The effects of UV-B and water deficit on grape amino acids and wine aroma compounds

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There are significant correlations between amino acid (AA) concentrations in grape must and wine aroma compounds. Solar radiation and water availability factors can affect fruit AA concentration and therefore influence the evolution of aroma compounds during alcoholic fermentation. This thesis investigates the effects of altering grapevine UV-B exposure and water availability on wine aroma compounds, focussing on the role of AAs in the process. Pinot noir vines growing in the Lincoln University vineyard and own-rooted, potted Pinot noir vines growing in a glasshouse were use in this study. In the glasshouse, different UV-B (+UV and -UV) and irrigation (+W and -W) treatments were established from veraison. A field trial water deficit treatment was not able to be put in place due to rainfall events, but fully exposed fruit (LR) was compared to fruit shielded from UV-B using PETG screens erected over the fruiting zone (PETG) and fruit shielded from full sun using shadecloth (SC). As small amounts of fruit were available for this study, micro-vinifications of 160g and 100g for the field and glasshouse study, respectively, were applied.

HPLC analysis of wine AAs indicated UV-B radiation significantly decreased total AA concentrations and most individual AAs by a range of 41% to 93%. Water deficit in the glasshouse study increased total and some individual AAs by a range of 56% to 90%. This provided a good basis on which to investigate impacts of fruit AAs on wine aroma compounds.

SPME-GCMS analysis of wine showed that concentrations of higher alcohols, fatty acids, esters and norisoprenoids were related to the consumption of AAs during yeast fermentation. From the pattern of results it can be concluded that direct catabolism of AAs into aroma compounds is less likely than their having an indirect effect on aroma compounds formation during alcoholic fermentation.

As relationships exist between AA consumption during fermentation and wine aroma compounds, the effects of UV-B and irrigation treatments on aroma compounds (via affecting grape AAs) deserved additional investigation. In the glasshouse trial higher alcohols, fatty acids, esters, monoterpenes and C_{13}-norisoprenoids were affected. +UV decreased the concentration of phenylethyl alcohol, hexanoic acid, ethyl acetate, ethyl isobutyrate, ethyl butanoate, ethyl isovalerate, ethyl hexanoate, and ethyl decanoate, and isoamyl acetate in wine, but did not affect isoamyl alcohol. UV-B exposure increased the concentration of linalool and decreased that of citronellol. On the other hand, water deficit in the glasshouse experiment increased most esters. For the field experiment β-damascenone in wines was unaffected by LR and PETG, but decreased by SC. Principal Components Analysis showed distinct separation of treatments: for example -UV-W tended to be more dominated by compounds related to fruity and floral aromas.

These results suggest sunlight (and thus UV-B) exposure and water deficit have direct effects on fruit AAs, which leads to significant differences in some wine aroma compounds. Moreover, UV-B radiation and water deficit combined had interactive effects on wine aromas. Based on these results, water deficit could amplify the positive effects of UV-B exclusion on the formation of aroma compounds.

Key words: UV-B radiation, water deficit, Pinot noir, amino acids, higher alcohol, fatty acids, ester, Ehrlich pathway, alcoholic fermentation, yeast metabolism, HPLC, GCMS.

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List of abbreviations

Ala	Alanine
Arg	Arginine
Asn	Asparagine
ASP	Aspartic acid
BSA	Bovine serum albumin
CO2	Carbon dioxide
Cys	Cysteine
GCMS	Gas chromatography mass spectrometry
Glu	Glutamic acid
Gln	Glutamine
Gly	Glycine
HPLC	High performance liquid chromatography
His	Histidine
Ile	Isoleucine
LR	Leaf removal
Leu	Leucine
Lys	Lysine
Met	Methionine
PETG	Glycol-modified polyethylene terephthalate
Phe	Phenylalanine
Pro	Proline
Ser	Serine
SC	Shade cloth
TA	Titratable acidity
Thr	Threonine
Trp	Tryptophan
Tyr	Tyrosine
UVI	UV Index
Val	Valine
+UV+W	UV-B exposure with regular irrigation
+UV-W	UV-B exposure with reduced irrigation
-UV+W	UV-B removal with regular irrigation
-UV-W	UV-B removal with reduced irrigation

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Chapter 1. Literature review

1.1 Introduction

Amino acids in grapes have potential influences on wine aroma profile. Amino acids serve as a major nitrogen storage compound, and are critical subunits and precursors of some aroma compounds in grape (Bisson, 1991; Bell & Henschke, 2005; Jackson, 2008; Jogaiah et al., 2010). Environmental factors can induce grapevine defence mechanisms, and influence the nitrogen metabolism in the grape (Martínez-Lüscher et al., 2014). As a result, the amino acids profile might be modified by environmental factors, such as UV-B and water deficit stress.

Yeast utilize amino acids as a nitrogen source to form aroma-active compounds during alcoholic fermentation (Bisson, 1991). Most important wine volatile compounds are generated during alcoholic fermentation as secondary metabolites of yeast metabolism. The formations of esters, higher alcohols and volatile fatty acids, and general fermentation kinetics are related to amino acids (Bisson, 1999; Garde-Cerdan & Ancin-Azpilicueta, 2008). Moreover, amino acids can act as aroma precursors to drive aroma compounds during fermentation (Ough et al., 1991; Jiranek, 1995; Bell & Henschke, 2005). This research focused on the effects of UV-B and irrigation treatments on wine amino acids and the changes of amino acids from grape to wine, how these effects can be transferred into wine aromas, establishing a relationship between the changes of amino acids and the concentrations of wine aroma compounds.

There are research reports where amino acid additions to must have been used to support the relationship between amino acids and wine aroma compounds (Garde-Cerdan & Ancin-Azpilicueta, 2008; Hernandez-Orte et al., 2002, 2005 & 2006). However, there appear to be no studies reporting the relationship between amino acid consumption and aroma compound development. Moreover, unlike addition of

amino acids into must, measuring amino acid consumption from starting materials to finished wine could provide more accurate information about the relationship between grape amino acids and wine aroma compounds. The present study focused on the correlation of amino acids consumption and aroma compounds development.

Further understanding of the influence of environmental factors or viticulture management on amino acids profile, and in turn their effect on wine aroma-active compounds is needed. To this end, different UV-B exposure treatments were applied in this research to alter grape amino acids, and in turn affect the alcoholic fermentation products, such as higher alcohol, ester and fatty acid compounds. Amino acid profiles of wine were evaluated by HPLC, and selected aroma compounds of finished wine were analysed by GC-MS.

M. Sun (a current PhD student in viticulture) has been studying the effects of UV-B and water deficit on grape composition, which included amino acids. The grape materials from Sun's experiments were used in this study to investigate treatment effects in wines, with a focus on amino acids and aromas compounds. An additional goal was to find links between amino acids consumed during fermentation and wine aroma compounds. M. Sun's thesis and this thesis are linked, but overlap between the data presented is kept as minimal as possible.

1.2 Grape berry development

Grape berry development can be divided into two sigmoid cycles separated by a lag phase: berry formation and berry ripening. Rapid cell division occurs in the first phase, along with cell enlargement and seed embryo development (Coombe & McCarthy, 2000). The total amount of cell division and extent of cell enlargement will determine final berry size and shape (Jackson, 2008). Moreover, malic acid is the primary metabolite accumulated via xylem and phloem transport during the first stage. Tartaric acid and hydroxycinnamic acids also accumulate in the first phase of

berry development (Coombe & McCarthy, 2000; Hrazdina et al., 1984).

The initiation of the second phase is associated with berry softening, colouring and sugar accumulation, and is usually called veraison (Coombe & McCarthy, 2000). Sugars are the primary organic soluble solids transported into the grape berry (Creasy and Creasy, 2009). Leaf photosynthesis is the major carbohydrate suppliers (Keller, 2010). During the fruit ripening stage, most flavonoid phenolic compounds, such as anthocyanins, accumulate in the berry skin. Non-flavonoid phenolic production tends to decline and may stop after veraison (Conde et al., 2007). The total amino acids content increase markedly at the end of maturation (Jogaiah et al., 2010). In addition, aroma compounds accumulate during secondary metabolism of berry development (Coombe & McCarthy, 1997).

1.3 Amino acids of grape

Amino acids are amine derivatives and major nitrogen (N) sources in grape. Moreover, amino acids serve as critical subunits in the generation of enzymes and proteins and are present as precursors of many aroma compounds (Kliewer, 1968 & 1970; Bisson, 1991; Jackson, 2008; Ough et al., 1991; Jiranek et al., 1995). Thus amino acids are key factors of alcohol fermentation and wine quality (Lamikanra & Kassa, 1999).

Most research has found an increase in free amino acid content of grapes during berry ripening (Kliewer, 1970; Kluba et al., 1978; Huang & Ough, 1991). Many reports suggested that arginine and proline are the major amino acids in most *V. vinifera* grape cultivars (Kliewer, 1968 & 1970; Huang & Ough, 1991). Arginine and proline increased significantly with grape maturity (Lamikanra & Kassa, 1999). Stines et al. (2000) suggested that arginine is a major N storage compound in the grape berry. It accumulates to a relatively high concentration prior to veraison, then increases slightly or stops accumulating during berry ripening (Jogaiah et al., 2010; Gregan et al., 2012). Arginine concentration can even decrease during ripening in some grapevine cultivars, possibly because arginine can be considered as a precursor in the

production of other compounds, such as polyamines, guanidines and other amino acids (Roubelakis-Angelakis and Kliewer, 1992; Gregan et al., 2012).

In most table and wine grape cultivars, over 90% of the total amino acids consisted of proline (Pro) and arginine (Arg), along with glutamic acid (Glu), phenylalanine (Phe), aspartic acid (Asp), serine (Ser), and threonine (Thr) (Jogaiah et al., 2010). The amino acid profiles of grape vary genetically in wide range among grape varieties (Huang & Ough; 1991; Lamikanra & Kassa, 1999). Each variety will have a more or less specific amino acid profile, especially regarding particular amino acids (Jogaiah et al., 2010; Hernández-Orte et al., 1999). For example, arginine (Arg) content is the highest in Pinot Noir (Table 1.1).

Table 1.1 Composition of amino acids related to the variety of grape (mg/l) (Hernández-Orte et al, 2002)

	Cabernet Sauvignon	Merlot	Tempranillo	Chardonnay	Pinot Noir	Riesling
Asp	22	22	87	39	52	76
Glu	47	52	85	114	139	102
Ser	35	47	60	86	60	89
Gly	4	5	6	3	6	9
His	315	24	137	39	39	181
Thr	40	36	72	66	96	71
Arg	80	29	673	159	333	225
Pro	1718	738	302	419	119	273
Met	44	11	25	11	13	45
Phe	6	19	8	28	24	12
Lys	0	15	14	10	15	0
Gln	72	5	177	84	71	248

Many nitrogenous compounds can be found in grape berries, such as amino acids, pyrazines, peptides and proteins (Jogaiah et al., 2010). According to Bisson (1991), the dominant amino acids （except proline） are the primary source of nitrogen for yeast metabolism during alcoholic fermentation. Furthermore, amino acids are considered as precursors of some volatile compounds produced during yeast fermentation (Bisson, 1991; Ough et al., 1991; Jiranek, 1995; Bell & Henschke, 2005).

1.4 The effects of UV-B and water deficit on grape amino acids

1.4.1 UV-B

UV-B is an environmental stress, which can cause significant negative influences on plants. Small amounts of solar electromagnetic radiation with a wavelength from 280 to 315 nm, named UV-B, can reach the Earth's surface (Jansen, 2012). Grapevines can suffer serious biological damage from UV-B exposure, but they will initiate related protective and repair responses (Schultz, 2000). For example, plants will generate more UV-absorbing compounds to inhibit UV penetration via up-regulation of several important enzymes, such as chalcone synthase, involved in flavonoid biosynthesis and phenylalanine ammonia-lyase involved in the phenyl-propanoid pathway (Jansen et al., 1998). In contrast, increased UV-B will decrease carotenoid pigment production and nitrogen incorporation into amino acids (Singh et al., 2012). As mentioned before, amino acids are involved in nitrogenous metabolism and act as key enzymes and proteins in grape formation. Thus, UV-B might affect accumulation of amino acids.

There are some mechanisms that may be able to explain how UV-B might have effects on amino acids:

(1) UV-B will reduce the energy available for nitrogen metabolism. UV-B inhibits the Photosystem II reaction centre, especially the photosynthetic electron transport chain (Kulandaivelu & Noorudeen, 1983; Jasen et al., 1998). Because the ATP pool from PS II will be decreased and the absorption and incorporation of nitrogen into amino acids requires this energy from photosynthesis, amino acid production will be decreased (Dohler et al., 1995; Schultz, 2000).

(2) UV-B will affect the key enzymes involved into nitrogen metabolism. In nitrogen assimilation, the reductions from NO_3^- to NO_2^-, and then to ammonium, are catalysed by nitrate reductase (NR) and nitrite reductase (NiR), respectively. Ammonium is assimilated into organic compounds and forms amino acids though the glutamate

synthase-glutamine oxoglutarate aminotransferase (GS-GOGAT) cycle. The activities of NR, NiR, GS and GOGAT will be suppressed by high UV-B, therefore, the nitrogen metabolism and amino acid biosynthesis can be restricted by UV-B (Singh et al., 2012; Martínez-Lüscher et al., 2014).

(3) UV-B will reduce the carbon skeleton supply for amino acid biosynthesis. The Calvin Cycle is damaged by UV-B (Dohler et al., 1995), resulting in decreased production of carbon skeletons. The resulting ammonium from NO_2 reduction must be incorporated rapidly into carbon skeletons, so amino acid biosynthesis is reduced by UV exposure.

The literature as a whole, however, is not entirely in agreement on these issues. For instance, Schultz et al. (1998) found that high UV reduced the total free amino acid concentration in grapes. However, Keller and Torres-Martinez (2004), and Gregan et al. (2012) observed there is no change in total amino acid content. This might be because amino acids are produced in leaves, and leaf removal has a more significant effect on amino acids than UV-B (Gregan et al., 2012). Moreover, Gregan et al. (2012) only applied a UV treatment to cover the fruit zone, not the whole grapevine. The leaves from the fruit zone were removed, but other leaves (which were above fruit zone and exposed to normal sun radiation) could produce the amino acids that were transported into the grapes. This might be a cause of the lack of change in their results. In the proposed research glasshouse trial, whole grapevines will be under UV-B treatment, although the field trial utilises the same treatment methods as Gregan et al. (2012).

Regarding individual amino acids, UV-B can decrease the concentration of arginine and glutamine, which are the major nitrogen sources during yeast fermentation (Schultz et al., 1998). Martínez-Lüscher's research (2014) indicated that some individual amino acids，such as isoleucine, methionine, threonine, serine and glycine, were decreased by UV-B exposure from berry veraison and they suggested that there are a potential effects of UV-B on amino acids metabolism. This is therefore evidence

to suggest that UV-B can alter the individual amino acids concentration of grape, which in turn may affect the formation of aroma compounds during alcoholic fermentation.

In addition, previous studies used grapevine cultivars such as Riesling, Chardonnay, Cabernet Sauvignon and Tempranillo, but not Pinot noir, and previous research has shown that different cultivars have different tolerances to UV-B (Nunez-Olivera et al., 2006). In the proposed research the same methods as reported in Gregan et al. (2012) will be used to investigate the response of field-grown Pinot noir to UV.

1.4.2 Water stress

Similar to exposure to UV-B radiation, water deficit can be considered as an environmental factor that alters physiological characteristics of vines, influencing the accumulation of secondary metabolites in ripening berries. *Vitis vinifera L.* are well adapted to high drought environments because of their large and deep root systems and physiological drought avoidance mechanisms (Rodrigues et al. 1993; Chaves et al. 2007). For example, in response to water stress, grapevines will control stomatal conductance to prevent xylem embolisms and promote osmotic adjustment (Lovisolo et al., 2002; Patakas & Noitsakis, 1999).

Many wine-making regions practice regulated deficit irrigation, because this cultural practice can improve berry and wine quality though increasing accumulation of phenolics, volatile compounds and sugar (Keller, 2010). As a result, wine aroma, colour, flavour and antioxidant activity can be improved (Matthews et al., 1990; German & Walzem, 2000). There are some reasons why water stress can increase amino acids:

(1) Proline is a can act as an osmoprotectant. Drought stress will induce subcellular osmotic adjustment of grapevine tissues through an increase in proline concentration, which is a common response of plants to dehydration (Yancey et al., 1982).

(2) Activity of the TCA cycle can be promoted by water deficit. The TCA cycle plays an important role in plant metabolism, because it can give rise to many primary and secondary metabolites, including some intermediates involved in amino acid metabolism in grapevine. There are two key steps in the TCA cycle to form aspartate and glutamate. Drought will significantly increase the key enzymes activities involved in this two steps (Cramer et al., 2013). In addition, threonine, glycine and cysteine biosynthesis are increased by water stress, because they can form an important antioxidant (glutathione) against stress (Cramer et al., 2013).

(3) Remobilization of nitrogenous compounds. Ndung'u et al. (1997) indicated that nitrogenous remobilization occurred when grapevines were under water deficit conditions, where nitrogen-containing compounds were translocated from dehydrated leaves into perennial tissue, such as cane, trunk and root. During this process, amino acids were transported into berries as well.

Previous studies have found that water deficit leads to specific accumulation of particular amino acids in berries, such as proline, arginine, and glutamate (Berdeja et al. 2014). Yamada et al. (2005) reported that higher proline concentration caused by overexpression of the gene P5CS can improve the tolerance of plants to drought and salinity (review as in Cramer et al., 2007). Cramer et al. (2013) found that the amount of two key enzymes (isocitrate dehydrogenase and malate dehydrogenase) involved in the TCA cycle were increased under water deficit, and that the concentration of glutamate, threonine, cysteine and glycine were increased as well.

To conclude, both UV-B and water deficit can alter grapevine physiology, inducing plant defensive responses and affecting nitrogen and secondary metabolite production. So it seems reasonable to suggest that these two environmental factors will have an impact on amino acids accumulation in grapes.

1.5 Aroma composition of wine

The most important families of wine aroma compounds are monoterpenoids,

C_{13}-norisoprenoids, aldehydes, ester, higher alcohols, fatty acids, volatile phenolic, and sulphur compounds (see Table 1.2). Tomasino et al. (2015) indicated that β-damascenone, β-ionone, C6 alcohols, higher alcohols, carboxylic acids and esters were important aroma compounds of New Zealand's Pinot noir wines. Fang and Qian (2005) suggested 2-phenylethanol and 3-methylbutanol play an important role in aroma of Oregon Pinot noir wine. In addition, although ethyl anthranilate, ethyl cinnamate, 2,3-dihydroxycinnamate and methyl anthranilate are present at low concentrations, they contribute greatly to the characteristic aroma of Burgundy Pinot noir, which might be because they can react with each other or other compounds to generate new aromas (Fang & Qian, 2006).

Table 1.2 Characteristics of the chemical classes of volatile compounds in wine (Gonzalez-Barreiro et al, 2015; Styger et al, 2011)

Terpenes	Monoterpenes:	-Generally originate in grapes
	(linalool, α-terpineol, nerol, geraniol)	-Most in non-volatile glycosylated form -Fruity/floral aromas
	C13-Norisoprenoids:	-Derived from grape carotenoids
	(β-ionone, β-damascenone, β-damascenone, TDN)	-Most in non-volatile glycosides -Fruity and fuel-like characters
Methoxyprazines	IBMP, IPMP	-Products of amino acid metabolism -Vegetal characteristic
Higher alcohols	Isoamyl alcohol, Isobutanol, phenylethyl alcohol	-Formed from amino acids metabolism during alcohol fermentation
Esters	Ethyl esters:	
	(Ethyl butanoate, Ethyl hexanoate, Ethyl octanoate)	-Formed from ethanolysis of acyl-CoA -Fruity aroma
	Acetate esters:	
	(isoamyl acetate, propyl acetate, hexyl acetate, phenethyl acetate)	-Result of the reaction of acetyl-CoA with alcohol (Formed from amino acids) -Fruity aroma
Volatile fatty acids	Octanoic acid, Decanoic acid, Hexanoic acid	-Formed via fatty acid syntheses pathway from acetyl-CoA during fermentation
Volatile phenolic	Flavonoids & non-flavonoids	-Produced by grapevine -Bitterness, astringency

Sulphur compounds	Hydrogen sulphide, Methanethiol, Dimethyl sulfoxide, Methionol	-Most are negative aromas

Three main sources of wine aroma profiles can be identified, namely, (1) Primary fruit aroma: grape-derived compounds (both primary and secondary metabolites); (2) Alcoholic fermentation products from yeast metabolism; (3) Wine ageing: aroma compounds formed as wine matures pre and post-bottling (Rapp & Versini, 1991, as cited in Bell et al., 2005).

(1) Primary fruit aroma

Although most grape varieties have similar primary metabolite composition, they can have significant differences in aroma and flavour. A few aroma compounds present in grape and must can directly contribute towards characteristic varietal aromas. Although they are low in concentration, they can be high in impact, as indicated by their odour activity value (OAV) (Polaskova et al., 2008; Kotseridis & Baumes, 2000). Most potential aroma compounds are formed during the second stage of berry development, but are present as odourless, non-volatile glycoconjugates, including some monoterpenes, norisoprenoids, benzene-derivatives and phenols (Bell et al., 2005).

Monoterpenes are produced during the ripening stage of berry development and most of them in bound (glycoside) form. Although most terpenes are present in the skin, such as geraniol and nerol, some terpenes accumulate more so in flesh than in skin, such as free linalool, diendiol, and norisoprenoids (such as vitispirane) (Styger et al., 2011). Yeast glycosidases and acidic pH conditions will lead to hydrolysis of monoterpene glycosides into their free form during alcohol fermentation. Similarly, C_{13}-norisoprenoids are present in grapes in glycosidic form and are released into their free form during fermentation (González-Barreiro et al., 2015). Recently, Hernandez-Orte et al. (2008) indicated that C_{13}-norisoprenoids can be produced though a transformation of aglycon, which can be mediated by yeast enzymes (Hernandez-Orte et al., 2008) and via the low pH and high temperatureas associated

with fermentation. In other words, C_{13}-norisoprenoids production is not only a simple hydrolysis process, but also a process of fermentation. Some low molecular weight phenolic alcohols (such as 2-phenylethanol and benzyl alcohol) may occur in non-volatile (glycoside form or conjugates form) during ripening. It was reported that linalool, 2-phenylethanol, β- damascenone and β-ionone as well as benzaldehyde, higher alcohols, fatty acids and ethyl esters are important aroma compounds in Pinot noir wines (Rutan et al., 2014; Tomasino et al., 2015; Fang & Qian, 2005).

(2) Alcoholic fermentation

Many aroma compounds found in wine are not directly from the fruit, but from alcoholic fermentation through yeast metabolism. There are three routes of aroma development during fermentation: (1) Some grape-derived odour compounds are extracted into wine with intact chemical status, (2) Some grape-derived compounds are released into flavour active form though hydrolytic or biotransformation, and (3) Others are metabolised by yeast to form volatile compounds. Fermentation-derived volatile products or by-products contribute to major wine aroma compounds. These important aroma compounds included: aldehydes, higher alcohols, esters, and fatty acids (Styger et al., 2011). Acetaldehyde is formed from pyruvate and acts as a precursor of acetate, acetoin and ethanol. Higher alcohols, volatile acids and esters are derived from amino acids or sugar during yeast fermentation (Bell et al., 2005; Rapp & Versini 1991; Styger et al., 2011; Swiegers et al., 2005).

(3) Wine ageing

Ageing of wines in bottle or oak barrels also will change the aroma profile of wine. Ageing generally reduces the characteristic varietal aroma of the grapes as well as fermentation-derived aromas and generates new aromas. It is reported that the ethyl esters content of branched-chain fatty acids were decreased, the concentration of fruity aroma compounds were decreased, but the long-chain alcohols and volatile fatty acids were increased during barrel ageing of muscadet wine (Diaz-Maroto et al., 2005, as reviewed by Styger et al., 2011).

In all, fermentation is a major stage for wine aroma development. Nitrogen compounds play a necessary role in flavour development during alcoholic fermentation, especially in the formation of higher alcohols, esters and fatty acids (Rapp & Versini, 1996; Henschke & Jiranek, 1993; Bella et al., 2005). Since amino acids can be considered as precursors of aroma compounds and a main nitrogen source for yeast fermentation, it is reasonable to suggest that there is a correlation between wine aromas and must amino acid.

1.6 The relation between amino acid and aroma compound

1.6.1 Amino acids serve as a nitrogen source

The amino acid fraction that will be utilized directly by yeasts includes Arg, Asp, Glu, Ser and Thr. This fraction contributes 35-40% of total grape juice nitrogen, but arginine has the largest contribution to nitrogen (6-44% of total nitrogen and 30-50% of total assimilable nitrogen) (Hirano et al., 2000, cited in Jogaiah et al., 2010). Jiraneck et al. (1995) also found that Arg, Ser, Gln, Thr, Asp and Lys provide 70-79% nitrogen utilized for yeast growth.

Yeast metabolism produces some volatile compounds during alcoholic fermentation. Low nitrogen concentration in must or juice will lead to nitrogen deficiency, resulting in stuck or sluggish fermentations (Bisson, 1999; Garde-Cerdán & Ancín-Azpilicueta, 2008). Low nitrogen levels may cause high hydrogen sulfide production as well, resulting in the formation of sulphurous off-flavours (Bisson, 1991; Gardner, Poole & Jiranek, 2002). Amino acids will release organic nitrogen compounds during crushing and pressing and provide nitrogen and energy for yeast metabolism (Bisson, 1991). Thus, amino acids play a necessary role in yeast growth and metabolism. On the other hand, amino acids also relate to wine aroma profile formation, either mediated by the yeast, or as starting materials for other enzymatic pathways.

1.6.2 Aroma compounds formed from amino acids

The catabolism of amino acids (except glycine) will synthesize keto-acids and their related alcohols (Styger et al., 2011). For instance, esters, higher alcohols, aldehydes, volatile fatty acids, ketonic acids, terpenes and S-compounds are the metabolites of sugar and amino acid metabolism during yeast fermentation (Garde-Cerdan & Ancin-Azpilicueta, 2008). Therefore, the aroma profile of different cultivars might be attributed to grape amino acid profiles and compositions.

According to Fraile et al. (2000), various alcohols were formed at the same time with significant consumption of most of the amino acids. Guitart et al. (1999) also indicated that there is a close relationship between amino acid content and wine volatile compounds, and more higher alcohols were produced by available amino acid nitrogen than by only ammonium as a nitrogen source. Hernandez-Orte et al. (2002) studied a multiple linear regression model which indicated wine aroma compounds are related to amino acid concentrations and found their relationship is highly multivariate.

1.6.3 Higher alcohol formation

Higher alcohols are formed by reduction of the equivalent aldehyde, which is derived from alpha-keto acids by yeast during alcoholic fermentation (Ugliana & Henschke, 2009). Some alpha-keto acids can be derived from amino acids, such as branched amino acids (e.g. Leu) and aromatic amino acids (e.g. Phe) via the Ehrlich pathway, or are derived from glycolysis (sugar) via a biosynthesis pathway (Hazelwood et al., 2008; Ugliana & Henschke, 2009), see Fig 1.1. For example, Leu, Ile, Val, Phe, Tyr and Trp can be synthesized into isoamyl, isobutyl and phenylethyl alcohol, tyrosol and tryptophol, respectively (Hazelwood et al., 2008; Lilly et al., 2006). Phe can be degraded into alpha-keto acids, catalyzed by Aro8p and Aro9p (aromatic amino acid transaminases), and then be decarboxylated and reduced to phenylethyl alcohol (Hazelwood et al., 2008). Leu can be metabolised into alpha-keto acids, which can then be catalyzed by Bap2p and Bap3p (branched chain amino acid transaminases), then followed by decarboxylation and reduction to form isoamyl alcohol via the

Ehrlich pathway (Begenberg et al., 1999). When amino acids are insufficient for yeast growth, the synthesis of alpha-keto acids from glycolysis will be induced. These alpha-keto acids can act as the intermediates in amino acid metabolism (Chen 1978), and surplus alpha-keto acids could be converted into higher alcohols (Ugliana & Henschke, 2009).

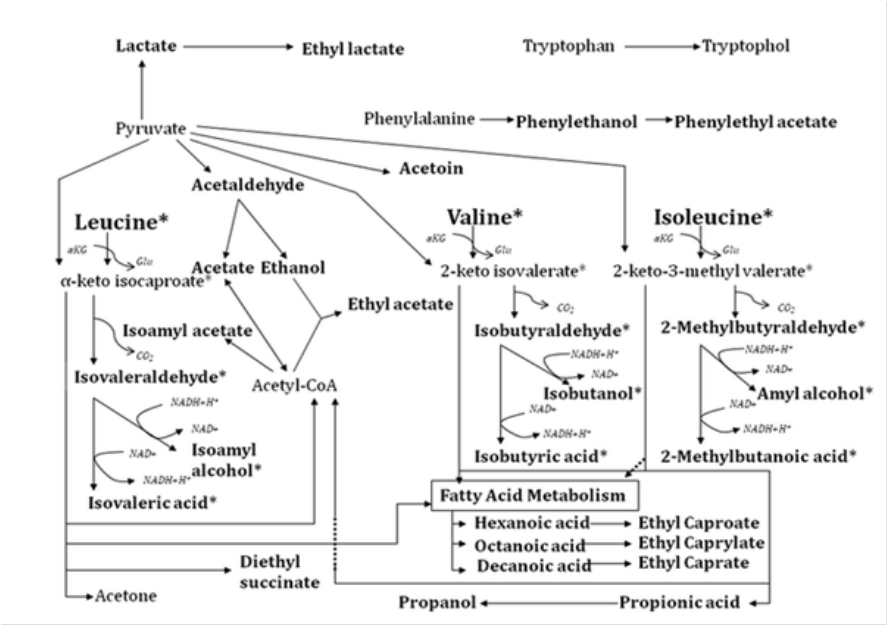

Fig 1.1 Metabolic map of yeast aroma compound production. Compounds marked with an asterisk constitute the Ehrlich pathway (Styger et al, 2011).

1.6.4 Fatty acids formation

Fatty acids can be produced during alcoholic fermentation (Ugliano & Henschke, 2009). Acetic acid is the most important fatty acid formed during fermentation, accounting for more than 90% of total wine volatile acidity (Eglinton & Henschke, 1999). Acetic acid is activated with coenzyme A (CoA) to produce acetyl-CoA for lipid biosynthesis, such as fatty acids. Acetic acid can also be produced from redox balancing reactions and then excreted into wine (Ugliano & Henschke 2009).

Branched chain fatty acids are produced from alpha-keto acids though an oxidation of aldehydes catalyzed by aldehyde dehydrogenases during amino acid metabolism (Ugliana & Henschke, 2009). The alpha-keto acids can be formed from branched

amino acids by yeast though the Ehrlich pathway, or derived from glycolysis (Styger et al., 2011). Isovaleraldehyde is derived from Leu, and it can be oxidized into isovaleric acid (Styger et al., 2011). Ile can be derived into 2-methylbutyraldehyde and further form corresponding acids. Isobytyric acid is derived from Val though oxidation of isobutyraldehyde, which derived from alpha-keto isovalerate (Styger et al., 2011). Many medium and long chain fatty acids are synthesized from acetyl-CoA (derived from glucose) via fatty acids metabolism during fermentation (Styger et al., 2011). Volatile medium chain fatty acids (hexanoic acid and octanoic acid) are by-products of long-chain fatty acids formation, which are required for cell membrane phospholipid biosynthesis. Uptake and storage of nitrogen by yeast is an adaptive response, because the fermentation medium, with increasing ethanol concentration, could inhibit the transport of amino acids (Bisson, 1991). The fatty acid compositions of yeast cell membranes can be altered by the predominant nitrogen source of fermentation medium (Torija et al., 2003).

1.6.5 Ester formation

Ester formation can be classified into two categories: enzyme-free formation and enzymatic formation. The former reaction acts as an equilibrium reaction between alcohol and acid, but this synthesis rate is slow and only accounts for a small percentage of the final ester (Lambreches & Pretorius, 2000). The second category occurs via yeast activity during alcoholic fermentation. The enzymatic formation is started with an activation of acid, combining the acid with coenzyme A (CoA). After that the activated acid will react with the alcohol by acetyltransferase to generate an ester (Styger et al., 2011). Both acetyl-CoA (from pyruvate) and acyl-CoA compounds (from enzyme acyl-ClA synthetase) can be the coenzyme donor (Park et al., 2009).
Acetate esters are produced enzymatically by the condensation of acetyl-CoA (from pyruvate) and ethanol or higher alcohols, which come from catabolism of amino acids, carbohydrates or lipids (Bisson & Karpel, 2010). The concentration of acetate esters in wine is the result of the balance between their synthesis and hydrolysis

(Plata et al., 2005). Ethyl acetate was produced though the condensation of ethanol with acetyl-CoA (Garde-Cerdan and Ancin-Azpilicueta; Ugliano & Henschke 2009). Moreover, it can be generated in the presence of ethanol and in presence of acetyl-CoA or acetic acid (Plata et al., 1998). Yoshimoto et al. (1998) indicated that there was a link between nitrogen availability and expression of ATF1gene (which encodes for alcohol acetyltransferase enzymes, which are responsible for acetate ester formation). Most ethyl esters of fatty acids are synthesized by the reaction of ethanol and acyl groups formed from activated fatty acids (acyl-CoA) during the early phase of lipid biosynthesis (Bisson & Karpel, 2010; Ugliano & Henschke 2009).

1.6.3 Amino acids impact higher alcohols, volatile fatty acids, and esters.

Amino acids do not only impact the kinetics of alcoholic fermentation, but also affect the formation of wine volatiles，because they can act as precursors to various volatile compounds (Hernández-Orte et al., 2002； Garde-Cerdan & Ancin-Azpilicuta, 2008). Past research has indicated that amino acid supplementation has effects on wine aroma compounds during fermentation, especially on higher alcohols, esters and volatile acids (Hernández-Orte et al., 2005; Hernández-Orte et al., 2006; Garde-Cerdan & Ancin-Azpilicuta, 2008; Bell et al., 2005; Hernández-Orte et al., 2002).

Higher concentrations of amino acids resulted in elevated amounts of esters (ethyl ester) but lower higher-alcohol compound concentrations (Ough & Bell, 1980; Ough & Lee, 1981). According to a study by Garde-Cerdan and Ancin-Azpilicueta (2008), adding amino acids can increase the formation of total esters, fatty acids, isoamyl acetate, 2-phenylethyl acetate, and tyrosol and 2-phenylethanol. Hernandez-Orte et al. (2005) observed that adding amino acids to must could increase the concentration of phenylethyl alcohol, methionol, and decrease isoamyl alcohol in the wine and increase the level of propanoic acid, γ-butyrolactone, and isobutanol.

Hernandez-Orte et al. (2006), found a positive relationship between amino acid content and concentrations of phenylethyl alcohol, benzyl alcohol, and phenylethyl acetates, ethyl hexanoate, ethyl octanoate, and most fatty acids. The relationship between amino acids and volatile compounds was reported by Hernández-Orte et al. (2002) using model wine solutions. They found that methionol, acetic acid, isoamyl alcohol, β-phenylethanol, hexanoic and octanoic acids, ethyl butyrate, isobutyl acetate and isoamyl acetate are correlated with Asp, Thr, His, Phe, Ser, Arg and Met. In summary there is enough evidence to suggest that there is a link between the amino acids in the starting material and concentration of higher alcohols, esters and fatty acids in wine.

Qualitative changes are also evident: wine sensory factors are also affected by the alterations to amino acids in the grapes, especially on sulphured, vegetal, fusel and floral notes (Hernandez-Orte et al., 2006), while the notes of spices and sweet fruits were affected only slightly. The sulphured note was decreased and the fusel and floral notes were increased by addition of amino acids. The fruity and floral notes of wine were closely related to juice nitrogen concentration during fermentation (Bell et al., 2005). Aromatic esters, such as 2-phenylethyl acetate, contribute floral, cherry, stone-fruit aroma to wine. Phenylethyl alcohol was described as rosy and honey aromas (Fang & Qian, 2006). Isoamyl alcohols contribute fusel notes, while fatty acids, such as octanoic acid and decanoic acid related to rancid and fresh notes (Song et al., 2014). Most ethyl esters of fatty acids and acetic acetate of higher alcohols are responsible for fruity and floral aromas (Fang & Qian, 2006). There is therefore the potential for amino acids to have an effect on Pinot noir wine aromas.

To conclude, amino acids are utilized by yeast as nitrogen compounds during alcoholic fermentation, and the metabolism of amino acids have effects on wine aroma development, particular on higher alcohols, esters and volatile fatty acids and the wine sensory attributes. Therefore, we suggest that if we change the amino acid profile in grapes, it might cause an alteration in finished wine aroma compounds.

1.7 A review of methodologies

1.7.1 UVI

UV intensity can be described by the UV Index (UVI, http://www.who.int/uv/intersunprogramme/activities/uv_index/en/). The scale was developed to inform people about the potential risks of exposure to UV solar radiation. For example, when the UVI is above 10, the intensity is considered extremely high to the point that people should avoid being outside. A UVI below 3 indicates there is minimal risk of UV damage when outside. Previous research reported that when UVI reached at 10.2, the exposed cluster of Shiraz was lower in berry weight, pH, TA and Brix, but higher in total phenolic, flavonoid (Ozden, 2014). Martinez-Luscher et al. (2014) also suggested that UV-B radiation had effects on Tempranillo grape phenolic and amino acids when the UV reached 5.98 kJ/m2 (equal to UVI 10.9 for 8h) per day during veraison. In the South Island of New Zealand, the UVI of most areas are around 8 in February, 5 in March and 3 in April, which are 40% higher than many other regions with same latitude (Liley & McKenzie, 2006).

This study applied UVI 6 in the glasshouse treatment, which was similar to the intensity of UV-B field-grown vines might experience during ripening.

1.7.2 Canopy management

Canopy management can affect the grape microclimate, such as light exposure, temperature, and humidity (Dokoozlian & Kliewer, 1995). Leaf removal (LR) has been widely applied in the cool climate viticultural areas, resulting in improvement of air circulation, exposure of fruit to solar radiation and higher temperatures (Keller, 2010). Shading of the cluster zone (SC) leads to a reduction in fruit exposure and berry temperature, consequently, altering berry growth and maturation (Rojas-Lara &

Morrison, 1989). Glycol-modified polyethylene terephthalate (PETG) was utilized on the fruit zone to exclude UV-B but transmit other solar radiation (Gregan et al., 2012). It provides the same exposure level, but different UV conditions, for the fruit zone.

1.7.3 Micro-vinification

Vinification begins after the grapes are harvested, with leaves and stems being removed. This is followed by crushing to release the juice and then macerating juice with the skins and seeds. Alcoholic fermentation is encouraged by yeast addition. During fermentation, phenolic compounds are extracted into wine and alcohol and aroma compounds are formed by yeast metabolism. After alcoholic fermentation, the wine may go through malolactic fermentation, which can reduce acidity and improve wine taste.

Due to the limited amount of research materials, micro-vinification was chosen to be applied in this study. Small-scale vinification has been applied for many research experiments. According to Dambergs & Sparrow (2011) small-scale fermentations can range from 100g to 1kg grapes through the use of commercially available coffee plungers. Compared to large-scale fermentations, microvinification is easier for control and less risk and cost, but have same efficient (Dambergs & Sparrow, 2011). Micro-fermentation can be efficiently monitored by CO_2 loss. The plunger can be utilized for cap management, promoting efficient extraction during fermentation. The efficiency of tannin extraction in these microvinification systems has been reported as being similar to large-scale fermentation (Dambergs & Sparrow, 2011) while adding the flexibility of using very small amounts of fruit.

1.7.4 Analysis of grape and wine amino acids by HPLC

HPLC is the most frequent method for analysis of amino acids in foods (Wang et al., 2014). O-phthaldialdehyde (OPA) was used for derivatisation the primary amino acids, and 9-fluorenylmethyl chloroformate (FMOC) was the fluorescence derivative for proline (Gregan et al., 2012). 21 amino acids can be analyzed by this method,

included Asp, Glu, Cys, Asn, Ser, Gln, His, Gly, Thr, Arg, Ala, Tyr, Val, Met, Trp, Phe, Ile, Lys, Leu, Pro and Tau.

1.7.5 Analysis of wine aroma compounds by GCMS

Head space solid phase micro extraction gas chromatography mass spectrometry (HS-SPME-GCMS) was used to quantify selected aroma compounds in Pinot noir wine samples (Tomasino et al., 2015). According to Tomasino et al. (2015), three different HS-SPME-GC-MS methods were required to determined the accurate quantitation of wine aroma compounds. The first method can analyze esters, alcohols, and aldehyde. The second method was applied for linalool, β-damascenone, geraniol, ethyl hydrocinnamate, β-ionone and ethyl cinnamate. The third method was developed to analyze the volatile fatty acids.

1.8 Conclusion

The accumulation of amino acids occurs during secondary metabolism of berry development. Amino acids play an important role in many of the biological functions and processes of the grapevine. They serve as major nitrogen storage compounds and critical subunits involved in grapevine nitrogen metabolism. Moreover, it was suggested that the generation of some individual amino acids are attributed to grapevine adaptive response. Thus, amino acids might be affected by environmental factors, such as UV-B and drought stress.

This review discussed the role of amino acids during alcoholic fermentation and the effects of amino acids on wine aroma compounds. Amino acids act as a nitrogen source and as aroma precursors during alcohol fermentation. As a result, fermentation kinetics and volatile metabolites, particularly higher alcohols, esters and fatty acids, will be affected by the amino acids profile of the must/juice. Thus, this suggests that the change of amino acids in grapes by environmental factors might result in different aroma compound profiles in finished wine. Also, it is

reasonable to suggest that there is a relationship between amino acid consumption during fermentation and aroma compound concentration in the final wine.

1.9 Research objectives

Little research has been published about the relationship between wine aroma compounds and grape amino acids. The understanding of how and to what extent the differences in grape can be transported into wines is lacking. Therefore, different treatments of UV-B exposure and water deficit were applied in this research to change grape amino acids profile, and in turn affect wine aroma compounds (higher alcohols, esters and fatty acids) produced during alcoholic fermentation. Amino acid profiles of grapes and wine will be evaluated by HPLC, and selected aroma compounds in finished wine will be analysed by SPME-GC-MS. This research will focus on how the changes of amino acids going into alcoholic fermentation results in changes of aroma compounds in the wine, such as phenylethyl alcohol, isoamyl alcohol, acetic acid, hexanoic acid, octanoic acid, ethyl esters, isoamyl acetate, and phenylethyl acetate. This could help to broaden our understanding of which amino acids can be affected by the treatments, and what the results of those changes are in the aroma compounds in the wine. Consequently, this knowledge can help prediction of the relationship between amino acids and wine aroma compounds, which could predict wine qualities based on pre-fermentation analysis of grapes.

Chapter 2. Materials and Methods

Note: The grape materials used in this research are part of M. Sun's PhD work. She has been studying the effects of UV and water deficit on grape composition, including amino acids. This study focuses on microvinification of the grapes and determination of wine parameters, including amino acids and aroma compounds.

2.1 Site

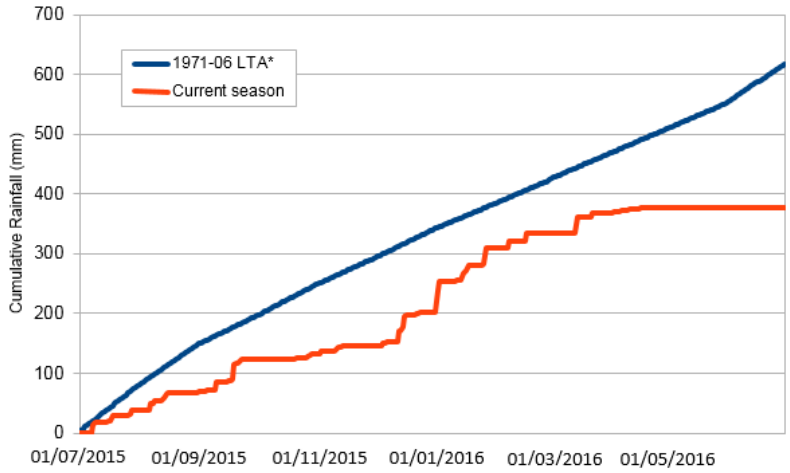

Figure 2. 3 Cumulative rainfall for the research site.

This study was conducted in the 2015/ 2016 growing season in the David Jackson vineyard and Nursery complex, Lincoln University, New Zealand. The vineyard is located at approximately 43° 39' south, 172° 28' east. The soil is predominantly Paparua and Wakanui series. The grape growing region has a cool climate with an average rainfall of 492 mm with the pattern for 2015-2016 shown in Figure 2.1 The growing degree day (GDD) accumulation at the research site from September 2015 through April 2016 was 1143 (10 ℃ base).

2.2 Material

The vineyard was planted in 1999 with *Vitis vinifera* L. cv. Pinot noir vines (clone 777 on 3309 rootstock) with a row and vine spacing of 2.5m * 1.2m, respectively. The

grapevine rows were oriented north-south and vines were trained on two bilaterally-opposed canes in a vertical shoot positioned system (VSP). All the grapes from trial were harvested by hand on 18 April, 2016 (six weeks post-veraison).

Vine material (Pinot noir clone 115) in the Nursery experiment were collected in August 2013 and rooted on a heating pad. The vines were transferred to 20L pots and grown outdoors at the Nursery with two shoots per vine. They were cane pruned in September 2015 to one cane each. Each vine was allowed to grow two shoots with one cluster per shoot. Each pot contained 80% composted bark and 20% pumice (1-7mm size), treated with Osmocote Exact 16-3.9-9.1 (NPK, 5g/L), horticultural lime (1g/L), and Hydraflo (1g/L) at the stage of budbreak (prior to the beginning of the experiment). A total of 24 vines were used in the experiment. All the grapes were harvested by hand on 23/Feb, 25/Feb, 01/Mar, and 08/Mar. Because clusters on each shoot were at slightly different developmental stages, the date of colour change was noted for each cluster, and each was harvested six weeks after that.

2.3 Treatment

2.3.1 Vineyard trial

Treatment (52 row)	Rep	Treatment (53 row)	Rep
	Buffer vines		Buffer vines
LR	R1	SC	R5
	Buffer vines		Buffer vines
SC	R1	LR	R5
	Buffer vines		Buffer vines
PETG	R1	PETG	R5
	Buffer vines		Buffer vines
LR	R2	SC	R6
	Buffer vines		Buffer vines
SC	R2	LR	R6
	Buffer vines		Buffer vines
PETG	R2	PETG	R6
	Buffer vines		Buffer vines
SC	R3	LR	R7
	Buffer vines		Buffer vines
PETG	R3	PETG	R7
	Buffer vines		Buffer vines
LR	R3	SC	R7
	Buffer vines		Buffer vines
SC	R4	LR	R8
	Buffer vines		Buffer vines
PETG	R4	PETG	R8
	Buffer vines		Buffer vines
LR	R4	SC	R8
	Buffer vines		Buffer vines

Figure 2. 4 Experimental design. SC: Shade cloth treatment; LR: Leaf removal treatment; PETG: Polyethylene terephthalate screen treatment

Three different UV-B treatments were applied in eight replicated blocks (four blocks in each row), as presented in Figure 2.2. It is a split plot design with three vines per plot. The vines were chosen for uniform leaf area and crop load. All treatments were applied from veraison.

In this experiment, shade cloth and PETG (glycol-modified polyethylene terephthalate, Mulford Plastics, Christchurch New Zealand) were used to block or transmit UV; shade cloth provides uniform shading in the fruiting zone and means that the leaf area is the same as in the other two treatments, LR and PETG.

(1) Shade cloth treatment (SC): all leaves and lateral shoots are removed in the cluster zone and the zone covered by green 70-80% shade cloth (Ultra-Pro Shadecloth, Cosio Industries Ltd, Auckland, NZ).

(2) Leaf removal treatment (LR): all leaves and lateral shoots are removed in the cluster zone.

(3) PETG: all leaves and lateral shoots are removed in the cluster zone and the zone covered by a PETG screen (240 cm*60 cm) mounted on an A-Frame. UV-B is excluded while longer wavelengths (such as UV-A) are transmitted.

2.3.2 Glasshouse treatment

2.3.2.1 UV-B treatment

Two UV-B treatments were applied to potted vines chosen for their similar leaf areas and crop loads. The vines were uniformly irrigated and under normal conditions in a glasshouse. All treatments were applied from veraison.

(1) UV-B control treatment (-UV): Twelve vines were exposed to normal sunlight and day length in the glasshouse. A UV filter was placed between –UV chamber and +UV chamber to block UV. The environment in the glasshouse was maintained at 22 °C/18 °C, day/night and relative humidity at 70–80%.

(2) UV-B treatment (+UV): UV-B-313 UV fluorescent tubes (313, Q-Lab Company, Westlake, OH, USA) were supported above the 12 vines. Fluence rates of UV-B (280–313 nm) were measured by a UV-B Biometer model 501 radiometer (Solar Light Company, Glenside, PA, USA). A plastic film was used in front of the UV lamps to

block radiation under 280 nm. The intensity of UV radiation was computer maintained at UVI-6 for eight hours per day. The environment in the glasshouse was the same as for the control.

2.3.2.2 Water treatment

Two water treatments were applied within each UV-B treatment group (Table 6.1).All treatments were applied from veraison:

(1) Well-watered control treatment: Six vines in each UV treatment were regularly irrigated (500 ml water every two days) to soil capacity.

(2) Water-deficit treatment: Six vines received 50% of the water used in the well-watered irrigation treatment (250 ml water every two days). The soil was dry to the touch and grape berry shrivelling was evident.

Table 2. 1 Four treatments of glasshouse trial

	UV-B radiation	UV-B exclusion
Well water	+UV+W	-UV+W
Water stress	+UV-W	-UV-W

2.4 Sample collection

All grapes from the vineyard and glasshouse trials were harvested at six weeks post-veraison. All the materials were frozen immediately in liquid nitrogen in the glasshouse or vineyard and stored at -20℃ prior to further processing for amino acids and aroma compounds analyses.

2.5 Micro-vinification

Very small-scale vinification was applied for research experiments due to the small amount of fruit generated in the trial. Following harvest, both field grapes and glasshouse grapes were prepared for fermentation. Any botrytis infected berries were removed from the bunches. Approximately 160g frozen grapes from each experimental plot were transferred into a plastic bag. When the grapes had thawed

(at room temperature) they were crushed by hand. The must was transferred into a 350 ml coffee plunger (Dambergs and Sparrow (2011) and left overnight at 4°C, then moved into a 26˚C chamber. Once the crushed grapes warmed to chamber temperature, each container was inoculated with 0.064g Maurivin PDM of *Saccharomyces cerevisiae* (manufacturer's recommended rate). Ferments were monitored initially using a balance to monitor weight loss and therefore fermentation progress. The plungers were pushed down and then back up gently twice per day until fermentation was complete. When the weight of the ferments stopped changing, wine density was measured with a density meter (DMA 35 Portable Density Meter, Anton Paar) and then covered with CO_2. The density measurement was repeated each day thereafter and if there were two consecutive Brix readings of <1.0, the primary fermentation was deemed complete (Weaver, 2007). The plunger was pushed down by hand, being careful to use similar pressure for all ferments, and the supernatant wine decanted and its volume measured. Following this, wines were transferred into 50ml plastic bottles, 35mg/L SO_2 solution added and the wines stored at -20˚C until analysis (Dambergs & Sparrow, 2011).

2.6 Measurement

2.6.1 General grape parameters

This analysis of grape characteristics is part of Sun's study. Total soluble solids of juice were measured by using a digital pocket refractometer (PAL-1, ATAGO, Tokyo, Japan). Titratable acidity (TA) and pH of grape juice were measured by pH meter (SP-701, Suntex Instruments CO., Ltd., Taipei, Taiwan) and titration to a pH 8.2 end point using 0.1N NaOH.

2.6.2 Amino acids of grape and wine analysis by HPLC

This HPLC analysis of grape was done as part of M. Sun's research. The method was described by Gregan et al. (2012).

(1) Sample preparation

Fifteen whole frozen grape berries of each treatment were ground to powder in liquid nitrogen. 2–3 g of frozen grape powder were measured into a 15 mL centrifuge tube, then thaw for about 20 min and centrifuged briefly (1960g for 10 min) to precipitate the solid material. The free-run juice (supernatant) was diluted 1:4 with distilled water in a new tube and. An internal standard (α-amino butyric acid) was added to a final concentration of 100 μmol/L. The sample was filtered via a 0.45 μmol/L nylon syringe filter into an HPLC glass vial and capped tightly.

(2) Equipment

Each sample was analysed using a Hewlett-Packard Agilent 1100 series HPLC system with a 250 × 4.6-mm, 5-μm prodigy column (Phenomenex).

(3) Chromatography

O-phthaldialdehyde and 9-fluorenylmethyl chloroformate were applied as fluorescence derivatives for primary amino acids and proline respectively. Cysteine sensitivity was increased by using Iodoacetic acid/mercaptopropionic acid. The fluorescence detector with an excitation of 335 nm and emission of 440 nm was applied in this study. The detector was switched into excitation 260 nm and emission 315nm at 25 min to test proline. A calibration curves can be formed by known concentrations of standards. The concentrations of unknown samples can be analyzed by this curves (Gregan et al., 2012).

Two solvents was utilized in this method: solvent A (0.01 mol/L Na_2HPO_4 with 0.8% THF, adjusted to pH 7.5 with H_3PO_4) and solvent B (20% solvent A, 40% methanol, 40% acetonitrile). Gradient: 0 min, 0% B; 14 min, 40% B; 22 min, 55% B; 27 min, 100% B; 35 min, 100% B; 36 min, 0% B, with a flow rate of 1 mL/min. Chemstation (Agilent) chromatography data system was used for data analysis.

2.6.3 General wine parameters

pH was measured by using calibrated pH electrode (SP-701, Suntex Instruments Co., Ltd., Taipei, Taiwan). 10 mL of wine sample was placed into 80 ml flask. Then the titratable acidity (TA) was measured by titration to pH 8.2 by standardised NaOH solution following the method from Iland et al (2000).

2.6.4 Wine volatile compounds analysis by GC-MS

These methods were adapted from Tomasino et al. (2015). Frozen wine samples were thawed to room temperature, and then 0.90 mL pipetted into 13-mL amber glass vials. A diluent solution of 5g/l tartaric acid in deionized water (pH 3.5) was used to dilute wine samples and standards by 10 fold. Standards were made up in dearomatized wine. 100 mL base wine was rotary evaporated (Buchi Rotavapor-R) at 30 °C for 2 hours (100 kPa). After evaporation, dearomatized wine was reconstituted in deionised water, adding 100 % HPLC grade ethanol to 14 %. The pH of dearomatized wine was adjusted to 3.5 (Song et al., 2015). All samples were kept at 8°C before injection in a stack cooler attached to the Combi-Pal autosampler (CTC-Analytics, Zwingen, Switzerland). All wine samples were analysed on a Shimadzu QP2010 GC-MS (Shimadzu Scientific Instruments, Japan) by duplicate in a randomised order (Tomasino et al., 2015).

Method 1

Analysis of esters, alcohols, aldehyde was carried out by adapting a method from Kemp (2010): benzaldehyde, trans-3-hexenol, cis-3-hexenol, hexanol, 1-heptanol, 3-methylbutanol, 2-phenylethanol, ethyl acetate, ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl-3-methylbutanoate, ethyl isobutyrate, ethyl pentanoate, ethyl lactate, ethyl heptanoate, hexyl acetate, and isoamyl acetate.

Method 2

Low concentration compounds were analysed using a similar procedure to Method 1

except that the acquisition mode was set to selected ion monitoring (SIM): linalool, β-damascenone, geraniol, ethyl hydrocinnamate, β-ionone and ethyl cinnamate.

Method 3

Analysis of volatile fatty acids (VFAs): acetic acid, butanoic acid, 3-methylbutanoic acid, 2-methylbutanoic acid, hexanoic acid and octanoic acid.

2.7 Statistical analysis

Statistical analyses were conducted using the SPSS. Amino acid data for berry and wine samples from field trial were analysed using One-Way ANOVA, Univariate, LSD, PCA, Duncan and Tukey's test at the 5% level. The analysis of amino acid from glasshouse trial samples was performed using Univariate and Tukey's test at the 5% level. The correlation between amino acids and aroma compounds were conducted Bivariate Correlations. The correlation coefficients were tested by the Pearson method, and the correlation significance tested by a two-tailed method at 5% ($p<0.05$) level. The parameters of the PCA are detailed in Chapter 3.

Chapter 3 Results

3.1 Introduction

Amino acids are accumulated in grapes during berry ripening, they serve as a nitrogen source for yeast fermentation and are considered as precursors of wine aroma compounds (Bisson, 1991). UV-B and water deficit can alter nitrogen and secondary metabolite production in grapevine (Martínez-Lüscher et al., 2014; Cramer et al., 2013), resulting changes in wine amino acids concentration and amino acids consumption.

The important families of wine aroma compounds include monoterpenoids, C13-norisoprenoids, higher alcohols, fatty acids and esters (Styger et al., 2011). UV-B and water treatments can affect these volatile compounds of wine differently. Monoterpenes and C13-norisoprenoids are present in bound form can be are released into their free form during fermentation (Bell et al., 2005). UV-B can promote the biosynthesis of terpenes and C13-norisoprenoids during ripening (Feng et al., 2014), while higher alcohols, fatty acids and their esters are derived from amino acids by yeast during alcoholic fermentation (Hernandez-Orte et al., 2002 & 2005). The composition and amount of amino acids present in fermentation can therefore influence the formation of volatile compounds.

Previous studies have investigated the relationship between the addition of amino acids in to musts and the concentrations of wine aroma compounds (Hernandez-Orte et al., 2006). In the present research the focus is on which and how much of each amino acid is utilized by yeast and how this consumption may be linked to higher alcohol, fatty acid, ester and grape-derived aroma formation in the wine.

The results consist of general grape characteristics, grape amino acids, general wine characteristics, wine amino acids, wine aroma compounds, amino acids

consumptions, correlations between amino acids and aroma compounds, and PCA results of the four treatments.

3.2 Grape results

3.2.1 General grape characteristics

Table 3. 1 Brix, titratable acidity (TA) (g/l) and pH of glasshouse grape (Sun, PhD student in viticulture)

Treatment	Brix	TA	pH
+UV+W	21.0	5.4	3.69
+UV-W	19.1	5.4	3.66
-UV+W	21.0	6.6	3.76
-UV-W	22.0	6.4	3.76
p value			
UV	0.05	n.s.	0.04
Water	n.s.	n.s.	n.s.
UV*Water	0.05	n.s.	n.s.

The data of grape Brix, TA and pH were obtained from M. Sun's study (Table 3.1). Brix and TA remained unaffected by both UV-B and water deficit treatments in the glasshouse study. The *p*-value for UV effect on Brix was close to 0.05, however, indicating that there may be some influence of UV on sugar accumulation, but the experiment was not robust enough to confirm this. UV-B slightly decreased the berry pH.

Compared to glasshouse trial, the results of field experiment were significantly different. In the field trial, there were significant differences in Brix, TA and pH among LR, PETG and SC treatments (Table 3.2). The SC fruit had significantly decreased Brix when compared with LR and PETG treatments, but there was no significant difference between LR and PETG. The PETG treatment decreased TA compared to LR and SC treatment. No significant differences were found between LR and SC

treatment. pH seemed to be decreased by SC. But unlike the glasshouse result, there was no difference between LR (equivalent to +UV) and PETG (-UV). This might be because of the different environmental UV-B intensity and other micro-climate conditions in the canopy, for example, berry temperatures can be directly increased by light exposure.

Table 3. 2 Brix, titratable acidity (TA) (g/l)) and pH of field grape (Sun, PhD student in viticulture)

Treatment	Brix	TA	pH
LR	21.5	8.4	3.57
SC	20.7	8.6	3.53
PETG	21.7	7.7	3.60
p value	0.03	0.03	0.04

3.2.2 Grape amino acids

The grape amino acids data were also part of M. Sun's results. The concentrations of grape amino acids in glasshouse grapes are shown in Table 3.3. The most abundant amino acids in grape berry at harvest were Arg, Pro, Gln and Thr. In the glasshouse trial, total free amino acids and almost all the individual amino acids concentrations were significantly lowered by UV-B treatment. Moreover, the concentration of individual amino acids showed a decrease (25-84%) with UV-B exposure in the majority of amino acids families: alpha-ketoglutarate group (Pro, Arg, Glu, Gln and His), aromatic amino acid (Phe), branched amino acid (Leu and Ile), aspartate group (Asp, Thr, Met) and 3-phosphoglycerate family (Ser and Gly). With regards to the field results (Table 3.4), no statistically significant effect of UV-B treatment (LR compared to PETG) was observed in the 21 amino acids or the total amino acids. These results are similar to those reported in Gregan et al. (2012), who found there was no significant effect of UV-B exclusion treatment compared to UV-B transmission treatment in the field.

Table 3. 3 Amino acid concentrations of fruit from the glasshouse experiment (μM)

	α-ketoglutarate					Aromatic			Branched			Aspartate				3-phosphoglycerate			Others		
Treatment	Pro	Arg	Glu	Gln	His	Phe	Trp	Tyr	Leu	Val	Ile	Asp	Thr	Met	Lys	Cys	Ser	Gly	Asn	Ala	Total
+UV+W	891	976	331	158	84	34	35	4	56	93	27	174	283	11	17	0	242	11	5	692	4124
+UV-W	608	494	253	127	72	26	52	0	46	95	39	99	206	5	28	0	198	20	15	490	2874
-UV+W	1765	2176	532	380	174	70	59	9	150	203	86	363	713	23	55	0	571	49	49	1532	8961
-UV-W	1869	1324	485	417	150	101	69	22	163	178	118	309	540	31	39	0	517	67	60	1238	7698
p value																					
uv	0.00	0.03	0.00	0.00	0.02	0.01	n.s.	0.00	0.00	0.00	0.00	0.02	0.00	0.01	n.s.	NA	0.00	0.01	0.04	0.00	0.00
w	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	NA	n.s.	n.s.	n.s.	n.s.	n.s.
uv*w	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.03	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	NA	n.s.	n.s.	n.s.	n.s.	n.s.

Table 3. 4 Amino acid concentrations of fruit from the field experiment (μM)

	alpha-ketoglutarate					Aromatic			Branched			Aspartate				3-phosphoglycerate			Others		
Treatment	Pro	Arg	Glu	Gln	His	Phe	Trp	Tyr	Leu	Val	Ile	Asp	Thr	Met	Lys	Cys	Ser	Gly	Asn	Ala	Total
LR	2589	5867	209	867	218	327	113	19	436	283	300	268	1141	71	63	0	677	32	53	1509	15041
SC	1827	6225	246	1855	283	443	144	29	515	332	368	281	1157	89	66	0	656	29	101	1646	16292
PETG	2196	5568	219	857	249	320	126	23	449	288	303	247	1161	71	64	0	656	34	66	1471	14370
p value	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s	n.s.	n.s.

3.3 Wine results

3.3.1 General wine characteristics

Neither wine TA nor pH was significantly affected by UV-B exposure or water deficit (Table 3.5). Although pH of field trial wines was not affected by canopy treatments, there were significant differences between LR, SC and PETG in wine TA.

3.3.2 Wine amino acids

The concentrations of amino acids in wines made from the glasshouse experiment are significantly different by treatments (Table 3.5). UV-B exposure significantly decreased (by a range of 41% to 93%) the concentrations of total amino acids and some individual amino acids groups as well: alpha-ketoglutarate group (Arg, Glu, Gln and His), aromatic amino acid (Phe), branched amino acid (Leu, Val and Ile), aspartate group (Asp, Thr, Met) and 3-phosphoglycerate family (Ser, Cys and Gly). Unlike grape amino acids, the water deficit treatment has significant effects on final wine amino acids. Asp, Glu, Asn, and Ser are increased by reduced irrigation by a range of 56%-90%.

The concentrations of total and individual amino acids in wines were significantly lower than that in grapes. The proline to arginine ratios of wine increased significantly, from 0.36 to 31.5 (field) and from 1.09 to 23.0 (glasshouse).

In this study, a weaker correlation (R^2= 0.294) can be found between grape and wine amino acids (Fig 3.1)，but this relationship was highly influenced by the lower three points, which are similar in wine concentrations, but significantly different in grape concentrations.

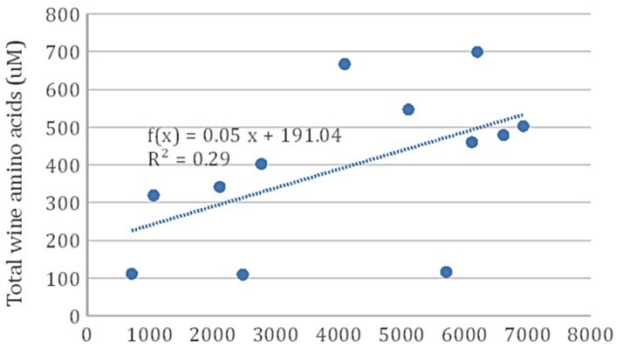

Fig 3. 5 Relationship between initial concentration of amino acids and residual amino acids

Table 3. 2 TA (g/l), pH and amino acid concentrations (μM), of glasshouse and field experiment wines.

	Glasshouse							Field			
					p value						
	+UV+W	+UV-W	-UV+W	-UV-W	UV	W	UV*W	LR	SC	PE	p value.
TA	7.1	5.9	7.2	6.0	n.s.	n.s.	n.s.	7.91	8.39	8.05	0.05
pH	4.4	4.0	4.4	4.7	n.s.	n.s.	n.s.	3.67	3.56	3.67	n.s.
α-ketoglutarate											
Pro	69.8	69.9	72.8	114.9	0.00	n.s.	n.s.	1374	1164	1384	n.s.
Arg	4.4	3.2	2.5	4.6	0.03	n.s.	n.s.	36.1	46.0	44.3	n.s.
Glu	17.7	40.3	49.6	67.2	0.00	0.00	n.s.	26.7	29.3	28.5	n.s.
Gln	2.8	18.5	19.0	24.5	0.00	n.s.	n.s.	26.8	28.7	29.8	n.s.
His	3.2	9.3	10.9	14.5	0.03	n.s.	n.s.	16.4a[i]	18.4ab	20.1b	n.s.
Aromatic											
Phe	1.2	10.5	16.4	22.7	0.02	n.s.	n.s.	8.2	10.4	10.3	n.s.
Try	0.8	1.2	1.4	2.2	n.s.	n.s.	n.s.	1.4a	0.8ab	1.5b	n.s.
Tyr	1.4	7.5	11.6	16.8	n.s.	n.s.	0.02	5.8	7.7	7.4	n.s.
Branched											
Leu	1.9	20.4	30.3	45.5	0.01	n.s.	n.s.	18.9	22.6	24.3	n.s.
Val	7.2	27.8	36.0	53.3	0.02	n.s.	n.s.	11.9a	13.2ab	14.9b	n.s.
Ile	1.5	13.1	18.9	29.3	0.01	n.s.	n.s.	6.0a	6.8ab	8.1b	0.03
Aspartate											
Asp	3.4	17.4	24.8	32.5	0.00	0.00	0.02	8.7	10.4	10.2	n.s.
Thr	11.1	31.6	35.2	52.1	0.00	n.s.	n.s.	12.4	12.0	13.3	n.s.
Met	0.6	3.4	4.8	7.3	0.02	n.s.	n.s.	3.8	4.6	4.5	n.s.
Lys	4.2	15.9	24.4	32.6	n.s.	n.s.	n.s.	17.9	22.0	22.2	n.s.
3-phosphoglycerate											
Ser	5.4	17.1	21.8	33.5	0.00	0.00	n.s.	11.4	12.5	13.1	n.s.
Cys	47.7	73.4	102.0	86.1	0.00	n.s.	0.00	76.9	76.3	74.0	n.s.
Gly	3.8	31.7	50.1	75.2	n.s.	n.s.	n.s.	22.5	23.6	25.6	n.s.
Others											
Asn	3.6	37.1	50.1	62.7	0.00	0.00	0.04	29.0	31.5	33.7	n.s.
Ala	7.3	48.4	77.1	119.9	0.01	n.s.	n.s.	71.7	80.8	80.6	n.s.

[i] Means followed by a different letter were significantly different by Tukey test ($p<0.05$)

3.3.3 GCMS results of wine aromas

Table 3.6 Lists the concentrations of the aroma compounds detected in Pinot noir wines in this research. Grape-derived terpenes, C13-norisoprenoid, as well as fermentation-derived esters, higher alcohols and fatty acids, were quantified.

3.3.3.1 Terpenes

UV exposure leads to a higher concentration of linalool (7%) and a lower concentration of citronellol (14%) in wine compared to the UV exclusion treatment under water deficit conditions. No significant differences can be found in the present field treatment. With regards to water treatments, the concentrations of linalool, geraniol and citronellol were affected by water deficit. The content of linalool and geraniol were decreased (by 29% and 34%, respectively) by water deficit without UV-B, while citronellol was increased (23%).

3.3.3.2 C_{13}-norisoprenoids

UV treatment did not influence the concentration of β-ionone, but did decrease the content of β-damascenone (27%). Unlike results from the glasshouse experiment, the concentration of β-damascenone in the shade treatment was 16% lower compared with leaf removal and PETG. As for water deficits, both β-damascenone (22%) and β-ionone (15%) were increased by reduced irrigation. In addition, UV-B together with water deficit had an effect on β-damascenone concentration, where the highest concentration can be found in UV-W- treatment and the lowest level in UV+W+.

3.3.3.3 Alcohols

UV radiation had effects on phenylethyl alcohol in this study. In comparison to the UV exclusion treatment, UV+ decreased phenylethyl alcohol by 8%. The level of isoamyl alcohol was not affected by UV treatment. Higher concentrations of isoamyl alcohol and phenylethyl alcohol were observed in wine from the water deficit treatment.

3.3.3.4 Fatty acids

The concentration of hexanoic acid (straight-chain fatty acids) in UV+ treatment is 19% lower than that in UV- treatment under reduced irrigation. Water deficit has effects on the concentrations of hexanoic acids. Under UV- conditions, reduced irrigation increased the level of hexanoic acids by 5%.

3.3.3.5 Esters

The UV treatment induced changes of wine ester concentrations. Isoamyl acetate was decreased (34%) by UV-B exposure. Moreover, the concentration of ethyl acetate was decreased (50%) by UV-B radiation. UV exposure treatment decreased ethyl esters of straight-chain fatty acids. UV exposure significantly decreased the concentration of ethyl butanoate, ethyl hexanoate, and ethyl decanoate by a range of 20% to 30% (under water deficit conditions). Ethyl esters of branched-chain fatty acid, such as ethyl isobutyrate, ethyl isovalerate, were decreased by UV+ treatment by a range of 5% to 42%. The aromatic esters, ethyl cinnamate and ethyl hydroxycinnamate, were not affected by UV radiation.

The concentration of isoamyl acetate was increased by water deficit, which was consistent with the trend of isoamyl alcohol. Ethyl esters of straight chain fatty acids were affected by water deficit. The concentrations of ethyl acetate, ethyl butanoate, ethyl hexanoate, ethyl decanoate, ethyl pentanoate were increased by reduced irrigation by a range of 9% to 51% (under the UV exclusion conditions). Ethyl esters of branched fatty acids, such as ethyl isobutyrate, ethyl isovalerate, were increased by limited irrigation as well. In addition, reduced irrigation significantly decreased the concentration of phenylethyl acetate (9%) and ethyl cinnamate (13%). Additionally, water deficit interacting with UV-B exclusion could increase the promotional effects on the concentrations of isoamyl and ethyl acetate, ethyl octanoate and ethyl hexanoate. Their concentrations were highest in -UV-W and lowest in +UV+W treatments. On the other hand, the effects of water deficit are opposite to the effects of UV-B exclusion, leading to similar results were obtained from +UV-W and -UV+W.

For the field experiment, there is no significant difference in most esters, alcohols and fatty acids in wine as a result of leaf removal, shade cloth or PETG treatments (Table 3.7). The content of phenylethyl acetate and ethyl cinnamate were affected by leaf removal and PETG treatments, where their concentrations were 17% and 33%, respectively, higher in sunlight exposure compared to the UV-B exclusion treatment.

Table 3. 3 The concentrations of aroma compounds in glasshouse experiment wines (μg/L)

	Treatment				p value		
	+UV+W	+UV-W	-UV+W	-UV-W	W	UV	W*UV
Ethyl isobutyrate	9.89	15.85	12.92	19.08	0.00	0.03	n.s.
Ethyl butanoate	12.06	22.48	15.88	32.51	0.00	0.02	n.s.
Ethyl isovalerate	0.28	0.45	0.38	0.64	0.00	0	n.s.
Isoamyl acetate	147	115	115	174	0.05	0.05	0.00
Ethyl pentanoate	0.35	0.4	0.36	0.43	0.00	n.s.	n.s.
Isoamyl alcohol	131416	160616	128260	194095	0.01	n.s.	n.s.
Ethyl hexanoate	67.13	71.06	66.04	90.71	0.01	0.04	0.03
Hexyl acetate	0.99	0.73	0.8	0.94	n.s.	n.s.	0.00
Hexanol	812	833	798	1120	n.s.	n.s.	n.s.
trans-3-hexen-1-ol	16.14	15.73	17.46	21.33	n.s.	n.s.	n.s.
Ethyl heptanoate	0.52	0.42	0.46	0.41	n.s.	n.s.	n.s.
cis-3-Hexen-1-ol	17.65	24.79	18.72	16.15	n.s.	0.04	0.02
1-Heptanol	15.73	16.22	14.89	17.25	n.s.	n.s.	n.s.
Ethyl octanoate	252	188	190	240	n.s.	n.s.	0.00
Benzaldehyde	12.97	10.53	13.84	11.96	0.03	n.s.	n.s.
Ethyl decanoate	394	235	266	292	0.00	0.04	0.00
Phenylethyl alcohol	39223	45285	44826	49417	0.00	0.01	n.s.
Ethyl acetate	44458	39546	42704	79278	0.00	0.00	0.00
Acetic acid	2727645	1265985	2040245	1499374	0.02	n.s.	n.s.
Isobutyric acid	3498	3864	3672	3009	n.s.	n.s.	n.s.
Butanoic acid	825	1038	1008	1025	0.04	n.s.	n.s.
Isovaleric acid	302	299	355	365	n.s.	n.s.	n.s.
2-Methylbutanoic acid	374	452	413	419	n.s.	n.s.	n.s.
Hexanoic acid	672	672	785	826	n.s.	0.00	n.s.
Octanoic acid	464	378	441	565	n.s.	n.s.	n.s.
Linalool	9.48	10.58	13.9	9.83	0.03	0.01	0.00
Citronellol	8.16	11.46	10.28	13.29	0.01	0.05	0.87
Phenylethyl acetate	22.68	14.94	21.68	19.82	n.s.	n.s.	n.s.
β-Damascenone	4.71	4.76	4.94	6.36	0.01	0.00	0.02
Geraniol	3.81	3.06	3.93	2.92	0.00	n.s.	n.s.
Ethyl hydrocinnamate	0.07	0.05	0.07	0.05	n.s.	n.s.	n.s.
β-Ionone	0.45	0.5	0.43	0.5	n.s.	n.s.	n.s.
Ethyl cinnamate	0.53	0.19	0.45	0.22	0.03	n.s.	n.s.

Table 3. 4 The concentrations of aroma compounds in field experiment wines (μg/L)

	LR	SC	PETG	Sig.
Ethyl acetate	28404	26829	28288	n.s.
Ethyl isobutyrate	30.2	32.2	31.1	n.s.
Ethyl butanoate	98.1	94.5	100.0	n.s.
Ethyl isovalerate	2.9	3.0	2.5	n.s.
Isoamyl acetate	65.4	62.7	62.9	n.s.
Ethyl pentanoate	1.0	1.0	1.1	n.s.
Isoamyl alcohol	133724	136867	125563	n.s.
Ethyl hexanoate	432	420	427	n.s.
Hexyl acetate	0.0	0.1	0.0	n.s.
Ethyl lactate	3305	3273	3107	n.s.
Hexanol	503	506	462	n.s.
trans-3-Hexen-1-ol	16.0	15.0	13.4	n.s.
Ethyl heptanoate	2.6	2.4	2.3	n.s.
cis-3-Hexen-1-ol	15.4	17.0	13.3	n.s.
1-Heptanol	27.7	28.5	28.3	n.s.
Ethyl octanoate	679	691	696	n.s.
Benzaldehyde	16.7	21.1	17.7	n.s.
Ethyl decanoate	207	200	209	n.s.
Phenethyl alcohol	40794	42110	39909	n.s.
Acetic acid	486512	485551	545441	n.s.
Isobutyric acid	2411	2439	2297	n.s.
Butanoic acid	1849	1873	2070	n.s.
Isovaleric acid	985.0	921.1	913.2	n.s.
2-Methylbutanoic acid	848.6	851.7	839.2	n.s.
Hexanoic acid	1849	1898	1816	n.s.
Octanoic acid	811	847	748	n.s.
Linalool	12.5	12.4	12.9	n.s.
Citronellol	13.8	14.3	15.2	n.s.
2-Phenylethyl acetate	10.1a	8.9ab	8.4b	n.s.
β-Damascenone	4.9b	4.1a	4.9b	n.s.
Geraniol	2.51	2.72	2.36	n.s.
Ethyl hydrocinnamate	0.23	0.20	0.20	n.s.
β-Ionone	0.34	0.34	0.35	n.s.
Ethyl cinnamate	0.9a	0.7ab	0.6b	n.s.

*Means followed by a different letter were significantly different by Tukey test ($p<0.05$)

3.4 Correlations between the consumption of amino acids and the concentrations of fermentation aroma compounds

Consumptions of individual amino acids are shown in Table 3.8. The significant correlations between the concentrations of higher alcohols, fatty acids, esters, C_{13}-norisoprenoids and the quantity of individual amino acids consumed are shown in Tables 3.9 to 3.16.

3.4.1 Amino acids consumption

Amino acids consumption was defined as the difference between amino acid concentrations in the initial grape materials and their amounts in the finished wine. The consumption of amino acids was significantly and positively correlated (R^2=0.99884) with the concentration of initial grape amino acids (Fig 3.2) for the glasshouse experiment.

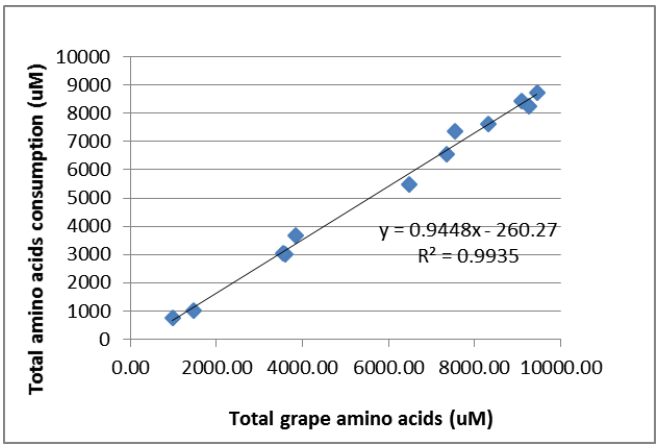

Fig 3. 6 Relationship between initial concentration of amino acids and consumed amino acids (glasshouse result)

The quantity of consumed amino acids is shown in Table 3.8, which were calculated from starting material (grape) and finishing wine. The consumption of alpha-ketoglutarate amino acids group (Glu, Gln and His), aromatic amino acids (Phe), branched amino acids (Leu, Val and Ile), aspartate amino acids group (Asp, Thr, and

Met), 3-phosphoglycerate family (Ser, Cys and Gly), and Ala, and total amino acids in -UV exposure treatments was greater than in the +UV treatments, while the concentration of Tyr was highest in -UV-W. All (100%) of Arg, 90-99% of Ala, and 82-98% of Asp were consumed by yeast. The proportion of consumed Glu, Thr and Ser was high (84%-95%, 85%-96%, and 91%-98%, respectively) as well. The aromatic amino acid Trp was also totally consumed, independently of the amount of must amino acids. The branched amino acids, Val and Leu were consumed by 70%-92% and 56%-97% respectively. Interestingly, Cys, because of the negative consumption rate, was likely synthesized and excreted by yeast throughout fermentation, but its concentration was affected by UV-B radiation treatment.

As for the field experiment, a positive correlation (r^2=0.991) was also found between amino acids consumption and total grape amino acids (Fig 3.3). No significant difference could be found in the concentrations of consumed amino acids between treatments (Table 3.9). However, similar to the glasshouse experiment, the consumed percentages of Arg, Ala and Asp were high during fermentation: 96%-97% for Asp, 99% for Arg and 95% for Ala. In addition, alpha-ketoglutarate group: Glu (86-88%), Gln (97-98%) and His (92-93%), branched amino acids: Val (95-96%), Ile (98%) and Leu (95%), aspartate amino acids group: Thr (99%) and Met (94-95%), 3-phosphoglycerate family: Ser (98%), were also highly utilized by yeast. The aromatic amino acids, Phe and Trp showed high consumed percentages: 97-98% for Phe, and 99% for Trp. Unlike these three aromatic amino acids, the consumed percentage of Tyr increased with the initial grape amino acids concentrations, from 63% to 75%. Asn (48-61%), Gly (12-34%), Lys (65-73%) were consumed in lower percentages in this study.

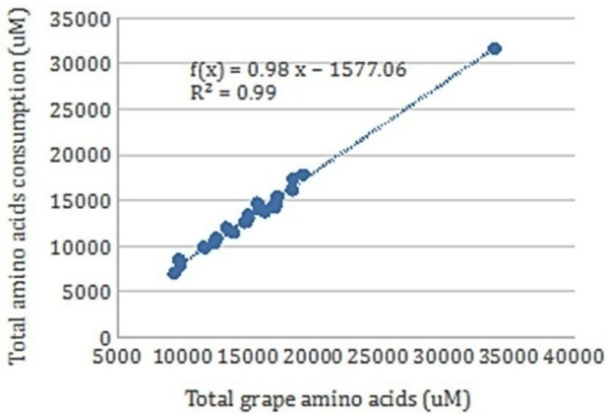

Fig 3. 7 The relationshp between initial concentration of total amino acids and consumed amino acids (field result)

Table 3.8 Amino acid consumption from the glasshouse and field experiment (μM)

Glasshouse:	Treatment				p value			Field:	Treatment		p value
	+UV+W	+UV-W	-UV+W	-UV-W	UV	W	UV*W	LR	SC	PE	
a-ketoglutarate											
Glu	314	213	482	418	0.00	n.s	n.s	197	184	208	n.s
Gln	155	109	361	393	0.00	n.s	n.s	928	832	1734	n.s
His	81	62	163	136	0.03	n.s	n.s	217	214	263	n.s
Arg	972	491	2173	1320	0.03	n.s	n.s	6065	5864	5605	n.s
Aromatic											
Phe	33	16	53	78	0.02	n.s	n.s	328	327	406	n.s
Trp	34	51	57	67	n.s	n.s	n.s	115	121	144	n.s
Tyr	2	-7	-2	5	n.s	n.s	0.02	15	13	22	n.s
Branched											
Leu	54	26	120	118	0.01	n.s	n.s	394	434	506	n.s
Val	86	67	167	125	0.02	n.s	n.s	258	271	333	n.s
Ile	26	25	67	88	0.01	n.s	n.s	272	297	381	n.s
Aspartate											
Asp	171	82	338	277	0.02	n.s	n.s	238	244	285	n.s
Thr	272	175	678	488	0.00	n.s	n.s	1094	1145	1182	n.s
Met	11	1	19	23	0.02	n.s	n.s	60	72	86	n.s
Lys	13	12	31	7	n.s	n.s	n.s	49	40	41	n.s
3-phosphoglycerate											
Ser	236	181	549	483	0.00	n.s	n.s	659	614	678	n.s
Cys	-48	-73	-102	-86	0.00	n.s	0.00	-77	-76	-74	n.s
Gly	7	-11	-1	-8	n.s	n.s	n.s	11	8	4	n.s
Others											
Asn	1	-22	-1	-3	n.s	n.s	n.s	45	29	52	n.s
Ala	685	442	1455	1118	0.01	n.s	n.s	1493	1438	1463	n.s
Total	3037	1800	6548	4942	0.00	n.s	n.s	12362	12073	13319	n.s

3.4.2 Correlations between amino acid consumption and wine aroma compounds

3.4.2.1 Higher alcohols

Phe and Leu can be synthesized into isoamyl and phenylethyl alcohol respectively (Hazelwood et al., 2008; Begenberg et al., 1999). However, there was no relationship between higher alcohols and their structurally corresponding amino acids in present study (Table 3.9 & Table 3.10). A correlation between Phe and phenylethyl alcohol was not observed in the present study. Moreover, no correlation between Leu and isoamyl alcohol was found in the present study. But there are some significant and positive correlations between isoamyl alcohols and the consumption of Asp, Ser, Thr, Arg, Ala, Tyr and Phe. In the field study, neither isoamyl alcohol nor phenylethyl alcohol showed a significant correlation between their formation and the quantity of consumed amino acids.

3.4.2.2 Fatty acids

(1) Branched-chain fatty acids

There were some correlations between branched-chain fatty acids and their corresponding amino acids (Table 3.11&3.12), although these fatty acids can also be connected with other amino acids.

A significant positive correlation ($p<0.05$) between Ile and 2-methylbutanoic acid can be found in the field experiment, but this was not the case in the glasshouse trial. In addition, a significant positive correlation ($p<0.01$) between Val and Isobytyric acid also can be found in field trail, while a negative correlation between them was found in glasshouse wine result. However, no correlation between Leu and isovaleric acid can be found in the present study, which was unlike the correlation found between Leu and isoamyl alcohol.

There are some correlations between amino acids consumption and branched fatty acids. Isovaleric acid was positively correlated with alpha-ketoglutarate amino acids (Glu and Gln), and aromatic amino acids (Tyr and Trp) in the glasshouse experiment, while these types of correlations were not been found in field. Isobutyric acid was related with alpha-ketoglutarate amino acids (Glu, Gln, Arg and His), aromatic amino acids (Phe , Tyr, and Trp), branched amino acids (Ile and Leu), aspartate amino acid group (Thr, Lys and Met), Asn and Ala. 2-methylbutanoic acid was correlated with Tyr, Met, Val, Trp, Phe, Leu in field results. However, with regard to the glasshouse results, 2-methylbutanoic acid and isovaleric acid are negatively related to His, Thr, Arg, Ala and Ser, Thr, Arg, Ala, Tyr, Phe and Leu, respectively.

(2) Straight chain fatty acids

There are significant correlations between hexanoic acid and Glu, Gln, Thr, Ser, Ala, Tyr, Met, Phe, Trp, Ile, Val, Leu and close relationships between octanoic acid and Ser, Thr, Arg, Ala, Tyr, Met, Phe, Leu in the present glasshouse study (Table 3.11&3.12).

(3) Acetic acid

In this study (Table 3.11&3.12), acetic acid was significantly correlated with Glu, Ser, Gln, His, Val, Met, Trp, Ile, Leu (glasshouse trail) and Asp, Glu, Asn, Gln, His, Ala, Tyr, Val, Met, Trp, Phe, Ile, Leu (field trial).

3.4.2.3 Esters

(1) Acetate esters

With regards to acetate esters and their structurally corresponding amino acids, a significant positive relationship between Thr and ethyl acetate was observed in the present study. Moreover, isoamyl acetate significantly related ($p<0.05$) with Leu in glasshouse results. However, no correlation between phenylethyl and Phe exists in the present study.

There was some correlation between amino acids consumption and acetate esters in the glasshouse study. Ethyl acetate was positively correlated with alpha-ketoglutarate amino acids (Glu and Gln), aromatic amino acids (Phe, Tyr and Trp), branched amino acids (Ile, Leu, and Val), Ser, Ala, and Met. Isoamyl acetate was related to the concentrations of consumed aspartate amino acids (Thr, Asp, and Met), alpha-ketoglutarate amino acids (Glu, Gln, Arg and His), Ser, Ala, Phe and Val. In the case of phenylethyl acetate, a positive correlation was found between its formation and the concentrations of consumed Glu, Gln, Ser, Met, Ile and Leu.

(2) Ethyl esters of fatty acids

In the present study (Table 3.13 & 3.14), medium chain fatty acids: ethyl octanoate was related to Ser, Gln, Tyr, Met, Phe, Ile, and Leu in glasshouse data, which was similar to what was found for octanoic acids; while ethyl hexanoate were correlated with Glu, Ser, Gln, Thr, Ala, Tyr, Val, Met, Trp, Phe, Ile and Leu, which was the same as found with hexanoic acid.

Esters derived from short chain fatty acids, ethyl butanoate and ethyl pentanoate were positively correlated with several similar amino acids, such as Glu, Asn, Gln, His, Tyr, and Phe (field results).

As for branched-chain esters, a significant correlation ($p<0.05$) between Val and ethyl isobutyrate was found in the field results, but this relationship was not found in glasshouse study, where ethyl isobutyrate correlated only with Asp. A correlation between Leu and ethyl isovalerate was observed in this study. Furthermore, branched chain esters were related with other individual amino acids, such as Asp, Glu, Ser, Gln, Thr, Arg, Ala, Tyr, Trp.

3.4.2.4 Grape-derived compounds

In this study (Table 3.15 & 3.16), β-damascenone was correlated with Glu, Gln, Trp, Phe, Ser, Met, Val, Ile and Leu. However, linalool was negatively correlated with Ser,

Thr, Ala and Tyr in the glasshouse study. A negative correlation between Asn and α-Ionone can be found in field results, while Lys was negatively related with linalool. With regards to other grape-derived aroma compounds, no correlation was found between them and amino acids consumption.

Table 3. 9 The correlations between the concentrations of higher alcohols and the consumption of individual amino acids (glasshouse result)

	Asp	Glu	Asn	Ser	Gln	His	Gly	Thr	Arg	Ala	Tyr	Val	Met	Trp	Phe	Ile	Lys	Leu
Phenylethyl alcohol	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Isoamyl alcohol	0.828*	n.s	n.s	0.931**	n.s	n.s	n.s	0.953**	0.867*	0.962**	0.984**	n.s	n.s	n.s	0.913*	n.s	n.s	n.s

Table 3. 10 The correlation between the concentrations of higher alcohols and the consumptions of individual amino acids (field result)

	Asp	Glu	Asn	Ser	Gln	His	Gly	Thr	Arg	Ala	Tyr	Val	Met	Trp	Phe	Ile	Lys	Leu
Phenylethyl alcohol	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Isoamyl alcohol	n.s	n.s	-0.328	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

Table 3. 11 The correlation between the concentrations of acids and the consumption of individual amino acids (glasshouse result)

	Arg	Glu	Gln	His	Phe	Tyr	Trp	Leu	Val	Ile	Asp	Thr	Met	Lys	Ser	Gly	Asn	Ala
Acetic acid	n.s	.902*	.952**	.879*	n.s	n.s	.902*	.944**	.948**	.911*	n.s	n.s	.825*	n.s	.990*	n.s	n.s	n.s
Isobutyric acid	-.825*	n.s	n.s	n.s	-.902*	-.928**	n.s	-.827*	-.835*	n.s	n.s	-.932**	n.s	n.s	-.927*	n.s	n.s	-.956**
Butanoic acid	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	-.887*	n.s	-.943**	n.s	n.s
Isovaleric acid	n.s	-.911*	.840*	n.s	n.s	.814*	.860*	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
2-Methybutanoic acid	-.903*	n.s	n.s	-.937**	n.s	n.s	n.s	n.s	n.s	n.s	n.s	-.852*	n.s	n.s	n.s	n.s	n.s	-.845*
Hexanoic acid	n.s	.907*	.937**	n.s	.911*	.928**	.880*	.935**	.861*	.888*	n.s	.843*	.936**	n.s	.933**	n.s	n.s	.865*
Octanoic acid	.816*	n.s	n.s	n.s	.953**	.945**	n.s	.848*	n.s	n.s	n.s	.904*	.848*	n.s	.913*	n.s	n.s	.943**

Table 3. 12 The correlation between the concentrations of acids and the consumptions of individual amino acids (field result)

	Arg	Glu	Gln	His	Phe	Tyr	Trp	Leu	Val	Ile	Asp	Thr	Met	Lys	Ser	Gly	Asn	Ala
Acetic acid	n.s	.488*	.559**	.555*	.505*	.582**	.523**	.496*	.532**	.502*	.558**	n.s	.491*	n.s	n.s	n.s	.534*	.421*
Isobutyric acid	.422*	.493*	.523**	.521**	.563**	.615**	.601**	.564**	.545**	.546**	n.s	.477	.561**	.545**	n.s	n.s	.564**	.421*
Butanoic acid	n.s	.497*	.677**	.566**	.691**	.690**	.669**	.609**	.628**	.639**	.520**	.466*	.602**	n.s	n.s	n.s	.615**	.429*
Isovaleric acid	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
2-Methybutanoic acid	n.s	n.s	n.s	n.s	.428*	.447*	.498*	.429*	.405*	.438*	n.s	n.s	.432*	n.s	n.s	n.s	n.s	n.s
Hexanoic acid	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Octanoic acid	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed)

	Arg	Glu	Gln	His	Phe	Tyr	Trp	Leu	Val	Ile	Asp	Thr	Met	Lys	Ser	Gly	Asn	Ala
Ethyl isobutyrate	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	.881*	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Ethyl butanoate	n.s	.955**	.941**	n.s	.864*	.927**	.906*	.925*	.853*	.855*	n.s	.885*	.871*	n.s	.965**	n.s	n.s	.880*
Ethyl isovalerate	n.s	.900*	.857*	n.s	n.s	.898*	.877*	.826*	n.s	n.s	.873*	.880*	n.s	n.s	.935**	n.s	n.s	.855*
Isoamyl acetate	.837*	.898*	.881*	.817*	.899*	.942**	n.s	.904*	.846*	n.s	.835*	.968**	.823*	n.s	.994**	n.s	n.s	.957**
Ethyl pentanoate	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Ethyl hexanoate	n.s	n.s	.897*	n.s	.886*	.941**	.875*	.905*	.880*	.841*	n.s	.887*	.864*	n.s	.952**	n.s	n.s	.904*
Ethyl heptanoate	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Ethyl octanoate	n.s	n.s	.862*	n.s	.907*	.855*	n.s	.880*	n.s	.857*	n.s	n.s	.953**	n.s	.822*	n.s	n.s	n.s
Ethyl decanoate	n.s	.854*	.886*	n.s	.880*	.850*	.800	.879*	n.s	.868*	n.s	n.s	.954**	n.s	.815*	n.s	n.s	n.s
Ethyl acetate	n.s	.941**	.926**	n.s	.896*	.931**	.859*	.933**	.863*	.828*	n.s	.933**	.867*	n.s	.988*	n.s	n.s	.924**
2-phenylethyl acetate	n.s	.969**	.911*	n.s	n.s	n.s	n.s	.884*	n.s	.833*	n.s	n.s	.844*	n.s	.822*	n.s	n.s	n.s

Table 3. 5 The correlation between the concentrations of esters and the consumption of individual amino acids (glasshouse result)

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

Table 3. 6 The correlation between the concentrations of esters and the consumption of individual amino acids (field result)

	Arg	Glu	Gln	His	Phe	Tyr	Trp	Leu	Val	Ile	Asp	Thr	Met	Lys	Ser	Gly	Asn	Ala
Ethyl isobutyrate	n.s	n.s	.514*	n.s	.489*	.489*	.506*	.411*	.426*	.446*	n.s	n.s	.423*	n.s	n.s	n.s	n.s	n.s
Ethyl butanoate	n.s	.465*	.475*	.486*	.438*	.424*	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	.430*	n.s	.499*	.466*
Ethyl isovalerate	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Isoamyl acetate	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Ethyl pentanoate	n.s	.455*	.580**	.562**	.523**	.522**	.490*	.436*	.477*	.450*	.491*	n.s	.435*	n.s	n.s	n.s	.537**	n.s
Ethyl hexanoate	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	.426*	n.s
Ethyl heptanoate	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Ethyl octanoate	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Ethyl decanoate	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Ethyl acetate	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
2-phenylethyl acetate	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

Table 3. 7 The correlation between the concentrations of grape-derived aroma compounds and the consumption of individual amino acids (glasshouse result)

	Arg	Glu	Gln	His	Phe	Tyr	Trp	Leu	Val	Ile	Asp	Thr	Met	Lys	Ser	Gly	Asn	Ala
Linalool	n.s	n.s	n.s	n.s	n.s	-.927**	n.s	n.s	n.s	n.s	-.875*	-.876*	n.s	n.s	-.865*	n.s	n.s	-.882*
Citronellol	n.s	.848*	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
2-Phenylethyl acetate	n.s	.969**	.911*	n.s	n.s	n.s	n.s	.884*	n.s	.833*	n.s	n.s	.844*	n.s	.822*	n.s	n.s	n.s
β-Damascenone	n.s	.989**	.952**	n.s	.816*	n.s	.849*	.932*	.814*	.878*	n.s	n.s	.900*	n.s	.884*	n.s	n.s	n.s
Geraniol	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	.881*	n.s	n.s	n.s	n.s
Ethyl hydroxycinnamate	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
α-Ionone	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Ethyl cinnamate	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s

Table 3. 8 The correlation between the concentrations of grape-derived aroma compounds and the consumption of individual amino acids (field result)

	Arg	Glu	Gln	His	Phe	Tyr	Trp	Leu	Val	Ile	Asp	Thr	Met	Lys	Ser	Gly	Asn	Ala
Linalool	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	-.458*	n.s	n.s	n.s	n.s
Citronellol	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
2-Phenylethyl acetate	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
β-Damascenone	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Geraniol	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Ethyl hydroxycinnamate	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
α-Ionone	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	-.443*	n.s
Ethyl cinnamate	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed)

3.5 PCA results

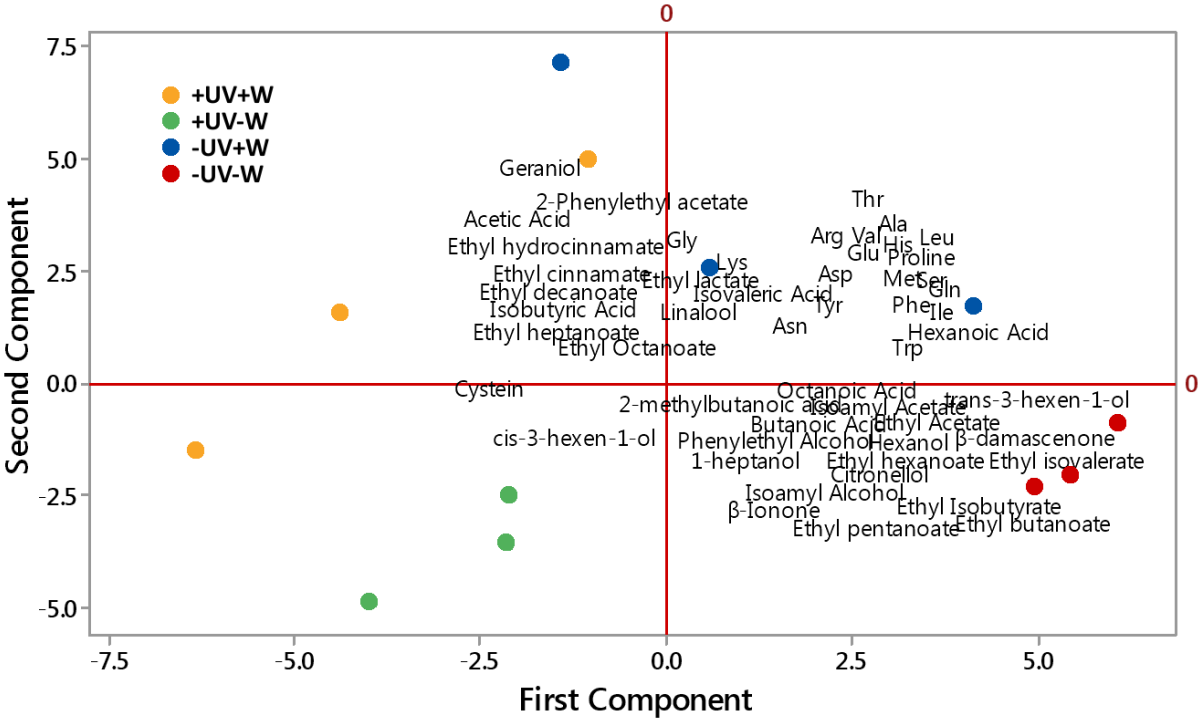

Fig 3. 8 Principal components analysis of wine aroma compounds and amino acids.

Figure 3.4 shows the results of the PCA. There were four groups, separated by treatment corresponding to: +UV+W, +UV-W, -UV+W, and -UV-W. The parameters chosen for PCA included trans-3-hexenol, cis-3-hexenol, hexanol, 1-heptanol, 3-methylbutanol, 2-phenylethanol, ethyl acetate, ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl-3-methylbutanoate, ethyl isobutyrate, ethyl pentanoate, ethyl lactate, ethyl heptanoate, isoamyl acetate, linalool, β-damascenone, geraniol, ethyl hydrocinnamate, β-ionone and ethyl cinnamate, acetic acid, butanoic acid, 3-methylbutanoic acid, 2-methylbutanoic acid, hexanoic acid and octanoic acid. Clear separation is evident, with +UV-W located in the lower left, –UV-W in the lower right quadrant and +UV+W and -UV+W being in the upper left and right quadrants, respectively.

The First Component was positively correlated with hexanoic acid, butanoic acid, isovaleric acid, octanoic acid, 2-methylbutanoic acid, isoamyl acetate, ethyl acetate, ethyl hexanoate, isoamyl alcohol, phenylethyl alcohol, hexanol, 1-heptanol, trans-3-hexen-1-ol, ethyl isovalerate, ethyl isobutyrate, ethyl pentanoate, ethyl

octanoate, ethyl butanoate, linalool, citronellol, β-damascenone, β-Ionone, Asp, Glu, Asn, Ser, Gln, His, Gly, Thr, Arg, Ala, Tyr, Val, Met, Trp, Phe, Ile, Lys, whereas this component was negatively related with ethyl cinnamate, ethyl hydrocinnamate, geraniol, ethyl decanoate, ethyl octanoate and ethyl heptanoate, phenylethyl acetate, cis-3-hexen-1-ol isobutyric acid, acetic acid.

The Second Component was negatively correlated with Cys, cis-3-hexen-1-ol, butanoic acid, octanoic acid, 2-methylbutanoic acid, ethyl pentanoate, ethyl isobutyrate, ethyl isovalerate, ethyl acetate, ethyl butanoate, ethyl hexanoate, 1-heptanol, hexanol, isoamyl acetate, isoamyl alcohol, phenylethyl alcohol, trans-3-hexen-1-ol, citronellol, β-damascenone, β-Ionone and positive with almost all amino acids, ethyl octanate, ethyl cinnamate, ethyl hydrocinnamate, geraniol, phenylethyl acetate, linalool, hexanoic acid, isobutyric acid, acetic acid, ethyl heptanoate and ethyl acetate.

3.6 Conclusion

To conclude, the concentrations of amino acids in wine and the consumption of amino acids during fermentation were affected by UV treatment. The data reveal that the reductions in amino acids could result from UV radiation. Total and most individual amino acids are decreased by UV-B.

The formation of aroma compounds in wine was affected by the change of grape amino acids induced by UV-B or water treatments. The +UV treatment decreased phenylethyl alcohol, hexanoic acid, ethyl acetate, ethyl isobutyrate, ethyl butanoate, ethyl isovalerate, ethyl hexanoate, ethyl decanoate and isoamyl acetate. In addition some grape-derived volatile compounds, such as terpene alcohols and C_{13}-norisoprenoids were affected by UV-B exposure.

Water deficit treatment had effects on few wine aroma compounds. Deficit irrigation increased isoamyl alcohol and phenylethyl alcohol, butanoic acid, ethyl acetate, and some ethyl esters of fatty acids, but decreased acetic acid.

There are some significant correlations between the concentrations of aroma

compounds and their structurally related amino acids, such as branched amino acids and Thr, could be implicated in the formation of some specific aroma compounds (e.g. branched-chain fatty acids, acetate esters, branched-chain esters). However, isoamyl alcohols and phenylethyl alcohol were not related to Leu and Phe respectively. Arg, Glu, Ser, Gln, Thr, Arg, Ala, Tyr and Phe are more closely related to the fermentation products than other amino acids. Correlations between β-damascenone and some individual amino acids were found in this study as well.

The PCA analysis shows that wines from -UV-W treatment grapes tended to have a more diverse set of aroma compounds, such as ethyl esters, higher alcohols and fatty acids compared with other treatments. Furthermore, UV-B radiation and water deficit had interactive effects on wine aromas. The highest level of aroma compounds can be found in -UV-W treatment, while the lowest value was found in +UV+W. On the other hand, the effects of -UV are opposite to the effects of +W, so regular irrigation could weaken the promotional effects of UV-B treatment. Similar results were obtained from +UV-W and -UV+W treatments in some aroma compounds.

Chapter 4 Discussion

4.1 Introduction

Amino acids concentration in grapes can be affected by environmental stress, UV-B radiation and water deficit, via nitrogen and secondary metabolisms. Amino acid availability can regulate yeast growth and metabolism because a large quantity of amino acids can be consumed by yeast during alcoholic fermentation (Arias-Gil et al., 2007) and they act as precursors of aroma compounds (Bell & Henschke, 2005).

Fermentation-derived volatile by-products, such as higher alcohols, fatty acids and esters are major contributors to wine aromas, and all of them can be formed during yeast fermentation (Bell et al., 2005; Styger et al., 2011). Amino acid consumption is a key factor for yeast fermentation and aroma compounds formation. Therefore, the variation in grape amino acids profiles by UV and water treatments can result in the different wine aroma compounds, and therefore these effects are worthy of research.

Monoterpenes and C13-norisoprenoids contribute to a number of characteristic varietal aromas. Their concentrations can be affected by UV-B. Moreover, C_{13}-norisoprenoids can accumulate though the transformation of the aglycone from glycosidically-bound precursors, a process that is affected by yeast enzymes (Hernandez-Orte et al., 2008). Due to amino acids playing an important role in yeast metabolism during fermentation (Ugliana & Henschke, 2009), the concentration of C_{13}-norisoprenoids in wine might be related to some amino acids.

The concentrations of aroma compounds in wines were analyzed to understand the effects of UV-B and water deficit on the concentrations of grape amino acids and the formation of wine aroma compounds.

The relationship between amino acids consumption and wine aroma compounds lacks a clear understanding. Amino acid consumption is a key factor for yeast fermentation and aroma compounds formation. For the present research, the starting materials of this study had differing amounts of amino acids, rather than the

concentrations being changed by addition into must, so measuring amino acids consumption may more accurately reflect the relationship between grape amino acids and wine aroma compounds.

4.2 General wine characteristics

Wine TA nor pH was not significantly affected by UV-B exposure or water deficit. There were significant differences between LR, SC and PETG in wine TA. Similar with Maya's results, shading significantly increased wine TA compared with leaf removal. Previous studies have indicated leaf removal reduced grape TA and wine TA, mainly due to altering canopy microclimate (Kozina et al., 2008; Qin, 2015). This might be because of the different environmental UV-B intensity and other micro-climate conditions in the canopy, for example, berry temperatures can be directly increased by light exposure. Bergqvist et al. (2001) also reported different results of grape Brix, TA and pH from those sampled from the north side vs. south side of vine canopies with different berry temperature. Higher temperature led to higher concentrations of soluble solids and pH, but lower TA. This could be because temperature was a more important factor affecting grape compositions than UV-B.

4.3 Amino acids were affected by UV-B and water deficit

UV-B exposure significantly decreased the concentrations of total amino acids and some individual amino acids. These results agree with Martinez-Luscher et al. (2014), where grapevines were grown in a controlled environment under different UV-B doses. There, they reported that UV-B radiation reduced the concentrations of Thr (34%), Met (40%), Ile (40%), Ser (52%) and Gly (83%) in berries. Nitrate and nitrite uptake rates and the activities of nitrogen assimilating enzymes were influenced by UV-B radiation though changes to nitrogen metabolism (Singh et al., 2012). However, there was no significant difference observed in the individual amino acids (except Lys) or the total amino acids between treatments in field wines. These results are similar to Gregan et al. (2012), who found there was no significant effect of UV-B exclusion treatment compared to UV-B transmission treatment. Gregan et al. (2012) and Keller and Torres-Martinez (2004) found UV-B had no effects on total amino acids in grape

berries. This may due to different cultivars being used, which may have different responses to UV-B radiation. Moreover, different treatment methods may lead to these varied results. Gregan et al. (2012) established UV treatments in the fruit zone rather than whole grapevines, and amino acids are produced in all leaves, such as those that are above the fruit zone, which were not included in the UV treatment in the present study. This may also explain why different results were obtained from glasshouse and field trials.

Water deficit treatment significantly increased Asp, Glu, Asn, and Ser. This agrees with Cramer et al. (2013), who indicated that the concentrations of Glu and Asp in grape were increased by drought. This is because the key enzyme activities of the TCA cycle involved in Glu and Asp formation could be increased by water deficit.

The concentrations of total and individual amino acids in wines were lower than that in grapes. The proline to arginine ratios of wine were higher than that in grape, and it can be concluded that proline was the most abundant wine amino acid. There is a direct relationship between the amino acid concentrations of final wine and that of the initial grape material for the glasshouse experiment, which has also been reported in the literature. Arias-Gil et al. (2007) altered must amino acids content via addition of proteic amino acids and found the concentration of wine amino acids were directly proportional to the concentrations of must amino acids (R^2=0.999). However, a weaker correlation was found in this study, which was highly influenced by three low AA points. All three points were similar in wine concentrations, but significantly different in grape concentrations. This might because that wine nitrogen was not increased with must amino acids when the yeast assimilable nitrogen levels are similar in wine, independently of must amino acid concentration (Arias-Gil et al., 2007).

4.4 Wine aroma compounds were affected by UV-B and water deficit

4.4.1 Terpenes

Monoterpenes exist in free and odorless glycosidically bound forms in berries. During

fermentation yeast can release bound forms into odour-contributing forms in wines (Styger et al., 2011).

UV-B exposure increased linalool concentration and decreased citronellol in the present study. This may be because the linalool synthesis enzyme is sensitive to sunlight, and the accumulation or biosynthesis of bound forms of terpenes can be mediated by exposure to the sun (Reynolds and Wardle, 1989), with linalool being one specifically affected (Skinkis et al., 2010). Linalool can be transformed into geraniol, nerol and further into citronellol through acid-catalyzed rearrangements (Fang & Qian, 2006). Song et al. (2015) found UV transmitting treatments significantly increased the concentrations of nerol (37%) and geraniol (17%) compared to UV exclusion treatments. This might be due to linalool being increased by UV and then transformed into geraniol and nerol.

No significant differences can be found in the present field treatment, but Song et al. (2015), who used a similar micro-ferment system, indicated that sunlight exposure in the field increased the concentrations of citronellol, nerol and geraniol in wine. These different results may be due to terpene concentrations changing during grape maturation. In the Song et al. (2015) study, there were significant differences in Brix between treatments, but only slight differences are found in this trial. It has been suggested that the levels of terpene alcohols increase with berry maturity. Terpenes are generated from carotenoids (lutein and β-carotene), and their concentration gradually increases during berry ripening (Fang and Qian, 2006; Camara et al., 2004; Wilson et al., 1984). Therefore, there may have been insufficient ripening time in this work for treatment differences to become apparent.

The concentrations of linalool, geraniol and citronellol were affected by water deficit. However, Qian et al. (2009) reported water deficit did not have effects on terpenes. McCarthy and Coombe (1984) found the accumulation rate of bound terpenes in Riesling grapes was reduced by irrigation. This fact could be related to abundant water supply increasing growth and yield, resulting in a delay in ripening, while water deficit generally has positive effects on grape ripening and phenolic compound accumulation (Ribereau-Gayon et al., 2006).

4.4.2 C13-norisoprenoids

UV treatment did not influence the concentration of β-ionone, but did decrease the content of β-damascenone. This could be due to decreasing berry maturity caused by UV-B, as the Brix of grape berry was decreased by UV treatment (Table 3.1). It was reported that higher maturity levels lead to higher levels of bound terpenols and C_{13}-norisoprenoids (Ribereau-Gayon et al., 2006). However, UV radiation was reported to increase β-damascenone in Riesling wine (Lafontaine et al., 2004). Unlike results from the glasshouse experiment, the concentration of β-damascenone in the shade treatment was 16% lower compared with leaf removal and PETG. Norisoprenoids are produced from carotenoid precursors, and the formation of norisoprenoid can be affected by sunlight (Oliveira et al., 2004). When leaves were removed, sun exposure has been found to promote the formation of C_{13}-norisoprenoids in grape berries during ripening, while leaf layer number also strongly affects norisoprenoid concentrations, independent of different levels of sunlight exposure (Lee et al., 2007). Thus the influences of temperature and microclimate on norisoprenoid synthesis or degradation in this experiment cannot be excluded. Moreover, Hernandez-Orte et al. (2008) indicated yeast enzymes could affect the formation of C_{13}-norisoprenoids during fermentation. Although the relationship between β-damascenone and amino acids can be seen in present study, the reduction in β-damascenone might be explained in that the amino acids level of must were decreased by UV-B, possibly resulting in an indirect effect on β-damascenone formation.

Water deficits increased both β-damascenone and β-ionone. The literature suggests water deficit affects the degradation of carotenoid contents of grapes, thereby possibly increasing C_{13}-norisoprenoids (Oliveira et al., 2003). This is important because a higher concentration of β-damascenone could contribute towards the sensory perception of floral, sweet and cooked apple (Song et al., 2015) in Pinot noir wines. In addition, the highest concentration of β-damascenone can be found in UV-W- treatment and the lowest level in UV+W+. This might due to reduced irrigation strengthening the promotional influence of UV exclusion on β-damascenone accumulation.

4.4.3 Alcohols

The reduction in phenylethyl alcohol concentration caused by UV-B may be a result of decreased concentrations of amino acids because amino acids act as nitrogen sources for yeast growth (Styger et al., 2011). Phenylethyl alcohol was correlated with amino acids in general, which could support this suggestion (see 4.6.1). However, Song et al. (2015) indicated that UV treatment significantly increased phenylethyl alcohol. Higher alcohols are produced from amino acids by the Ehrlich pathway or from sugar by glycolysis during alcoholic fermentation (Ugliano & Henschke, 2009; Styger et al., 2011), but there was no correlation between higher alcohols and their related amino acids in present study, suggesting the Ehrlich pathway is not the main pathway for higher alcohol biosynthesis. It is possible that more higher alcohols are produced from glycolysis via a biosynthesis pathway (Ugliana & Henschke, 2009). The level of isoamyl alcohol, however, was not affected by UV treatment, which is in agreement with one previous study (Song et al., 2015).

Water deficit increased the concentrations of isoamyl alcohol and phenylethyl alcohol. The level of irrigation may have effects on the amount of the amino acids involved into biosynthesis of isoamyl and phenylethyl alcohol. Moreover, limiting irrigation could increase the must nitrogen content, which will then affect yeast fermentation (Berdeja et al., 2014). Moreover, if must nitrogen is low, surplus alpha-keto acids can only be excreted as higher alcohols due to lack of available nitrogen (Ugliana & Henschke, 2009).

Higher alcohols are regarded as a positive factor for the desirable complexity of wine, when at concentrations below 300mg/l (Rapp and Mandery, 1986). At concentrations above 400mg/l, however, the fusel alcohols can contribute negative aroma to wine (Garde-Cerdan and Ancin-Azpilicueta, 2008).

4.4.4 Fatty acids

UV-B treatment decreased the concentration of hexanoic acid (a straight-chain fatty acid). Song et al. (2015) reported the content of octanoic acid and decanoic acids in wines were decreased by UV+ treatment. Although it has been reported that

straight-chain fatty acids were affected by the availability of unsaturated fatty acids and fermentation conditions, it was suggested must nitrogen content could affect fatty acids metabolism as well (Torija et al., 2003). Wang et al. (2016) have observed that the formation of medium chain fatty acids was increased by addition of branched amino acids and Phe in must (Wang et al., 2016). In the present study, there were some significant correlations between medium chain fatty acids and several individual amino acids, which could suggest amino acids have effects on helping yeast cells to adapt their membrane fatty acids and increase yeast growth and glycolysis rate, resulting in greater medium chain fatty acid formation (Bely et al., 1990; Torija et al., 2003). Reduced irrigation increased the level of hexanoic acids, which might be because water deficit could increase the amino acids level in the must, in turn affecting the formation of this medium chain fatty acid.

Previous studies have found that the concentration of acetic acid was increased with berry maturity (Houtman et al., 1980). But in this study, the higher grape maturity (Brix) caused by reduced irrigation corresponded to a lower level of acetic acid, which consistent with Qin (2015).

Fatty acids can contribute a fresh flavour to wine at moderate concentrations, and fatty acids are associated with fatty, cheese, rancid and fresh notes when their level is above 20 mg/l. They contribute to the complexity of wine aroma when at low concentrations (Lambrechts & Pretorius, 2000; Ribereau-Gayon et al., 2006).

4.4.5 Esters

The UV-B treatment affected wine ester concentrations. Isoamyl acetate was decreased by UV-B exposure, which was consistent with Wang et al. (2016) who suggested that acetate esters were increased by addition of branched amino acids and Phe. This might have been because UV-B decreased the level of amino acids (including Leu) involved in acetate ester formation (as found in the relationship between Leu & isoamyl acetate), which indicated Leu might involved in isoamyl acetate formation (Styger et al., 2011). Moreover, although the concentration of ethyl acetate was decreased by UV-B radiation, Song et al. (2015) reported no effects

of UV-B on this ester. The reduction of ethyl acetate might be due to decreased general amino acid concentration caused by UV-B. Thr is the corresponding amino acid of ethyl acetate, and these two were related in the present study. That the total concentration of amino acids able to be consumed will generally affect yeast fermentation supports the relationship found between isoamyl acetate, ethyl acetate and general amino acid concentrations. Yoshimoto et al. (1998) indicated that nitrogen availability could affect expression of the ATF1 gene (encoding alcohol acetyltransferase enzymes), which in turn could affect acetate ester formation. These acetic esters of higher alcohols contribute to fruity notes (banana, acid drops and apple) and aromatic complexity of wines (Simpson, 1979; Song et al., 2014).

UV exposure decreased ethyl esters of straight-chain fatty acids, which is in accordance with the results of Song et al. (2015). UV exposure also significantly reduced the concentration of ethyl butanoate, ethyl hexanoate, and ethyl decanoate. This decrease in ethyl esters could be supported by the reduction of straight chain fatty acids induced by UV-B exposure. This is consistent with the close relationship between medium chain fatty acids and ethyl esters of medium chain fatty acids found in the present study. Ethyl esters of branched-chain fatty acids, such as ethyl isobutyrate and ethyl isovalerate, were decreased by UV+ treatment. Song et al. (2015) also observed a decrease in ethyl esters of branched-chain fatty acids, which could be related to UV-B exposure affect the amino acids or amino acids derivatives which involved in their formation. These ethyl esters of fatty acid acetates can be important because they contribute global “fruity” aroma and complexity to wine (Perestrelo et al., 2006).

The aromatic esters, ethyl cinnamate and ethyl hydroxycinnamate, were not affected by UV radiation. Song et al. (2015) found the concentration of phenylethyl acetate stayed constant under UV treatments, but the concentration of ethyl cinnamate was decreased (26%) by UV+ treatment. These ethyl esters of fatty acids acetates contribute global “fruity” aroma and complexity to wine (Perestrelo et al., 2006).

The concentration of isoamyl acetate was increased by water deficit, which was consistent with the trend of isoamyl alcohol. Ethyl esters of straight chain fatty acids

were affected by water deficit. The concentrations of ethyl acetate, ethyl butanoate, ethyl hexanoate, ethyl decanoate, ethyl pentanoate were increased by reduced irrigation. Ethyl esters of branched fatty acids, such as ethyl isobutyrate, ethyl isovalerate, were increased by limited irrigation as well. Most likely, the increase in esters could, again, be supported by the increase of amino acids induced by water deficit: the amino acids which are involved in the metabolism of branched fatty acids could be increased under those conditions. In addition, reduced irrigation significantly decreased the concentration of phenylethyl acetate and ethyl cinnamate. Deficit irrigation may promote the synthesis of volatile compounds and their precursors in the grapes, which, combines with reduced vine vigor and vegetative growth that promotes berry ripening. In addition, drought may have other effects on grape maturity, leading to variations of a range of aroma compounds in wines (Qian et al., 2009). Water deficit may also interact with UV-B exclusion to cause an increase in the concentrations of isoamyl and ethyl acetate, ethyl octanoate and ethyl hexanoate. Their concentrations were highest in -UV-W and lowest in +UV+W treatments. On the other hand, the effects of water deficit are opposite to the effects of UV-B exclusion, leading to similar results being obtained from +UV-W and -UV+W treatments.

For the field experiment, there is no significant difference in most esters, alcohols and fatty acids in wine as a result of leaf removal, shade cloth or PETG treatments. That may due to no significant differences being found between wine amino acids with these three treatments. Similar results were reported by Song et al. (2015), where sunlight exposure did not significantly affect the concentrations of acetates of higher alcohol and fatty acid ethyl ester, and did not significantly influence fusel alcohols, 2-phenylethanol and fatty acid alcohols (except hexan-1-ol) either. Conversely, they found octanoic and decanoic acids could be decreased by sun exposure. However, the straight-chain fatty acids in wine largely depend on the unsaturated fatty acids in the grapes and fermentation conditions (Lambrechts & Pretorius, 2000). However, unlike in the glasshouse, the content of phenylethyl acetate and ethyl cinnamate were increased by leaf removal compared to PETG treatments. UV-B radiation might have increased these acetate concentrations in the

field experiment wines.

These aroma compounds play an important role in the sensory profiles of Pinot noir wine. They exhibit distinct red fruity aromas, especially the notes of stone fruits (plum and cherry) (Fang & Qian, 2006). The most important aroma compounds in Pinot noir wine are higher alcohols, fatty acids, ethyl esters and acetates, methionol, benzaldehyde, phenylethyl alcohol, linalool and damascenone (Fang & Qian, 2005). Higher alcohols are regarded as a positive factor of the desirable complexity of wine at concentrations below 300mg/l (Rapp and Mandery, 1986). Isoamyl alcohols contribute fusel notes to wine, and hexanol contributes herbaceous notes (Song et al., 2014). Fatty acids produce fresh notes when they are at a high level (20 mg/l), while they contribute to the complexity of wine aroma when they are at low concentrations (Lambrechts & Pretorius, 2000). Esters are important wine sensory contributors and largely responsible for the fruity and floral notes of Pinot noir wines (Fang & Qian, 2006). The acetate esters of higher alcohols contribute the unusual odours of banana, acid drops and apple, and contribute aroma complexity as well (Simpson, 1979). The esters of fatty acids, such as ethyl hexanoate, octanoate, and decanoate, are also described as fruity and floral (Garde-Cerdan and Ancin-Azpilicueta 2008). As a result, the higher consumption of amino acids, brought about by increased amounts in the grape material, could increase the fruity, fusel, floral, and vegetal notes and complexity of wine aroma.

4.5 Amino acid consumption

During fermentation, yeast utilize a great quantity of amino acids. The consumption of amino acids in this study was significantly and positively correlated with the concentration of initial grape amino acids for both the glasshouse and field experiments. This result was consistent with previous findings (Arias-Gil et al., 2007).

The consumption of alpha-ketoglutarate amino acids (Arg, Glu, Gln and His), branched amino acids (Leu, Val and Ile), Phe, aspartate amino acids (Asp, Thr, Met), 3-phosphoglycerate (Ser and Cys), Ala, and their total in -UV exposure treatments was greater than in the +UV treatments (Table 3.5). Arg, Ala, Asp, Glu, Thr and Ser

were consumed in a high proportion, likely because these forms of amino acids are preferred nitrogen sources for wine yeasts (Henschke & Jiranek, 1993). The aromatic amino acid Trp was also totally consumed, independently of the amount of must amino acids, a result consistent with those of Arias-Gil et al. (2007). Most of the branched amino acids, Val and Leu, were consumed during alcoholic fermentation. Interestingly, Cys consumption was negative, which means its concentration increased, not decreased, during fermentation. This could be due to Cys being synthesized and excreted by yeast throughout fermentation (Henschke & Jiranek, 1993). If the concentrations of Cys in grape must are not sufficient for yeast cell growth, the sulfate reduction sequence (SRS) pathway can be activated to meet this demand. If the nitrogen supply is sufficient/suitable, this pathway can transfer HS- to form methionine and cysteine (Henschke & Jiranek, 1993).

As for the field experiment, no significant differences were found in the concentrations of consumed amino acids between treatments. The consumed percentage of Arg, Ala, Asp, Glu, Ser, Gln, Thr, and Met were high during fermentation, because these are also regarded as good nitrogen sources for yeast (Henschke & Jiranek, 1993). The branched amino acids (Val, Ile and Leu) and the aromatic amino acids (Phe, Trp and His) showed high consumed percentages, which consistent with Arias-Gil et al. (2007).

4.6 Correlations between amino acid consumption and wine aroma compounds

The discussion starts with those amino acids and aroma compounds where there has been a link already described in the literature. This is followed by the relationships between other aroma compounds and amino acids where a link is not so obvious.

4.6.1 Higher alcohols

In present study, there was no relationship between higher alcohols and their structurally corresponding amino acids, but there was a relationship to other individual amino acids.

It was reported that there was a relationship between the addition of Phe prior to fermentation and the appearance of phenylethyl alcohol in wine (Wang et al., 2016), most likely via the Ehrlich pathway (Styger et al., 2011). However, a correlation between Phe and phenylethyl alcohol was not observed in the present study.

Similarly, Leu is regarded as a precursor of isoamyl alcohol (Styger et al., 2011). However, no correlation between Leu and isoamyl alcohol was found in the present study.

These results suggest that the Ehrlich pathway is not the main pathway for higher alcohol biosynthesis. Higher alcohols can also be produced from glycolysis via a biosynthesis pathway (Ugliana & Henschke, 2009). When amino acids are insufficient for yeast growth, the synthesis of alpha-keto acids from glycolysis will be induced. These alpha-keto acids can act as the intermediates in amino acid metabolism (Chen 1978), and surplus alpha-keto acids could be converted into higher alcohols (Ugliana & Henschke, 2009). Higher alcohols synthesized from the biosynthesis pathway account for greater proportion of all higher alcohols during fermentation (Chen, 1978). As a result, the correlations between the consumption of structurally related amino acids and the concentrations of corresponding higher alcohols are not generally found (Ugliana & Henschke, 2009).

However, there are some significant and positive correlations between isoamyl alcohols and the consumption of Asp, Ser, Thr, Arg, Ala, Tyr and Phe, suggesting that these amino acids have effects on isoamyl alcohol production. Asp and Ala are involved in aminotransferase activities, which could catabolize amino acids into alpha-keto acids which could go further to produce higher alcohols (Ardö, 2006). According to Hernandez-Orte et al. (2002), the concentration of must Thr can also affect the proportion of isoamyl alcohol. Moreover, higher alcohol formation was strongly affected by assimilable nitrogen content and composition of the must (Ugliano & Henschke, 2009), because nitrogen promotes biomass production and increases the rate of glycolysis (Arias-Gil et al., 2007). Asp, Arg, Thr, Ala, Tyr are regarded as the preferred nitrogen source for yeast metabolism (Henschke & Jiranek et al., 1993). But past studies have reported different patterns of isoamyl alcohol

production as affected by amino acids content. Garde-Cerdan & Ancin-Azpilicueta (2008) found there was a positive effect of increasing amino acid concentrations on isoamyl alcohol, consistent with the results of the present study. However, Hernandez-Orte et al. (2006) reported that the greater the concentration of amino acids in grape juice, the less isoamyl alcohol formation. These converse trends may be reconciled in that the assimilable nitrogen concentration of must can affect the formation of higher alcohols. When the must nitrogen is low, surplus alpha-keto acids can only be excreted as higher alcohols and cannot be converted into amino acids because of the lack of available nitrogen. If the initial nitrogen concentration is high, increased nitrogen could lead to more surplus alpha-keto acids being transferred into amino acids rather than higher alcohols (Ugliana & Henschke, 2009).

In the field study, neither isoamyl alcohol nor phenylethyl alcohol showed a significant correlation between their formation and the quantity of consumed amino acids. That might due to the consumption of related amino acids and the concentrations of higher alcohols being similar between treatment groups i.e. a larger range of starting concentrations is required to see a trend. It is worth noting that the field UV treatments were applied only over the fruit zone, which may have limited their effect on fruit amino acids (M. Sun, personal communication).

4.6.2 Fatty acids

(1) Branched-chain fatty acids

There were some correlations between branched-chain fatty acids and their corresponding amino acids, although these fatty acids can also be connected with other amino acids.

It was reported that Leu is the structurally related amino acid for isovaleric acid (Wang et al., 2016). However, like in the case for Leu and isoamyl alcohol, no correlation between Leu and isovaleric acid can be found in the present study.

Ile is a precursor of 2-methylbutanoic acid (Styger et al., 2011). A significant positive correlation ($p<0.05$) between them can be found in the field experiment, but this was

not the case in the glasshouse trial. The reasons for this remain unknown, but may centre around the magnitude of differences in AA among the treatments, as well as uncontrolled factors in the field experiment.

Isobutyric acid is derived from Val though oxidation of isobutyraldehyde, which derived from alpha-keto isovalerate (Styger et al., 2011). A significant positive correlation ($p<0.01$) between them can be found in field trial. A negative correlation was found in glasshouse wine results, which was consistent with Guitart et al. (1999). These researchers found no correlation between amino acids and higher alcohols, but did find a negative correlation between amino acids and isobutyric acid. This might be because more alpha-keto isovalerate is converted into amino acids rather than forming isobutyric acid (similar to higher alcohols).

These different results might be explained by the fact that branched chain fatty acids are not only produced from amino acids via the Ehrlich pathway, but also derived from glycolysis. The source of alpha-keto acids in branched-chain fatty acid formation seems to depend on the nitrogen concentrations of the fermentation medium, and the concentrations of grape amino acids in the field experiment were much higher than those in the glasshouse trial. However, the regulation mechanism of branched-chain acids biosynthesis remains unclear (Clarke & Bakker 2004; Ugliano & Henschke, 2009), so it is difficult to discuss in more detail.

There are some correlations between amino acids consumption and branched fatty acids. Isovaleric acid was positively correlated with Glu, Gln, Tyr and Trp in the glasshouse experiment, while these types of correlations were not been found in field. Isobutyric acid was related with alpha-ketoglutarate amino acids (Glu, Gln, His, Arg) , aromatic amino acids (Phe, Tyr and Trp), branched amino acids (Leu and Ile), aspartate amino acids (Thr, Lys and Met), Asn, and Ala. 2-methylbutanoic acid was correlated with Tyr, Met, Val, Trp, Phe, Leu in field results. It was reported that Glu, Gln, Asp, Thr, His, Ala, Tyr and Arg are good nitrogen sources for yeast fermentation (Henschke & Jiranek, 1993), so they may have indirect effects on branched-chain acid formation. However, with regard to the glasshouse results, 2-methylbutanoic acid and isovaleric acid are negatively related to His, Thr, Arg, Ala and Ser, Thr, Arg, Ala, Tyr,

Phe and Leu, respectively. Due to the lack of information about the regulation mechanisms (Clarke & Bakker 2004; Ugliano & Henschke, 2009), the negative relationship of 2-methylbutanoic acid and isovaleric acid cannot be explained in more detail.

(2) Straight chain fatty acids

Straight-chain fatty acids are generated from saturated fatty acid metabolism (Ugliano & Henschke 2009). It seems amino acids are not directly involved in fatty acids metabolism, however, there were significant correlations between hexanoic acid and Glu, Gln, Ala, Phe, Trp, Tyr, Thr, Ser, Met, Ile, Val, Leu and there is a close relationship between octanoic acid and Ser, Thr, Arg, Ala, Tyr, Met, Phe, Leu in the present study. Although it has been reported that straight-chain fatty acids were affected by the availability of unsaturated fatty acids and fermentation conditions, previous studies have observed that the formation of medium chain fatty acids was affected by the must amino acids quantity (Wang et al., 2016).

Volatile medium chain fatty acids (hexanoic acid and octanoic acid) are by-products of long-chain fatty acids formation, which are required for cell membrane phospholipid biosynthesis. Uptake and storage of nitrogen by yeast is an adaptive response, because the fermentation medium, with increasing ethanol concentration, could inhibit the transport of amino acids (Bisson, 1991). The fatty acid compositions of yeast cell membranes can be altered by the predominant nitrogen source of fermentation medium (Torija et al., 2003). Therefore, the production of fatty acids can be promoted by addition of amino acids due to their impact on helping yeast cells to adapt their membrane fatty acids (Wang et al., 2016; Torija et al., 2003). It was suggested that the concentrations of must amino acids have effects on the formations of straight chain fatty acids (Wang et al., 2016). This is likely found because yeast assimilable nitrogen (such as from amino acids) indirectly influences the yeast growth, the rate of glycolysis and fatty acids formation (Bely et al., 1990; Torija et al., 2003)

(3) Acetic acid

According to Ylva Ardö (2006), Thr can be catabolized into acetaldehyde and eventually form ethanol or acetic acid. However, this correlation was not found in the present research, possibly because the acetaldehyde of acetic acid synthesis is mainly derived from pyruvate via decarboxylation, rather than derived from Thr.

In this study, acetic acid was significantly correlated with Glu, Gln, His, Val, Ile, Leu, Met, Trp, Ser (glasshouse trail) and Glu, Gln, His, Phe, Trp, Tyr, Leu, Ile, Val, Asp, Met, Asn, and Ala (field trial). Due to acetic acid biosynthesis being involved in the carbohydrate metabolism pathway (Lambrechts & Pretorius, 2000), these amino acids might be indirectly related to acetic acid, because most of them are the preferred sources of nitrogen for yeast growth (Henschke & Jiranek, 1993). Previous studies have suggested that the yeast assimilable nitrogen concentrations of must could strongly affect acetic acid accumulation (Bely et al., 2003; Vilanova et al., 2007).

Notably, the acetic acid concentrations of glasshouse wines were high, and this could be caused by non-*Saccharomyces* species of yeasts in the ferment (Ugliano& Henschke, 2009), for example *Kluyveromces thermotolerans* is an acid-producing yeast that can produce large concentrations of acetic acid (Ciani et al. 2006; Kapsopoulou et al. 2007). *Hanseniaspora uvarum* is another yeast found in musts characterised by high production of acetic acid, but populations reduce quickly during alcoholic fermentation due to its low tolerance to ethanol (Ugliano & Henschke, 2009). The very small-scale (100g) of the ferments in the glasshouse study may have contributed to acetic acid production through relatively high surface area to volume ratios and potential acetic acid bacteria contamination, though this was not measured.

4.6.3 Esters

Aroma-active esters can be classified into two groups: acetate esters and ethyl esters of fatty acids. Significant correlations between amino acid consumption and ester concentrations were found in the present study.

(1) Acetate esters

Thr can be catabolized into ethanol and acetic acid (Ardö, 2006), which are involved in ethyl acetate formation. A significant positive relationship between Thr and ethyl acetate was observed in the present study, consistent with Hernandez-Orte et al. (2002). It was reported the higher the Thr concentration in the fermentation medium, the higher the concentration of ethyl acetate obtained (Hernandez-Orte et al., 2002). However, Thr was not correlated with acetic acid in the present study.

Leu is the structurally related amino acid of isoamyl acetate (Styger et al., 2011), and a significant relationship was found ($p<0.05$) between them in glasshouse results. This would seem to indicate that isoamyl acetate might be formed from isoamyl alcohol derived from Leu, but there is insufficient evidence to show this conclusively, as the correlation between Leu and isoamyl alcohol was not found in this study.

It was suggested Phe could participate into the formation of phenylethyl alcohol (Hazelwood et al., 2008) and the further production of phenylethyl acetate (Styger et al., 2011). However, no correlation between them exists in the present study, as found with the lack of correlation between Phe & phenylethyl alcohol.

These significant correlations between acetate esters and amino acids might indicate that Leu and Thr possibly are involved in the formation of related aroma compounds formations. However, no relationship could be found between Leu & isoamyl alcohol and Thr & acetic acid, which seems support that the higher alcohols or acetic acids involved in the formation of acetate esters are not primarily produced from amino acids, but are formed from glycolysis (Lee et al., 2004).

There was some correlation between amino acids consumption and acetate esters in present study. Yoshimoto et al. (1998) indicated that there was a link between nitrogen availability and expression of ATF1gene (which encodes for alcohol acetyltransferase enzymes, which are responsible for acetate ester formation). Ethyl acetate was positively correlated with alpha-ketoglutarate amino acids (Glu and Gln), aromatic amino acids (Phe, Tyr, Trp), branched amino acids (Leu, Val, Ile), Ser, Ala,

and Met. Isoamyl acetate was related to the concentrations of consumed alpha-ketoglutarate amino acids (Glu, Gln, His, Arg), Phe, Val, aspartate amino acids (Thr, Asp, Met) Ser, and Ala. It was suggested must nitrogen level had effects on the production of isoamyl acetate (Rapp and Versini, 1991; Saerens et al., 2006; Wang et al., 2016). In the case of phenylethyl acetate, a positive correlation was found between its formation and the concentrations of consumed Glu, Gln, Ser, Met, Ile and Leu, which agrees with Garde-Cerdan and Ancin-Azpilicueta (2008). Those researchers reported that the formation of 2-phenylethyl acetate increased with the addition of amino acids. Wang et al. (2016) reported addition of branched amino acids and Phe slightly increased phenylethyl acetate compared to addition of Phe on its own. This might indicate that branched chain amino acids seem to be able to attenuate the positive effects of Phe on phenylethyl acetate formation.

(2) Ethyl esters of fatty acids

Medium chain fatty acids are the precursors for the equivalent ester compounds, and their biosynthesis and concentration are important factors for ethyl esters formation (Saerens et al., 2006 & 2008). In the present study, ethyl octanoate was related to Ser, Gln, Tyr, Met, Phe, Ile, Leu, which was similar to what was found for octanoic acids; while ethyl hexanoate were correlated with Glu, Ser, Gln, Thr, Ala, Tyr, Val, Met, Trp, Phe, Ile and Leu, which was the same as found with hexanoic acid. This is consistent with Saerens et al. (2008), who stated that ethyl fatty acid esters are closely related to the corresponding fatty acids. As mentioned before, although medium chain fatty acids metabolism does not directly involve amino acids, it can be indirectly affected by must amino acids level (Torija et al., 2003; Wang et al., 2016).

Esters derived from short chain fatty acids, ethyl butanoate and ethyl pentanoate were positively correlated with several similar amino acids, such as alpha-ketoglutarate amino acids (Glu, Gln, His), aromatic amino acids (Tyr, Phe), and Asn. This may be because ethyl propanoate is produced from propanoic acid, which is derived from alpha-ketobutyrate (Eden et al., 2001), while ethyl butanoate is formed from butanoic acid derived from alpha-ketobutyrate as well (Ugliano & Henschke, 2009).

With regards to branched-chain esters, according to Ugliano & Henschke (2009), Val is the structurally related amino acid of ethyl isobutyrate. A significant correlation ($p<0.05$) between them was found in the field results. However, this relationship was not found in glasshouse study, where ethyl isobutyrate correlated only with Asp. The reasons remain unknown, but could be because branched-chain esters were not all derived from amino acids but from sugar. Also according to Ugliano & Henschke (2009), Leu is related to ethyl isovalerate based on structure. A correlation between the two was observed in the present study, however, these correlations cannot be discussed more due to a lack of understanding about the metabolism of branched-chain esters. Furthermore, branched chain esters were related with other individual amino acids, such as alpha-ketoglutarate amino acids (Glu, Gln, Arg), aromatic amino acids (Tyr, Trp), aspartate amino acids (Asp, Thr), Ser, and Ala, which are also good sources of nitrogen source for yeast growth (Henschke & Jiranek et al., 1993).

4.6.4 Grape-derived compounds

In this study, β-damascenone was correlated with Glu, Gln, Trp, Phe, Val, Ile and Leu Ser, and Met. Most of these amino acids are preferred nitrogen sources for yeast growth during fermentation (Henschke & Jiranek et al., 1993). This result could be supported by the work of Hernandez-Orte et al. (2008), who indicated that acid-catalyzed transformations of glycosidically-bound precursors is a possible pathway for the formation of β-damascenone during fermentation. Therefore, it was suggested formation of β-damascenone is not a simple hydrolytic process, but is a part of yeast metabolism and can be affected by the availability of yeast assimilable amino acids.

Although monoterpenes are considered to be similarly derived from the grape (Gonzalez-Barreiro et al., 2015), Carrau et al. (2005) proposed that under micro-aerobic conditions monoterpenes might be produced by yeast in mitochondria, related to Leu metabolism. They found linalool production was significantly promoted by high assimilable nitrogen concentrations (400 mg/l) in the fermentation medium. However, linalool was negatively correlated with Ser, Thr, Ala and Tyr in the

present study. The reason for this remains unknown, but deserves clarification. A possible reason might be because the nitrogen content was much lower in the present study, compared to Carrau et al. (2005). With regards to other grape-derived aroma compounds, no correlation was found between them and amino acids consumption.

4.7 PCA

According to PCA figure, there were clear separations between four treatments: +UV+W, +UV-W, -UV+W, and -UV-W. In general, wines from -UV-W treatment grapes tended to have a more diverse set of aroma compounds, such as ethyl esters, higher alcohols and fatty acids compared with other treatments, potentially resulting in more fruity and floral sensory characteristics. The PCA analysis shows that the treatment effects are distinguishable, therefore, the influence of environmental factors or viticulture management (such as UV-B radiation and deficit irrigation) on grape amino acids profiles appears significant and measurable. This has translated to changes to wine amino acid profiles and wine aroma-active compounds.

4.8 Conclusion

To conclude, the concentrations of amino acids in wine and the consumption of amino acids during fermentation were decreased by UV-B treatment. This result is supported by similar results obtained from other research into nitrogen metabolism, uptake rates of nitrate and nitrite and the activities of nitrogen assimilating enzymes where they were shown to be influenced by UV-B radiation (Singh et al., 2012). On the other hand, amino acids are the major nitrogen source for yeast during alcoholic fermentation and act as precursors of fermentation-derived aroma compounds, so the aroma compounds of wines made from the grapes were affected as well.

The +UV treatment decreased wine aroma compounds formation. This was likely due to higher alcohols, fatty acids and esters being produced from amino acids or from sugar by glycolysis during yeast fermentation. UV-B could decrease the aroma precursors and nitrogen sources for alcoholic fermentation (Ugliano & Henschke, 2009; Song et al., 2015). In addition, some grape-derived volatile compounds were

affected by UV-B exposure, as UV-B may promote some of their accumulation during berry ripening (Lee et al., 2007) and UV-B could reduce the grape maturity and must nitrogen level, which in turn, can affect the level of C_{13}-norisoprenoids.

Water deficit treatment had effects on few wine aroma compounds. This may be due to water deficit decreasing amino acids in must and limiting vine vigor and promoting the accumulation of secondary metabolites in ripening berries. Limited irrigation can also affect berry maturity, consequently increasing the concentrations of aroma glycosides of grapes (Qian et al., 2009).

These two factors alter the concentrations of some aroma compounds. Water deficit could amplify the positive effects of UV-B exclusion on the formation of aroma compounds. Thus, the highest level of aroma compounds can be found in -UV-W treatment, while the lowest value was found in +UV+W. On the other hand, the effects of -UV are opposite to the effects of +W, so regular irrigation could weaken the promotional effects of UV-B treatment. Similar results were obtained from +UV-W and -UV+W treatments in some aroma compounds.

In addition, wine sensory profiles could also be changed by the concentrations of higher alcohols, fatty acids and esters. Higher alcohols can contribute fusel and green notes; fatty acids are related to the fresh notes in wines and aroma complexity. Esters are responsible for fruity and floral flavour. Thus, more formation of higher alcohols, fatty acids and esters may increase the fruity, floral, green, fresh notes and, potentially, aroma complexity in Pinot noir wines. Higher UV-B radiation induced a decrease in grape amino acids, which in turn reduced the overall consumption of amino acids by yeast. As a result, fewer aroma compounds are formed in UV-B treatment wines with lower concentrations of fruity and floral compounds. Water deficit could increase the concentrations of most wine aroma compounds, leading to more fruit, freshness and aroma complexity. The interactive effects of combination of UV-B exclusion and water deficit treatment could lead to highest value of aroma compounds in corresponded wine.

In this study, higher alcohols were not related to their structurally related amino

acids, likely because the Ehrlich pathway is not the main pathway for higher alcohol biosynthesis during fermentation, but from the biosynthesis pathway, which agrees with the literature (Chen 1978). But there were some correlations between higher alcohols and other amino acids, again suggesting that amino acids have effects on higher alcohol formation. Amino acids could influence general fermentation activity, aminotransferase activity, biomass production and glycolysis rate (Ardö, 2006; Arias-Gil et al., 2007; Ugliano & Henschke, 2009).

The different results of the correlations between branched chain acids, ethyl esters of branched chain acids and their corresponding amino acids seems to indicate that branched chain aroma compounds might be formed from amino acids, but also can be produced from (likely) pyruvate.

There was significant relationship between acetate esters and their related amino acids. However, there is insufficient evidence to show this conclusively, as the correlation between higher alcohols and related amino acids was not found in this study. Acetate esters were still related to other amino acids, however. A possibility for this may be due to the expression of the ATF1 gene, responsible for acetate ester formation, which could be affected by general amino acid availability. Most ethyl esters of medium chain fatty acids and their corresponding fatty acids are related to the same amino acids, which could indicate a close relationship. Amino acids appear to have indirect effects on ester formation, which might be because they can alter yeast cell membranes and be otherwise utilized during fermentation.

Correlations between β-damascenone and some individual amino acids were found in this study, which suggest that the formation of β-damascenone is not just a simple hydrolytic step, but is a part of yeast metabolism. However, linalool was negatively correlated with amino acids consumption, and the reason for this still needs to be explored.

The PCA analysis shows that the treatment effects are distinguishable, therefore, the influence of environmental factors or viticulture management (such as UV-B radiation and deficit irrigation) on grape amino acids profiles appears significant and

measurable. This has translated to changes to wine amino acid profiles and wine aroma-active compounds. Further research will be required to confirm that the quantitative changes are perceived using sensory methods.

In summary, the effects of UV or water treatments on wine aroma compounds can be attributed to the effects on the grape amino acids, because they serve as a nitrogen source and as precursors of volatile compounds during alcoholic fermentation. Higher alcohols, fatty acids and esters are produced by yeast during alcoholic fermentation. Thus it was suggested that there was a significant correlation between the concentrations of consumed amino acids and the formation of wine aroma compounds. The actual utilisation of amino acids involved in aroma development has not been made clear, however. This work is correlative because the synthesis and degradation of amino acids has not been measured, but since there appear to be significant relationships between wine aroma compounds and some grape amino acids, further research into identifying the specific relationships seems worthwhile.

Chapter 5. Summary

The correlation of consumed amino acids (calculated from grape and their corresponding wine) and wine aroma compounds was investigated in experiments taking place in the Lincoln vineyard with VSP trellising system and in a glasshouse. The contents of consumed amino acids were directly proportional to the concentrations of grape amino acids. The initial grape amino acid concentrations were altered by +UV/ -UV and +W/-W treatments in glasshouse, and were also changed by leaf removal, shade cloth and PETG treatment in field, which led to changed wine amino acids and aroma compound composition.

The concentrations of total and most individual amino acids in glasshouse wine were significantly decreased by UV-B radiation, consistent with Sun's results (personal communication). This is most likely because UV-B radiation affects nitrogen metabolism via changing nitrate and nitrite uptake rates and the activities of nitrogen assimilating enzymes (Singh et al., 2012). The concentrations of total and some individual amino acids (Asp, Glu, Asn, Ser) were significantly increased by water deficit, however, there was no significant difference found in the grape results (M. Sun, personal communication). But the reason for these different results remains unknown. It could be due to different sample sizes: for example, the analysis of grape amino acids was done from a separate fifteen grape berry sample and not from the grapes used for fermentation. Using a larger sample size could result in less variation, which may improve the signal to noise ratio sufficiently to discern significant differences by water treatment. There was no significant difference found between LR, SC and PETG treatments in field wine amino acids, which is consistent with M. Sun's result in grapes (personal communication).

In the present study, the was no relationship between higher alcohols and their structurally corresponding amino acids, while only isoamyl acetate was related with its corresponding amino acid. A poor relationship can be found between branched chain fatty acids and their related amino acids. These results might be explained by the Ehrlich pathway (i.e. that they are derived from amino acids) not being the main

pathway for aroma compound development in this type of situation. Most higher alcohols appear to be produced from sugar via the biosynthesis pathway (Chen, 1978). However, significant relationships have been found between these aroma compounds and some individual amino acids. It can be suggested that amino acids have effects on higher alcohol formation via affecting general fermentation activity, aminotransferase activity, biomass production and glycolysis rate. Medium chain fatty acids and their ethyl esters are related to the same amino acids, which appear to have indirect effects on ester formation and could indicate a close relationship between medium chain fatty acids and their ethyl esters. Amino acids also have effects on ester biosynthesis, because they could affect the expression of the *ATF1* gene and be otherwise utilized during fermentation. Moreover, β-damascenone and monoterpenes were related with some individual amino acids, which could suggest there is a possible pathway for their association during yeast fermentation.

Due to the significant correlations between amino acid consumption and wine aroma compounds, the changes in amino acids induced by UV and irrigation treatments will therefore translate into alterations in wine aroma compounds.

The results show that concentrations of wine aroma compounds were affected by +UV/-UV treatment. +UV treatment decreased higher alcohols, fatty acids and esters, especially phenylethyl alcohol, hexanoic acid, ethyl acetate, ethyl isobutyrate, ethyl butanoate, ethyl isovalerate, ethyl hexanoate, ethyl decanoate and isoamyl acetate (Table 4.6). Decreased concentrations of amino acids caused by +UV treatment may have had effects on the formation of fermentation-derived aromas（Song et al., 2015）.

In the field experiment, aroma compounds in wines were generally not affected by LR, SC and PETG treatments. On the other hand, some grape-derived volatile compounds, such as terpene alcohol, were increased by UV-B exposure, which could be due to their accumulation being sensitive and promoted by UV-B during berry ripening (Lee et al., 2007). An additional factor could be fruit maturity at harvest, as UV-B could reduce the rate of grape ripening, leading to a decrease in the level of β-damascenone.

With regards to irrigation treatments, drought increased the concentrations of higher alcohols, fatty acids and esters, such as isoamyl alcohol and phenylethyl alcohol, butanoic acid, ethyl acetate, citronellol, C_{13}-norisoprenoids and some ethyl esters of fatty acids, but decreased linalool and geraniol (Table 4.7). Water deficit may affect the accumulation of amino acids in ripening berries and also affect berry maturity, which then changes the relative concentrations of aroma glycosides of grapes (Fang & Qian, 2006). Moreover, the effects of combining UV-B and water deficit can be different than the individual effects. These two factors together alter the concentrations of ethyl acetate, ethyl hexanoate, Isoamyl acetate, cis-3-hexen-ol, and C_{13}-norisoprenoids. Water deficit could strengthen the promotional effects of UV-B exclusion on wine aroma compounds, while regular irrigation could fortify the negative effects of UV-B. Details around these interactions are still unclear.

In these studies, the effects of UV or water treatments on wine aroma compounds can be attributed to the effects on the grape amino acids, because they serve as a nitrogen source and as precursors of volatile compounds during alcoholic fermentation. Higher alcohols, fatty acids and esters are produced by yeast during alcoholic fermentation. Thus it was suggested that there was a significant correlation between the concentrations of consumed amino acids and the formation of wine aroma compounds.

This study suggested that there were positive correlations between the amounts of amino acids consumed and the formation of aroma compounds in general. The sensory perception of wine might be modified by the change of aroma compounds. For example, higher alcohols could contribute a masking effect of fruity aromas in wines when their concentrations are above 400mg/l (Garde-Cerdan and Ancin-Azpilicueta, 2008). Isoamyl alcohols can contribute fusel notes to wine, while hexanol contributes grassy notes (Song et al., 2014). Fatty acids are partly responsible for the complexity of wine, and also contribute a fresh flavour (Garde-Cerdan and Ancin-Azpilicueta, 2008). Esters also play an important role in wine aroma. Acetate esters generate fruity flavours and the ethyl esters of fatty acids contribute fruity, floral and sweet notes to wine (Song et al., 2014; Perestrelo et al., 2006). Thus, higher

concentrations of grape amino acids promote greater consumption of amino acids during fermentation, potentially leading to increased fusel, fruity, floral and green notes, as well as the aroma complexity in wines.

In a general sense, this study has found that the effects of UV-B radiation and water deficit on grape amino acids can be transferred into wine amino acids and their consumption during fermentation. Due to these significant correlations between the consumption of amino acids and the formation of aroma compounds, UV-B and water deficit could affect the formation of wine aroma compounds. PCA of data generated in this thesis has shown there is a distinct grouping pattern of the different treatments, highlighting the chain of events: environment influences grape, grape influences fermentation, which influences wine aromas. The understanding of the links between grape and wine could potentially establish a method of predicting the quantity of key wine aroma parameters based on grape composition, though at present there remain a number of uncertainties around the exact relationship.

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