar *et al.*, 2015; Martin *et al.*, 2016). However, once each individual component is understood in its entirety, the integrated effect of all changes should also be studied throughout the winemaking process as everything is connected.

Several studies have been completed on Sauvignon blanc in our lab where a Field-Omics approach (Alexandersson *et al.*, 2014) was implemented in a model vineyard in the Elgin area of South Africa. This approach, where a site is subjected to extensive characterisation to identify and quantify sources of variability before further experimentation is conducted, proved very successful and lead to a number of datasets that confirmed the following: Sauvignon blanc grapes display significant phenotypic plasticity and berries acclimate to their light environments by adapting their metabolism. Berries will acclimate differently to shade (low light) than to sun exposure (high light) (Young *et al*., 2016). The carotenoid pathway was found to be centrally linked to these adaptations, and responses in green berries were found to strongly determine the aromatic profile of the ripe berries (Young *et al*. 2016; Du Plessis *et al*., 2017). Moreover, several classes of volatile aroma compounds and precursors, as well as polyphenolics accumulated in the sun exposed berries, due to their biological roles as stress protective molecules and antioxidants (Young *et al*. 2016; Du Plessis *et al*., 2017). Interestingly, it was also shown that many of these effects were not only linked to the quantity of the light, but actually occurred in response to the UVB component in the light, confirming that light quality and quantity can strongly influence the metabolic state and volatile profiles of Sauvignon blanc grape berries (Joubert *et al*., 2016). In addition to the vineyard and berry studies, wines were made from

the grapes from the low and high light microclimates, using standardised white wine making techniques, confirming that the two different wine styles were achieved from the different microclimates.

These prior studies were the building blocks leading to the current study where the aim was to study the aromatic potential of the SB berries in the HL and LL microclimates, as well as profile and measure grape- and fermentation-derived aroma compounds in different wine matrices under standard or modified winemaking procedures, chosen to enhance extraction of volatiles.

Some technical problems were experienced in the study with some of the analyses. As outlined below:

- All the metabolite profiles of the berries were generated with whole, ground berries. The aroma linked metabolites were not quantified in the different sub-tissues of the grape berry (skin, seeds and pulp), and we relied on well-known distribution patterns that have shown which compounds accumulate in which sub-tissues from literature (Jackson, 2014). The addition of particularly skin-specific analysis would have been beneficial to the determination of aromatic potentials of the grape berries and is recommended for future studies.

- The pomace-fraction was also not analysed for aroma-linked metabolites before or after skin contact and pressing, limiting the link(s) that can potentially be made between aromatic potential loss with the removal of grape solids, and/or the of extraction of aromatic compounds from grape tissues. The pomace matrix should be explored for volatiles in further studies.

- The juice sediment matrix (collected after clarification) produced highly variable data. It was clear that there were significant matrix interference with some of the analysis methods applied to this sediment that would require some method optimisation, particularly for the amino acids of which the data was excluded in our analysis due to unacceptable high variation in the sample extracts and repeats.

- The juice yield of berries and the proportion of juice that settled out as sediment was not calculated, limiting the direct comparisons that could be made between the aromatic potentials of (clarified) juice and sediments. This will provide valuable insights into the “fate” of grape derived aromatic potentials during juice processing in future studies.

- There is a vast number of chemical compounds in wine which could contribute to the different effects observed in the samples and only a portion of these were chosen to be analysed. It

is possible that there are other compounds which could have contributed to the differences seen in the samples, but were not analysed in this study.

- More sampling points during fermentation is recommended, as well as analysis of the yeast-nutrition related metabolites such as nitrogen, lipids and sterols (Smit, 2013) (in the different matrices), to determine the impact of this factor on the final product. Additionally the same approach to investigate aroma extractability could be used to study a red-wine cultivar.

Despite these technical and logistical issues, the study could deliver on the objectives set out in the planning of the project and provided important insights. Figure 1.3 represents an overview of selected pertinent results obtained in the study as contextualised in the discussion below:

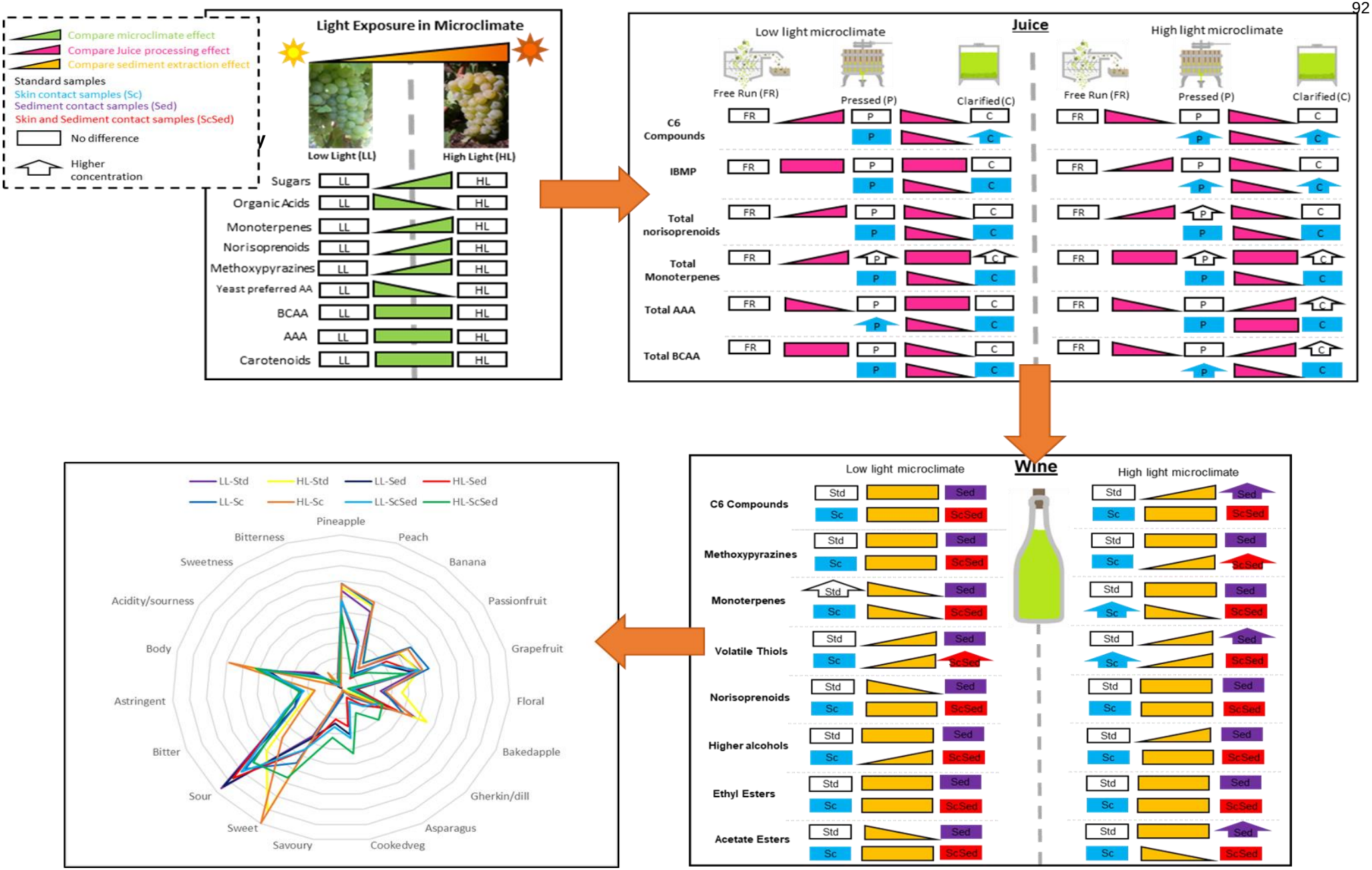


Figure 3.1: Summary of all results in the study including Sauvignon blanc ground up berry, juice and wine chemical data and wine descriptive sensory data. The tapering of the triangle indicates significantly higher/lower concentrations. This is only a visual representation of the relative concentrations, and the size of the triangle is no indication of the quantitative value. The arrowheads indicate (per compound group) significantly higher compound concentrations, comparing in *Juice:* the effect of skin contact between **Std** and **Sc** samples and in *Wine:* the effect of skin contact between **Std** & **Sc** samples and between **Sed** & **ScSed** samples. This figure excludes sediment data.

High aromatic potential grapes from a high light microclimate and low aromatic potential from low light microclimate?

Analysis of Sauvignon blanc whole berries confirmed what has previously been established in literature: exposure to high light (HL) microclimates during grape development increased the concentration of sugars, monoterpenes (linalool) and norisoprenoids (6-MHO) in berries and decreased yeast preferred amino acids and organic acids (Gregan *et al.*, 2012; Šuklje *et al.*, 2014; Joubert *et al.*, 2016; Young *et al.*, 2016; Du Plessis, 2017). The increased presence of many of the aroma-linked metabolites quantified in this study can be attributed to the physiological response of the grape berry to increased light exposure that is characteristic of the HL microclimates, as was reported in Young *et al.* (2016) and du Plessis *et al.* (2017). This was further confirmed by the analysis of photosynthetic pigments in the grape berries, which yielded significantly higher concentrations of the xanthophyll, zeaxanthin, in the HL samples. This was indicative of the upregulations of the xanthophyll cycle , a photoprotective response in plants (Siefermann-Harms, 1977). Some compounds were specifically interesting in their response to microclimates, as with the concentrations of methoxypyrazines which were found to be higher in HL versus LL berries - in contrast to what was observed in literature (Šuklje *et al.*, 2014; Martin *et al.*, 2016; Honeth, 2018). The pattern of methoxypyrazine concentrations persisted throughout the matrices (juice, sediment and wine) and was possibly due to the early leaf removal (before flowering) of all vines designated for both HL and LL treatments in the commercial vineyard where this study was conducted. Therefore, taking into consideration the significantly higher concentrations of various aroma compounds and precursors in the HL berries, it can be said that grapes from a HL microclimate had a higher aromatic potential (HAP) compared to the lower aromatic potential (LAP) in berries from a LL microclimate. The initial establishment of the aromatic potential of the berries of the two microclimates outlined here were crucial to the success of the study as it provided a platform to compare how the aromatic potential was affected by processing and winemaking techniques. The characterised LAP and HAP berries indicated that microclimate was a definitive aromatic predictor that resulted in two distinct groups in terms of aromatic potential, each of which were used in the subsequent production of wine.

Juice metabolic profiles were labile, with many aroma impact compounds diminishing towards clarification, and settling out with the juice sediment.

Our data showed a steady decrease in specific aroma-linked metabolites as juice processing progressed, with significantly lower concentrations of hexyl formate, esters, norisoprenoids, hotrienol, IBMP, tryptophan and isoleucine (HL only) in clarified juice samples when compared to free run samples under standard winemaking conditions (Figure 3.1).

Overall the results obtained from the different juice preparation stages were variable, alluding to the dynamic nature of the juice, and how compounds are continually interacting, degraded and/or extracted during juice processing (Marais, 1998; Van Rensburg *et al.*, 2000; Lukić *et al.*, 2017; Honeth, 2018). The decrease of aroma-linked metabolites from free run to pressed juice and from pressed juice to clarified juice could suggest that a portion of the aroma-linked metabolites reside/remain in the pomace (which is typically discarded after pressing) or in the sediment (that settles out during juice clarification) – resulting in a "loss" of aromatic potential. Preliminary analysis of the sediment matrix revealed that it did indeed contain a significant proportion of some important aroma-linked metabolites, including linalool, hexyl formate and IBMP. These results contribute to our understanding in terms of the juice sediment matrix, confirming that it contains a pool of "untapped" aromatic potential which could be utilised with increased extraction (Houtman & Du Plessis, 1981; Ancín *et al.*, 1996; Nicolini *et al.*, 2011).

Grapes with a higher aromatic potential resulted in more aromatic juices and wines

The distinction between LAP and HAP berry samples persisted throughout juice processing to clarified juices, especially with regards to hotrienol, IBMP, and aromatic amino acids. Alcoholic fermentation of these standard juices (i.e. no skin or sediment contact) resulted in higher aromatic potential wines from HAP juice and lower aromatic potential wines which are from LAP juice. The difference in the aromatic profile between HAP wine and LAP wine was mainly in the concentrations of grape-derived aroma compounds. The grape-derived aroma compounds showed strong responses to light exposure, and HL-Std wines contained significantly higher concentrations of IBMP, hotrienol, linalool and β-damascenone, compared to the LL-Std wines and this difference was perceived in descriptive sensory analysis. Interestingly there were no significant difference observed in the fermentation-derived aroma compounds (total esters and alcohols produced) between LL-Std and HL-Std wines. The sensory profiles of the wines made with the standard protocol agreed to what was found in previous studies (Šuklje *et al.*, 2014) from berries of a LL and HL microclimate in the same viticultural plot – "fruity" HL-Std wines and "vegetative" LL-Std wines. Although HL-Std wines contained significantly higher concentrations of IBMP, the volatile thiols were present in much greater concentrations with particularly high OAV values and therefore driving the "fruity" perception of the HL wines. The "fruit" forward perception of HL-Std wines, regardless of significantly higher concentrations of IBMP, could be due to the cumulative effect of three possible aromatic interactions: (1) The "vegetative" aroma of IBMP was supressed due to mutual masking effects of high concentrations of volatile thiols and methoxypyrazines (van Wyngaard, 2013), (2) the suppression of the thiol driven aromas were counteracted by the enhancing effect that esters have in the presence of high concentrations of volatile thiol aromas (Campo *et al.*, 2005; King *et al.*, 2011); or (3) the "fruity" character was enhanced by the additive effects of the high concentrations of various monoterpenes in HL-Std wines (Ribéreau-Gayon *et al.*, 1975).

The extraction treatments were effective in impacting the aromatic potentials of the juices and the wines

The implementation of skin contact mitigated the loss of some aroma-linked metabolites (C_6 compounds and methoxypyrazines) in both LAP and HAP juices. This mitigation effect of skin contact on the aroma losses that occurred during juice processing and fermentation confirmed that skin contact is a controllable way to increase the aromatic potential of the juice from which the wine was made (Cabaroglu *et al.,* 2002; Selli *et al.*, 2006; Maggu *et al.*, 2007). It was seen in wine samples that skin contact resulted in increased extractions of grape-derived aroma compounds – specifically linalool and 3MH–and it was found that LL-Sc wines grouped closely together with HL-Std and HL-Sc wines on a chemical and especially on a sensory level; scoring high for fruit driven descriptors. This was again probably due to the same interaction effects stated above. This proved that although the wine was made from LAP berries, that these berries contained an adequate pool of "residual aromatic potential" which was retained in the skin. The addition of skin contact resulted in increased extraction from this additional pool of aromatic potential and thereby "lifted" the aromatic profile of LAP wine to the level of HAP wine.

Fermentative sediment contact resulted in increased extraction of grape-derived aroma compounds like C_6 compounds (hexyl formate), volatile thiols (3MH) and volatile alcohols, and resulted in a decrease of ester concentrations. Furthermore, the sediment probably provided additional nutrients to the yeast, potentially influencing their metabolism and utilisation of precursors and production of aroma compounds, possibly also influencing the concentration of aroma compounds quantified in wine samples. The decrease in ester concentrations with increasing juice turbidity was also found by Houtman *et al.* (1980), although it was attributed to unknown factors. The same grape-derived compounds were identified in analysis of the sediment matrix, however, no increased extraction of methoxypyrazines were observed except in HL-ScSed wines. These findings established that the sediment does contain a "pool" of residual aromatic potential, and that this can be further extracted with fermentative sediment contact. The investigation furthermore revealed that the sediment contact was the most effective treatment in terms of increased extractability of aroma linked metabolites during wine making. Although many desired aroma-linked metabolites were extracted, less aromatically appealing compounds were also extracted, and sediment contact particularly resulted in lower concentrations of esters and resulted in wines perceived with some strong malodours (e.g. "cooked vegetable" and "asparagus" notes). These sensory perceptions could possibly be linked to the presence of the C_6 alcohols and aldehydes, as well as other compounds that were not quantified with the analyses used in this study.

It was found that HL wines contained higher concentrations of residual sugar and glycerol and LL wines more acidic, which was supported by "full-bodied", "sweet" and "acidic" attributes identified,

during the sensory analysis. The bitter notes identified in the sediment contact wines, seemed to have had a masking effect on the sweetness in HL-Sed and HL-ScSed wines. The descriptive sensory analysis of the wines found that there was a correlation between “fruity” aromas and perceived “sweetness” and “body”. The higher perceptions of the “body” was probably due to higher concentrations of glycerol in the HL wines, which is known to contribute to the mouthfeel of wine (Jackson, 2000). Wines that had sediment contact displayed less prominent “fruity” aromas, scored lower for both “body” and “sweetness” and higher for “bitterness”, “acidity”, “astringency” and “vegetative” aromas. This could be due to the reduction in aromatic metabolites observed in the chemical analysis (Figure 1.3 – acetate esters, norisoprenoids and monoterpenes). This is further supported by Williams (2019), who in a parallel study on the same samples, investigated the effects of increased extraction methods on the polyphenol contents of the juices and wines. He found significantly higher concentrations of catechin in fermentative sediment- contact samples, which could result in the perceived astringency. However, only sediment contact led to the negative impacts generally associated with enhanced extraction treatments. Furthermore, Williams (2019) found that a pre-fermentative skin contact treatment was strongly affected by juice processing and that most of the phenolic compounds settled out in the pomace and juice sediments. Interestingly, sediment contact led to an increase in volatile thiol concentration in all sediment contact wines of both LL and HL samples; however these wines did not receive high scores for “fruity” characteristics in sensory analysis. The low perception of “fruity” aromas in the sediment contact wines was possibly due to two-fold interaction effects: the lower concentrations of esters in the sediment contact wines, preventing known enhancing effects on thiol-related aromas (Campo *et al.*, 2005; King *et al.*, 2011) and the masking effect of either high concentrations of methoxypyrazines on thiol-related aromas (van Wyngaard (2013). Furthermore the presence of reductive aroma compounds which has been known to exhibit a “cooked vegetable” aroma could mask the thiol driven aromas (which were unfortunately not quantified in this study (Coetzee & Du Toit, 2015).

In conclusion, the study has shown that low light and high light microclimates result in berries with two distinct aromatic profiles, and that berries grown in high light microclimates have a comparably higher aromatic potential. The vineyard driven aromatic potentials of certain grape-derived aroma-linked metabolites persisted throughout standard wine making conditions to the final wine, resulting in standard wines with distinctive sensory profiles: ”sour” for LL and “sweet” and “fruity” for HL. We have shown that juice processing resulted in significant decreases in the concentration of important aroma-linked metabolites and that these losses were somewhat mitigated by pre-fermentative skin and fermentative sediment contact. Moreover, it was found that LAP berries contained significant aromatic potential, that with the addition of skin contact resulted in HAP-like wine at both sensory and chemical levels. Furthermore, it was found that sediment contact during fermentation resulted in the most drastic changes to wine aroma profiles that were reflected in the sensory profiles of the wines with resulting vegetative aromas and perceived bitterness and astringency. Finally, this study

provided a better understanding of the integrated release and fate of Sauvignon blanc aroma compounds from grapes to wine.

References

Alexandersson, E., Jacobson, D., Vivier, M. a, Weckwerth, W. & Andreasson, E., 2014. Field-omics-understanding large-scale molecular data from field crops. Front. Plant Sci. 5(June), 286.

Ancín, C., Ayestarán, B., Corroza, M., Garrido, J. & González, A., 1996. Influence of prefermentation clarification on the higher alcohol contents of wines. Food Chem. 55(3), 241–249.

Cabaroglu, T. & Canbas, A., 2002. The effect of skin contact on the aromatic composition of the white wine of Vitis vinifera L. cv. Muscat of Alexandria grown in Southern Anatolia. Acta Aliment. 31(1), 45–55.

Campo, E., Ferreira, V., Escudero, A. & Cacho, J., 2005. Prediction of the wine sensory properties related to grape variety from dynamic-headspace gas chromatography-olfactometry data. J. Agric. Food Chem. 53(14), 5682–5690.

Coetzee, C. & du Toit, W.J., 2012. A comprehensive review on Sauvignon blanc aroma with a focus on certain positive volatile thiols. Food Res. Int. 45(1), 287–298.

Costa, J.M., Vaz, M., Escalona, J., Egipto, R., Lopes, C., Medrano, H., Chaves, M.M., Allen, T., Herbst-Johnstone, M., Girault, M., Butler, P., Logan, G., Jouanneau, S., Nicolau, L., Kilmartin, P.A., Tominaga, T., Furrer, A., Henry, R. & Dubourdieu, D., 2016. Identification of new volatile thiols in the aroma of Vitis vinifera L. var. Sauvignon blanc wines. Agric. Water Manag. 13(3), 10641–10650.

Dubourdieu, D., Tominaga, T., Masneuf, I., Des Gachons, C.P. & Murat, M.L., 2006. The role of yeasts in grape flavor development during fermentation: The example of Sauvignon blanc. Am. J. Enol. Vitic. 57(1), 81–88.

Green, J.A., Parr, W. V., Breitmeyer, J., Valentin, D. & Sherlock, R., 2011. Sensory and chemical characterisation of Sauvignon blanc wine: Influence of source of origin. Food Res. Int. 44(9), 2788–2797.

Gregan, S.M., Wargent, J.J., Liu, L., Shinkle, J., Hofmann, R., Winefield, C., Trought, M. & Jordan, B., 2012. Effects of solar ultraviolet radiation and canopy manipulation on the biochemical composition of Sauvignon Blanc grapes. Aust. J. Grape Wine Res. 18(2), 227–238.

Gregan, S.M., Winefield, C. & Jordan, B., 2017. Amino acid metabolism and accumulation in 'Sauvignon Blanc' grapes – investigating berry composition in response to canopy manipulation. Acta Hortic. 1188 9–14.

Honeth, C., 2018. The impact of UV-light on grapevine berry and wine metabolites. (PhD), Stellenbosch University, Stellenbosch, South Africa.

Houtman, A.C. & Du Plessis, C.S., 1981. The effect of juice clarity and several conditions promoting yeast growth on fermentation rate, the production of aroma components and wine quality. South African J. Enol. Vitic. 2(2), 71–81.

Houtman, A.C., Marais, J. & Du Plessis, C.S., 1980. The possibilities of applying present-day knowledge of wine aroma components: Influence of several juice factors on fermentation rate and ester production during fermentation. South African J. Enol. Vitic. 1(1), 27–33.

Jackson, R.S., 2000. Specific and Distinctive Wine Styles.

Jackson, R.S., 2014. Chemical constituents of grapes and wine.

Joubert, C., Young, P.R., Eyeghe-Bickong, H.A. & Vivier, M.A., 2016. Field-grown grapevine berries use carotenoids and the associated xanthophyll cycles to acclimate to UV exposure differentially in high and low light (shade) conditions. Front. Plant Sci. 7(June), 786.

King, E.S., Osidacz, P., Curtin, C., Bastian, S.E.P. & Francis, I.L., 2011. Assessing desirable levels of sensory properties in Sauvignon Blanc wines - consumer preferences and contribution of key aroma compounds. Aust. J. Grape Wine Res. 17(2), 169–180.

Lacey, M. & Allen, M., 1991. Methoxypyrazines in Sauvignon blanc grapes and wines. Am. J. Enol. Vitic. 42(2), 103–108.

Lukić, I., Lotti, C. & Vrhovsek, U., 2017. Evolution of free and bound volatile aroma compounds and phenols during fermentation of Muscat blanc grape juice with and without skins. Food Chem. 232 25–35.

Maggu, M., Winz, R., Kilmartin, P.A., Trought, M.C.T. & Nicolau, L., 2007. Effect of skin contact and pressure on the composition of Sauvignon blanc must. J. Agric. Food Chem. 55(25), 10281–10288.

Maggu, M., Kilmartin, P.A., Trought, M.C.T. & Nicolau, L., 2007. Effect of skin contact and pressure on the composition of Sauvignon blanc must. 13th Aust. Wine Ind. Tech. Conf. 379.

Marais, J., 1998. Effect of grape temperature, oxidation and skin contact on Sauvignon blanc juice and wine composition and wine quality. South African J. Enol. Vitic. Stellenbosch 19(1), 10–16.

Martin, D., Grose, C., Fedrizzi, B., Stuart, L., Albright, A. & McLachlan, A., 2016. Grape cluster microclimate influences the aroma composition of Sauvignon blanc wine. Food Chem. 210 640–647.

Naor, A., Gal, Y. & Bravdo, B., 2002. Shoot and cluster thinning influence vegetative growth, fruit yield, and wine quality of Sauvignon blanc grapevines. J. Am. Soc. Hortic. Sci. 127(4), 628–634.

Nicolini, G., Moser, S., Román, T., Mazzi, E. & Larcher, R., 2011. Effect of juice turbidity on fermentative volatile compounds in white wines. Vitis - J. Grapevine Res. 50(3), 131–135.

Olejar, K.J., Fedrizzi, B. & Kilmartin, P.A., 2015. Influence of harvesting technique and maceration process on aroma and phenolic attributes of Sauvignon blanc wine. Food Chem. 183 181–189.

Patel, P., Herbst-Johnstone, M., Lee, S.A., Gardner, R.C., Weaver, R., Nicolau, L. & Kilmartin, P.A., 2010. Influence of juice pressing conditions on polyphenols, antioxidants, and varietal aroma of Sauvignon blanc microferments. J. Agric. Food Chem. 58(12), 7280–7288.

du Plessis, K., Young, P.R., Eyéghé-Bickong, H.A. & Vivier, M.A., 2017. The transcriptional responses and metabolic consequences of acclimation to elevated light exposure in grapevine Berries. Front. Plant Sci. 8(July),.

Du Plessis, K., 2017. The evaluation of the impact of microclimatic factors on grapevine berries in a vineyard setting through molecular profiling. (PhD), Stellenbosch University, Stellenbosch,

South Africa.

Van Rensburg, P. & Pretorius, I.S., 2000. Enzymes in winemaking: Harnessing natural catalysts for effiecent biotransformations- A review. South African J. Enol. Vitic. 21(Special Issue), 52–73.

Ribéreau-Gayon, P., Boidron, J.N. & Terrier, A., 1975. Aroma of Muscat grape varieties. J. Agric. Food Chem. 23(6), 1042–1047.

Selli, S., Canbas, A., Cabaroglu, T., Erten, H., Lepoutre, J. & Gunata, Z., 2006. Effect of skin contact on the free and bound aroma compounds of the white wine of Vitis vinifera L. cv Narince,. Food Control 17(1), 75–82.

Siefermann-Harms, D., 1977. The Xanthophyll Cycle in Higher Plants. In: Lipids Lipid Polym. High. Plants. Springer Berlin Heidelberg, Berlin, Heidelberg 218–230.

Šuklje, K., Antalick, G., Coetzee, Z., Schmidtke, L.M., Baša Česnik, H., Brandt, J., du Toit, W.J., 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. 20(2), 223–233.

Swiegers, J.H., Kievit, R.L., Siebert, T., Lattey, K.A., Bramley, B.R., Francis, I.L., King, E.S. & Pretorius, I.S., 2009. The influence of yeast on the aroma of Sauvignon Blanc wine. Food Microbiol. 26(2), 204–211.

Williams, D.L., 2019. Linking the phenolic potential of grape berries with polyphenol distribution during wine making : A Sauvignon Blanc case study. (MSc), Stellenbosch University, Stellenbosch, South Africa.

van Wyngaard, E., 2013. Volatiles playing an important role in South African Sauvignon blanc wines. (MSc), Stellenbosch University, Stellenbosch, South Africa.

Young, P., Eyeghe-Bickong, H.A., du Plessis, K., Alexandersson, E., Jacobson, D.A., Coetzee, Z.A., Deloire, A. & Vivier, M.A., 2016. Grapevine plasticity in response to an altered microclimate: Sauvignon Blanc modulates specific metabolites in response to increased berry exposure. Plant Physiol. 170(March), 1235–1254.