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Volatile and Color Composition of Young and Model-Aged Shiraz Wines As Affected by Diammonium Phosphate Supplementation Before Alcoholic Fermentation

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A Shiraz must with low yeast assimilable nitrogen (YAN) was supplemented with two concentrations of diammonium phosphate (DAP) and then fermented with maceration on grape skins. The nonvolatile, volatile, and color composition of the final wines were investigated. Ethanol and residual sugars were not affected by DAP supplementation, while glycerol, SO_2, and residual YAN increased and acetic acid decreased. DAP-supplemented treatments gave rise to higher concentrations of acetates, fatty acids, and fatty acid ethyl esters but lower concentrations of branched-chain fatty acids and their ethyl esters. No major difference between treatments was observed for higher alcohols, monoterpenes, norisoprenoids, and low-molecular-weight sulfur compounds. DAP-supplemented fermentations resulted in wines with higher concentrations of malvidin-3-glucoside, higher color intensity, and altered color tonality. Model aging studies indicated that higher concentrations of esters are still present in wines from the DAP-treated fermentations after aging. DAP supplementation also resulted in increased concentrations of dimethyl sulfide after model aging. It can be concluded that DAP treatment of a low YAN must fermented by maceration on skins can significantly affect wine color, aroma, and flavor.

KEYWORDS: Wine aroma; esters; DMS; malvidin-3-glucoside; nitrogen; DAP; fermentation; wine color

INTRODUCTION

Fermenting grape must is a harsh environment in which suboptimal levels of different nutrients can interfere with the ability of yeast to complete alcoholic fermentation. Among the various nutrients contained in grape juice, yeast assimilable nitrogen (YAN), defined as the nitrogen contained in the ammonia and free α-amino acid (FAN) fractions of juice, provides nitrogen for protein biosynthesis of the cell and is therefore of primary importance for correct functioning of cell metabolism (reviewed in ref *1*). Several studies have shown that supplementing grape must with YAN in the form of ammonium salts is a useful tool for lowering the risk of slow and stuck fermentation and can also decrease the formation of unwanted sulfur volatiles by yeast (*1, 2*). These observations have led to the generalized practice of supplementing grape must with ammonium salts, in some cases without the knowledge of initial YAN content. However, excessive ammonium additions, although improving fermentation rates, can lead to a high concentration of residual nitrogen in the wine, which could promote the growth of unwanted spoilage microorganisms, such as lactic acid bacteria and various yeasts including *Dekkera*/*Brettanomyces* strains (*1*). Moreover, more detailed studies on stuck and sluggish fermentation have highlighted the role played by other factors, such as temperature management and the degree of clarification of the must, with the latter directly affecting lipids and oxygen availability (*3*). It is now widely accepted that the risk of incomplete fermentation of a highly clarified grape juice with a sugar concentration of around 200 g/L is minimized when the YAN concentration exceeds 140 mg/L (*4*).

Anecdotal observations suggest that, in the case of red wine fermentation, which involves maceration, typical conditions, such as warmer temperatures, presence of grape solids, aeration of the fermenting must during cap management operations, and extraction of assimilable nitrogen and lipids from grape skins during maceration, can reduce the risk of slow and incomplete fermentations. Nevertheless, several surveys carried out in different countries have shown that the YAN concentration in red grape juice can be well below 140 mg/L (*5*−*7*). This indicates that, during the fermentation of musts prepared from red grapes, yeast can also be exposed to severe nitrogen limitation, although to a lesser extent than in clarified juice because additional YAN can be extracted from grape skins and possibly seeds (*5*). Besides its effects on fermentation kinetics, YAN can regulate yeast metabolism at several other levels,

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Table 1. Sugar and YAN Concentration of 51 Shiraz Grape Juice Samples

	average	minimum−maximum
° Brix	22.2	20.8–23.4
NH_3 (mg/L)	59	16–102
FAN (mg/L)	118	60–237
YAN (mg/L)	166	87–321

including the production of volatile compounds responsible for wine aroma properties. It is well-known that diammonium phosphate (DAP) supplementation can lower the production of H_2S and other sulfur volatiles by yeast (reviewed in ref *1*). Early studies have also shown the existence of a link between YAN availability and the production of higher alcohols (*8*). More recently, a relationship between nitrogen supplementation of the must and formation of volatile compounds, such as esters, has been suggested (*6, 9*−*11*). However, most of these studies used model grape juice (*9, 10*), or in the case of studies using real grape juice, they were carried out with white grape juices having already a relatively high YAN content (*11*). This focus is surprising in view of the fact that, in the winery, the demand for optimizing the nitrogen composition of juice is generated by the frequent occurrence of low nitrogen juices showing poor fermentation kinetics and/or undesirable aroma characters. Moreover, very little work has been carried out on nitrogen supplementation during red winemaking, and the effects of DAP supplementation on red wine aroma composition are mostly unknown. Also, the relationship between nitrogen supplementation of juice and volatile composition of aged red wines has been unexplored thus far, even though aging is a key step in the production of red wine.

Along with aroma, color is another key quality parameter for red wine. Yeast metabolites, including acetaldehyde, pyruvic acid, and vinyl phenol, are involved in reactions with anthocyanins and various flavanols, to produce stable pigments in red wine (*12, 13*). The extent of these various reactions will depend upon many fermentation factors, including yeast strain characteristics and the modulation of appropriate metabolites by growth conditions and nutrients. However, we are not aware of any studies that have specifically investigated the effect of nitrogen supplementation of red wine ferments on wine color and phenolics composition.

In the present study, the effects of nitrogen supplementation of Shiraz aroma and color composition was investigated by means of DAP addition to a low YAN Shiraz must. Because of the chemical instability of several aroma compounds at wine pH, the main changes in the volatile fraction of the experimental wines during aging was also investigated in a model aging study.

MATERIALS AND METHODS

Winemaking. One of the aims of this study was to investigate the effect of inorganic nitrogen additions on the composition of wine obtained from YAN-deficient musts, which are more likely to require YAN correction in the winery. A total of 51 samples from different vineyards located in the McLaren Vale region (South Australia) were collected and analyzed during the 2006 vintage to determine their YAN content (**Table 1**). Among the 23 samples that were found to have a YAN content of less than 140 mg/L, a sample with YAN of 103 mg/L [free α-amino nitrogen (FAN) = 71 mg/L, ammonium = 32 mg/L], 23.8° Brix, and pH 3.4 was selected. Grapes were hand-picked and collected in 20 kg crates. Once in the winery, different crates were pooled together to obtain a homogeneous mass. Individual 30 kg lots were then destemmed and crushed, and the must was collected in 34 L "wide mouth" glass demijohns. Potassium metabisulfite was added at 100 mg/kg to each fermentation lot. DAP additions were performed according to an experimental design consisting of three YAN concentrations, with each one fermented in triplicate, for a total of nine fermentations. A control that did not receive any DAP addition represented the lowest nitrogen concentration (103 mg/L YAN), while in the two additional treatments, the final YAN concentration was 250 and 400 mg/L YAN, respectively. After 2 h from DAP additions, pH of the samples was measured and readjusted to 3.4 by means of 1 N HCl. Then, the samples were inoculated with *Saccharomyces cerevisiae* AWRI 796 (Maurivin, Australia) at a rate of 1×10^6 cells/mL, following rehydration in water at 40 °C for 30 min. Fermentations were carried out at 22 °C, with the cap plunged 3 times per day. The fermentation progress was monitored by enzymatic analysis of the residual sugars. Dominance of the inoculated strain in all of the treatments was confirmed by transposon polymerase chain reaction (PCR) analysis (*14*). The wines were left to macerate on grape skins until the slowest treatment reached dryness (residual sugars ≤ 2 g/L), after which the fermented musts were pressed and the wines were collected in 20 L glass containers and placed at 4 °C under inert headspace to accelerate clarification. No malolactic fermentation was carried out. After 4 weeks, 150 mg/L of potassium metabisulfite was added to the wines, which were then filtered through 0.45 μm membranes (Sartorius, Gottingen, Germany) and bottled under ROTE closures. Analysis of the volatile fraction of the wines was carried out after 2 months of storage at 12 °C, except for the aging study (see below).

Wine Chemical Parameters, Phenolic Composition, and Color Parameters. Ethanol, titratable and volatile acidity, and free and total SO_2 were measured as described by Parker et al. (*15*). Glycerol, FAN, and ammonia were measured as previously described (*10*). YAN was calculated as the sum of ammonia-derived nitrogen and FAN and, therefore, did not include proline. Malvidin-3-glucoside, tannin, and pigmented polymers were quantified by reverse-phase high-performance liquid chromatography (HPLC), performed on an Agilent 1100 LC (Agilent, Australia) using the method described by Mercurio et al. (*16*). Because of high anthocyanin concentrations, the injection volume was reduced to 5 μL for all wine samples. Samples were centrifuged at 13 000 rpm and 20 °C to remove any solid present prior to injection. Aqueous (−)-epicatechin (Sigma-Aldrich, Sydney, Australia) solutions were used to establish a calibration curve for reporting tannin concentration. Aqueous malvidin-3-glucoside (Sigma-Aldrich, Sydney, Australia) solutions were used to establish a calibration curve for reporting malvidin-3-glucoside and pigmented polymer concentrations. Color parameters were analyzed according to the CIELab method as described previously (*15*).

Volatile Compounds. All solvents were Merck Suprasolv grade (Merck, Kilsyth, VIC, Australia) nanopure grade and verified for purity by gas chromatography/mass spectrometry (GC/MS) prior to use.

Fatty acids and their ethyl esters, higher alcohols and the corresponding acetate esters, and branched-chain acids and the corresponding ethyl esters were quantified using a stable isotope dilution assay (SIDA), as described previously (*17*). Ethyl propanoate, ethyl 2-methylpropanoate, ethyl butanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, 2-methylpropyl acetate, 2-methylbutyl acetate, 3-methylbutyl acetate, 2-methylbutanol, 3-methylbutanol, ethyl hexanoate, hexyl acetate, ethyl lactate, hexanol, propanoic acid, ethyl decanoate, ethyl dodecanoate, butanoic acid, 2-methylbutanoic acid, 3-methylbutanoic acid, 2-phenylethanol, and decanoic acid were supplied by Sigma-Aldrich. Ethyl acetate, butanol, and 2-phenylethyl acetate were supplied by Merck (Kilsyth, Victoria, Australia). 2-Methylpropanol was supplied by Riedel-de Haën (Seelze, Germany). Ethyl octanoate, hexanoic acid, and octanoic acid were supplied by Hopkin and Williams (London, England). Labeled standards purchased from Sigma-Aldrich were d_8-ethyl acetate, d_{10}-butanol, d_{13}-hexanol, d_5-propanoic acid, d_7-butanoic acid, d_{11}-hexanoic acid, d_{15}-octanoic acid, and d_{19}-decanoic acid. All had >98 atom % deuterium. The rest of the standards used were prepared as described in ref *17*.

The monoterpenes linalool, geraniol, and α-terpineol were analyzed using a stable isotope dilution assay (SIDA), as described previously (*18*). Unlabeled reference standards were obtained from Sigma-Aldrich, while labeled compounds were prepared as described in ref *18*. For the analysis of the norisoprenoids β-damascenone and β-ionone, an aliquot (100 μL) of a solution of $^2H_4$ β-damascenone and $^2H_3$ β-ionone

Table 2. Wine Chemical Parameters

	treatment[a]		
	control	250	400
alcohol (%)	14.2 a	14.2 a	14.1 a
residual sugars (g/L)	0.2 a	0.2 a	0.2 a
pH	3.53 a	3.47 a	3.46 a
titratable acidity (g/L)	8 a	7.7 a	7.4 a
residual ammonia (mg/L)	nd	nd	nd
residual YAN (mg/L)	12 c	22 b	46 a
volatile acidity (g/L)	0.4 a	0.3 b	0.3 b
free SO_2 (mg/L)	45 c	55 b	60 a
total SO_2 (mg/L)	75 c	86 b	90 a
glycerol (g/L)	11 c	12 b	12.6 a

[a] Control, 100 mg/L YAN; 250, same must as the control but initial YAN increased until 250 mg/L by means of DAP addition; 400, same must as the control but initial YAN increased until 400 mg/L by means of DAP addition. For each compound, different letters denote significant differences between treatments, at $p < 0.05$.

(*19*) in ethanol (10 μg/mL) was added to the wine samples (50 mL) in a 50 mL volumetric flask, using a glass syringe (100 μL of SGE). The sample was shaken and transferred to a 100 mL glass measuring cylinder with a ground glass stopper; pentane/ethyl acetate (2:1, 3.5 mL) was added; and the mixture was shaken briefly. A portion of the organic layer was then transferred to a vial for GC/MS analysis. To calculate the concentration of the analytes in the wine samples, replicate standards were prepared at the same time as the wine samples, by adding the same amount of internal standard as above (100 μL) to a solution of β-damascenone (Firmenich, Switzerland) and β-ionone (Sigma-Aldrich) in ethanol (each 5 μg/mL), diluted with dichloromethane (1800 μL) and analyzed by the GC/MS method (see below) to calculate the relative response factors. Samples were analyzed as described in ref *19*, with the following ions added in the selective ion monitoring (SIM) mode, *m/z* 180 and 195 for [$^2H_3$] β-ionone and *m/z* 177 and 192 for β-ionone; the underlined ion for each compound was the ion typically used for quantitation, having the best signal-to-noise and the least interference from other wine components. The other ions were used as qualifiers.

The low-molecular-weight sulfur volatiles hydrogen sulfide (H_2S), dimethyl sulfide (DMS), and carbon disulfide (CS_2) were determined by static headspace analysis with atomic emission detection, as described by Siebert and Pollnitz (*20*). All of these compounds were identified by means of coinjection with pure reference compounds obtained from Sigma-Aldrich. H_2S was obtained by dissolving sodium hydrosulfide hydrate (Sigma-Aldrich) in water adding top model wine at pH 3.2. 3-Methylthio-1-propanol was extracted and analyzed as described by Ugliano and Moio (*21*).

Aging Experiment. A model aging study was carried out to evaluate the effects of nitrogen on the volatile composition of the wines during longer term aging. For this, wines were transferred into glass flasks, pH was adjusted to 3.5 with either 1 M NaOH or 1 M HCl, the headspace was flushed with N_2, and the samples were stored at 30 °C for 6 weeks. Volatile compounds at the end of the experiment were analyzed as described before.

Statistical Analysis. Analysis of variance and least significant difference (LSD) test were used to interpret the differences in means at the 95% confidence level. The data were processed using JMP 5.0.1 (SAS, Cary, NC).

RESULTS AND DISCUSSION

Fermentation Performance and Wine Chemical Composition. The addition of ammonium salts to media low in YAN prior to fermentation resulted in a decrease of the fermentation length, with DAP-treated fermentations reaching dryness in 8 days (400 mg/L YAN) and 10 days (250 mg/L YAN), respectively, while 14 days were required for the control (100 mg/L YAN). The main chemical parameters of the final wines are given in **Table 2**. The final concentration of ethanol and residual sugars was not affected by DAP supplementation. Small variations in ethanol yield by different strains in response to DAP supplementation have been reported by others (*11*). However, as we observed previously, AWRI 796 showed insignificant differences to a wide range of initial ammonium concentrations of a synthetic medium (*10*). Furthermore, in contrast to fermentations carried out in clarified media, residual sugars in the control wines of these high solid fermentations were similar to those of DAP treatments, suggesting that sugar transport is more protected against inactivation by grape solids (a source of lipids) as has been observed in lipid supplementation experiments (*3*).

No residual ammonia was detected in any of the treatments, indicating that the uptake of preferential nitrogen sources, such as ammonia, was complete even under the conditions of high DAP supplementation, probably because of the low concentrations of FAN present in the grapes used for this study. However, a positive relation was observed between residual YAN and initial DAP, which was due to increased concentrations of residual FAN. The fact that residual FAN was also observed in the non-DAP supplemented treatments suggests the release of α-amino nitrogen by yeast in the later stages of fermentation, as observed in clarified media by others (*11*). This release might also explain the higher residual FAN observed in the DAP-supplemented fermentations, which were only racked off yeast lees when the control fermentations had reached dryness. Amino acid release could be due to increased ethanol-induced membrane permeability (*22*).

DAP supplementation was negatively correlated with volatile acidity but positively correlated with glycerol and free and total SO_2. Glycerol production by AWRI 796 in response to nitrogen supplementation of synthetic media (*10*) showed a similar pattern, except that the concentrations were markedly higher in this study. This might be due to the presence of grape solids, which could be acting by increasing osmotic stress. The decrease in volatile acidity in response to increasing initial DAP is consistent with the results of Bely et al. (*23*). They suggested that growth stimulation by increased nitrogen availability increases NADH production, lowering the need for the cell to generate NADH through other redox reactions, such as in the oxidative formation of acetic acid from acetaldehyde. Alternatively, the nitrogen-induced growth stimulation could be acting by increasing lipid synthesis, which increases acetyl-CoA demand, thereby limiting acetic acid accumulation. The effect of nitrogen supplementation observed on SO_2 is in agreement with previous findings (*24*).

Polyphenolics and Color Parameters. The concentration of malvidin-3-glucoside, the principal anthocyanin in *Vitis vinifera* grapes, was higher in the two DAP-supplemented fermentations compared to the control, while no difference was observed for pigmented polymers and tannin concentration (**Table 3**). The values of the CIELab coordinates of the wines, *L* (a measure intensity, with higher values corresponding to lighter color), *a** (positive values related to redness), and *b** (positive values related to yellowness), are reported in **Figure 1**. Wines made under conditions of DAP supplementation of the must showed higher color intensity (lower *L* values) compared to the control and had more red−blue component. A direct comparison between wine samples based on their ΔE^*_{ab} (a measure of color differences between samples made with different DAP supplementations) indicated that the color differences between the DAP-treated fermentations and the control were higher than 1, and therefore potentially perceived by the human eye.

To our knowledge, this is the first time that a relationship between the anthocyanin concentration of red wines and nitrogen

Table 3. Phenolic Composition of the Final Wines[a]

	treatment[b]		
	control	250	400
malvidin-3-glucoside	1224 c	1427 b	1578 a
pigmented polymers	58 a	55 a	60 a
tannin	785 a	723 a	746 a

[a] Malvidin-3-glucoside and pigmented polymer results are expressed as mg/L malvidin-3-glucoside, and tannin results are expressed as mg/L epicatechin equivalents. [b] Control, 100 mg/L YAN; 250, same must as the control but initial YAN increased until 250 mg/L by means of DAP addition; 400, same must as the control but initial YAN increased until 400 mg/L by means of DAP addition. For each compound, different letters denote significant differences between treatments, at $p < 0.05$.

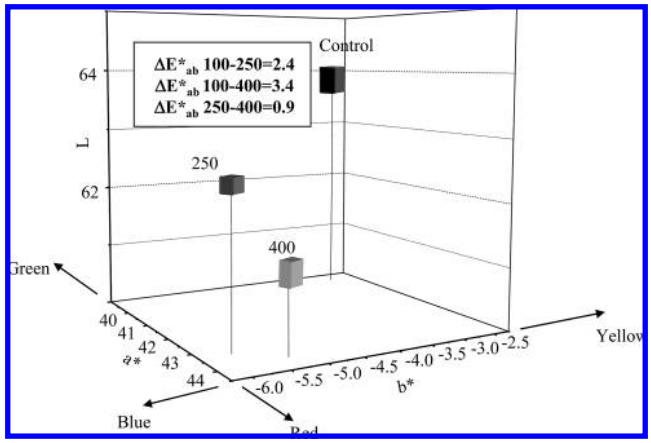

Figure 1. Effect of DAP supplementation of the must on CIELab coordinates of the young experimental wines.

supplementation of the must has been reported. Maceration variables, such as temperature and duration of pomace contact, have been reported to affect the levels of malvidin-3-glucoside in wine (*25*). In the present study, maceration length was the same for all treatments, although DAP-associated variations in the fermentation rate resulted in a longer exposure of grape solids to higher ethanol concentrations, which might have caused the increase in malvidin-3-glucoside observed. Alternatively, longer exposure to high ethanol concentrations in the DAP-supplemented fermentations may have protected anthocyanins against degradation reactions. Furthermore, although fermentations were carried out under controlled temperature and the cap was plunged 3 times daily, higher fermentation rates in the DAP-supplemented treatments resulted in increased cap maximum temperature (maximum difference recorded in the cap was 3.5 ± 0.3 °C immediately before plunging), which can also affect final anthocyanin extraction (*25*). Yeast can also influence the final color composition of the wines by, for example, absorption of anthocyanins on yeast cell walls (*26*), degradation by glycosidases (*27*), or reactions between yeast-derived metabolites, such as acetaldehyde or pyruvic acid and proanthocyanidins (*13*), to form pigmented polymers (*28*). It is not clear however how these yeast-mediated processes might be affected by DAP supplementation of the must, also considering that no difference was observed between treatments for pigmented polymers in this study. Furthermore, the tannin data indicate that initial YAN does not affect extraction of tannins. The formation of pigmented polymers appears therefore to be more linked to tannin concentration rather than to anthocyanins. Accumulation of SO_2 in response to DAP supplementation might also have contributed to increased anthocyanins extraction (*29*). Regardless of the nature of the processes responsible for the differences in color observed in this study, the values obtained for ΔE^*_{ab} by CIELab analysis indicate that the differences in color composition of the experimental wines can be perceived by the human eye. Because the pH of the samples was similar, these differences were not due to changes in wine pH. Considering the importance of color as a quality parameter for red wine, the factors linking DAP supplementation of the must to the color composition of Shiraz wine are worthy of further investigation.

Table 4. Concentration (μg/L) of Different Volatile Compounds in the Final Wines

		treatment[a]		
		control	250	400
2-methylpropanol	A[b]	36359 b	39570 b	46005 a
butanol	A	1442 a	1392 a	1347 a
2-methylbutanol	A	63412 a	61702 a	54169 b
3-methylbutanol	A	279686 b	295366 a	274322 b
2-phenylethanol	A	59869 a	53745 a	54151 a
2-methylpropanoic acid	B	1442 a	1181 b	1050 c
3-methylbutanoic acid	A	1069 a	506 b	326 c
2-methylbutanoic acid	A	1265 a	782 b	589 c
ethyl acetate	A	56790 c	64710 b	85930 a
2-methylpropyl acetate	A	24 c	49 b	110 a
2-methylbutyl acetate	A	134 c	271 b	377 a
3-methylbutyl acetate	A	1069 c	2618 b	4846 a
hexyl acetate	A	22 c	42 b	98 a
phenylethyl acetate	A	50 c	85 b	230 a
ethyl 2-methylpropanoate	A	40 a	33 b	30 b
ethyl 2-methylbutanoate	A	7 a	4 b	2 c
ethyl 3-methylbutanoate	A	10 a	7 b	1 c
ethyl propanoate	A	369 b	367 b	400 a
ethyl butanoate	A	259 c	390 b	453 a
ethyl hexanoate	A	680 b	893 a	952 a
ethyl octanoate	A	604 b	756 a	804 a
ethyl decanoate	A	131 c	198 b	303 a
ethyl dodecanoate	B	62 c	212 b	354 a
hexanoic acid	A	2889 b	4205 a	4177 a
octanoic acid	A	2531 b	3389 a	3325 a
decanoic acid	A	505 c	707 b	922 a
linalool	A	5.3 a	4.8 a	5.1 a
α-terpineol	A	4.6 ab	6.2 a	2.9 b
nerol	A	0.4 a	0.3 a	0.2 a
geraniol	A	8.6 a	6.8 a	6.3 a
β-damascenone	A	1.2 a	1.4 a	1.2 a
β-ionone	A	0.12 a	0.14 a	0.13 a
H_2S	C	4 a	2 a	6 a
DMS	C	18 a	18 a	17 a
CS_2	C	4 a	5 a	4 a
3-methylthio-1-propanol	A	184 a	156 b	138 c

[a] Control, 100 mg/L YAN; 250, same must as the control but initial YAN increased until 250 mg/L by means of DAP addition; 400, same must as the control but initial YAN increased until 400 mg/L by means of DAP addition. [b] Identification: A, identification confirmed by comparing mass spectrum and linear retention indices with commercially available pure reference compounds; B, identification confirmed by comparing mass spectrum and linear retention indices with pure reference compound synthesized in the laboratory; C, compound identified by comparing retention times and linear retention indices with commercially available pure reference standards. For each compound, different letters denote significant differences between treatments, at $p < 0.05$.

Volatile Compounds of Young Wines. A total of 36 volatile compounds were detected and quantified in the samples through the use of different analytical methods. The results are given in **Table 4**, with several fermentation-derived volatile compounds showing a strong relationship with nitrogen supplementation of the must.

Higher Alcohols and Acetates. DAP supplementation of the must had a minor influence on the final concentration of higher alcohols, with the only significant variation being 3-methylbutanol, which occurred at higher concentrations in the 250 mg/L YAN treatment. Higher alcohols are formed from the α-ke-

toacids formed as intermediates during amino acid metabolism. Availability of YAN can affect the rate of amination of α-keto acids, modulating the balance between α-keto acids that are transformed into amino acids and those that are converted into alcohols (*30*). There is a general acceptance that increased YAN can lower the formation of higher alcohols (*9, 10, 31*). However, according to Äyräpää (*8*), nitrogen additions result in decreases in higher alcohols production only when the initial YAN concentration is higher than 200−300 mg N L^{-1}, whereas for lower values of initial YAN, the formation of higher alcohols increased as YAN increased. Similar observations have been made in our laboratory for the *S. cerevisiae* strain used in this study (*10*). Gene expression studies have shown that this pattern of higher alcohols produced reflects the expression of genes involved in the amino acid biosynthesis (*32*). These observations are consistent with the behavior observed in this study for 3-methylbutanol, which peaked at intermediate nitrogen levels. Nevertheless, the other higher alcohols measured in this study showed small but different trends at increasing nitrogen levels, suggesting that further research is needed to understand the factors controlling higher alcohol formation during fermentation of red grapes, particularly in relation to the presence of high levels of grape solids (*33*). The elevated concentrations of higher alcohols noted in this study when compared to wines made from clarified musts or filtered media suggest that grape solids impose a dominant control over the synthesis of higher alcohols.

Ethyl acetate and other acetates were generally increased with increasing nitrogen, with more than 4-fold increases over the control for compounds such as 2-methylpropyl acetate, 3-methylbutyl acetate, hexyl acetate, and phenylethyl acetate in the 400 mg/L YAN treatments. Acetates are formed through condensation of alcohols with acetyl-CoA, catalyzed in the cell by alcohol acyl transferase enzymes (*34*). According to Yoshimito et al. (*32*), increased nitrogen availability can increase transcription of *ATF1* and *ATF2*, which encode the two main alcohol acyl transferases in yeast. Noteworthy, significant differences in acetate production were still observed between 250 and 400 YAN treatments, indicating that saturation of alcohol ester conversion was not reached even for more than a 5-fold increase of ammonium (initial ammonium concentration in the must was 32 mg/L). Under conditions such as those used in this study, DAP additions appeared therefore to be a powerful tool to modulate higher alcohols/acetate esters ratio during red wine production, shifting the balance toward increased formation of acetates. This might have positive implications for wine aroma characteristics, because acetate esters are among the compounds mainly responsible for wine fruitiness, with compounds such as 3-methylbutyl acetate recently indicated as important odorants of red wines (*35*).

Medium-Chain Fatty Acids and Their Esters. The addition of DAP to the must also resulted in an increase in the final concentration of medium-chain fatty acids (MCFAs) and MCFA ethyl esters, with the exception of ethyl propanoate, for which the final concentration values were not affected by initial nitrogen concentrations. Interestingly, for the main constituents of these two classes of compounds, namely, the C_6 and C_8 compounds, differences were not significant between the two higher nitrogen levels. This suggests a nitrogen saturation effect on the net synthesis of these groups of compounds. While considerable knowledge has been generated on the formation of acetate esters during fermentation, the mechanisms of formation of MCFA and their ethyl esters during fermentation are still poorly understood. Data from different model fermentation studies have shown that ammonium supplementation can have an impact on the MCFA and MCFA ethyl esters profile of wine, although the type of effect seems to vary with yeast strain and experimental conditions employed (*9, 10*). MCFAs are formed through acylation by coenzyme A during the early stages of fatty acid biosynthesis, with the exception of propanoic and, in part, butanoic acid, which are formed from α-ketobutyrate. The corresponding esters are then formed enzymatically through the action of an esterase or an acyl-CoA ethanol transferase, with the latter being responsible for a large part of the MCFA ethyl esters produced during fermentation (*34*). The observation that, in this study, the patterns of formation of MCFAs and MCFA ethyl esters were very closely linked suggests that the regulation mechanisms controlling ester formation, although still poorly understood, might be active at the level of acid production rather than esterification. Interestingly, in the case of the C_6 and C_8 compounds, differences were not significant between the two higher initial YAN concentrations, showing that, under our experimental conditions, the maximum increase in the production of these metabolites was already achieved at 250 mg/L YAN. Conversely, significant differences were observed for the formation of longer chain fatty acids and their esters between intermediate and high YAN concentrations. Increased relative losses of the lower molecular-weight compounds in the high YAN treatments, characterized by higher fermentation rates, could account for this behavior. Alternatively, it is possible that biosynthesis of the longer chain compounds is more efficiently stimulated by increased nitrogen availability, because of yeast growth stimulation increasing fatty acid synthesis.

The sensory implications of the positive relationship between YAN availability and MCFA ethyl esters observed here could be of practical interest, because these compounds are among the most important contributors to the fermentation bouquet of wine (*35*). However, although MCFA ethyl esters are characterized by sweet-fruity odors, which are generally considered positive for wine quality, a recent study on the aroma quality of red wines produced in the Bordeaux region has indicated a negative relationship between wine quality and a high concentration of compounds such as ethyl butanoate, ethyl hexanoate, and ethyl octanoate (*35*). As is the case with other odorants in wine, their concentration in relation to other odorants might also be important.

Branched-Chain Acids and Ethyl Esters. Branched-chain acids (2-methylpropanoic, 2-methylbutanoic, and 3-methylbutanoic) and their ethyl esters were found to decrease with increasing levels of nitrogen. Branched-chain acids are formed from α-ketoacids via decarboxylation and subsequent oxidation of the aldehyde formed (*36*), and therefore, they have in α-ketoacids the analogous precursor as higher alcohols. It is therefore interesting to observe that, while variations in alcohol concentrations at different YAN concentrations were mostly nonsignificant, the formation of branched-chain acids was reduced by increasing YAN, consistent with recent findings in model grape juice fermentations (*10, 11*). Moreover, under our experimental conditions, the final concentration of branched-chain acids seemed to determine the concentrations of the branched-chain ethyl esters, ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, and ethyl 3-methylbutanoate, which all decreased with increasing YAN. While previously considered of marginal importance for wine flavor, recent findings have shown that, because of their particularly low odor threshold (between 1 and 18 μg/L, *37*), ethyl esters of branched-chain acids can be important

contributors to wine flavor, being characterized by strawberry-like aromas. Further research is therefore needed to understand the factors controlling branched-chain acid metabolism in yeast.

Monoterpenes and Norisoprenoids. Availability of YAN during fermentation had no effect on the final concentration of grape-derived volatiles, such as the monoterpene alcohols linalool, nerol, geraniol, and α-terpineol. Recent studies have indicated that fermentation of low-terpene grape must can affect the monoterpene profile of wine by *de novo* biosynthesis of monoterpenes (*38*) or through hydrolysis of glycosylated monoterpene precursors (*18*). A positive relationship between YAN availability and monoterpene production has also been reported (*38*). Although in the present study a general increase in monoterepene concentration was observed with fermentation (concentration of the four monoterpenes measured in the must before inoculation was <1 μg/L), no variation in the concentration of monoterpenes was observed among the different treatments at the end of fermentation. Analysis of the enzyme hydrolysates obtained from the glycosidic material extracted from the final wines also indicated that the concentration of residual monoterpene glycosides did not vary with nitrogen availability (data not shown). These results indicate that in the present study none of the pathways that determine the monoterpene profile of wine was affected by initial YAN availability.

As for the norisoprenoids β-damascenone and β-ionone, no association was found between YAN supplementation of the must and final concentration of these compounds, although recent studies have indicated that yeast plays a determinant role in the release of these compounds from their glycosidic precursors (*39*).

Sulfur Compounds. The low-molecular-weight sulfur compounds H_2S, DMS, and CS_2 detected in wines after fermentation were generally not affected by prefermentative addition of DAP. The concentration of 3-methylthio-1-propanol was found to decrease with increasing YAN.

There is clear evidence in the literature of an inverse correlation between the concentrations of ammonium in the fermenting medium and production of H_2S during fermentation (*1, 2*). However, no relationship was observed here between the final concentration of H_2S and initial YAN availability. Rauhut et al. (*40*) reported that the final concentration of H_2S observed in wines does not reflect the amount of this compound synthesized by yeast during fermentation, because of the high volatility of H_2S. This might explain the fact that no difference was observed in this study for the final concentration of H_2S in wines produced from musts with different initial concentrations of YAN. Moreover, the use, in this study, of a must containing low methionine (2.8 ± 0.2 mg/L) might have contributed to mitigating the effect of ammonium addition on H_2S production (*41*).

DMS was also found to not vary in response to YAN supplementation of the must. The mechanism of formation of DMS during grape must fermentation is not completely understood, although there is evidence that this compound is formed from cysteine, cystine, methionine, or glutathione (*42, 43*). Studies on beer fermentation have shown that yeast can also form DMS through enzymatic reduction of dimethylsulfoxide. According to Gibson (*44*), this enzymatic activity is induced under nitrogen-limiting conditions, but such a response was not observed in this study.

A significant decrease in the concentration of 3-methylthio-1-propanol (methionol), a volatile compound characterized by a boiled potato odor, was observed with increasing YAN, consistent with previous reports (*9, 31*).

Table 5. Concentration of Different Volatile Compounds after the Model Aging Experiment

	concentration after aging (μg/L)			percent variation		
	control[a]	250	400	control	250	400
2-methylpropyl acetate	20 c	42 b	77 a	−18	−24	−26
2-methylbutyl acetate	55 c	121 b	171 a	−23	−26	−19
3-methylbutyl acetate	802 c	2136 b	3493 a	−22	−21	−20
hexyl acetate	12 c	38 b	69 a	−34	−24	−22
phenylethyl acetate	41 c	80 b	193 a	−19	−15	−20
total acetate esters	929 c	2417 b	4002 a	−22	−21	−20
ethyl propanoate	311 c	343 b	448 a	−7	+6	+14
ethyl hexanoate	593 b	897 a	881 a	−30	−28	−23
ethyl octanoate	489 b	763 a	696 a	−15	−7	−16
ethyl decanoate	101 c	152 b	185 a	−30	−36	−65
ethyl dodecanoate	31 c	51 b	79 a	−73	−85	−87
total MCFA ethyl esters	932 c	2597 b	2705 a	−13	−14	−27
ethyl 2-methylpropanoate	77 a	68 b	62 b	+90	+92	+89
ethyl 2-methylbutanoate	13 a	8 b	5 c	+109	+107	+168
ethyl 3-methylbutanoate	22 a	17 b	11 c	+98	+168	+412
total iso-acids ethyl esters	112 a	93 b	78 c	93	104	111
linalool	9.3 a	7.8 b	7.6 b	+75	+62	+49
α-terpineol	7.1 a	7.5 a	5.2 b	+54	+20	+79
nerol	0.5 a	0.2 a	nd	−25	−33	
geraniol	4.2 a	1 c	2.1 b	−51	−85	−66
total monoterpenes	21 a	16 b	14 b	+1	+2	+1
β-damascenone	2.5 a	2.7 a	2.5 a	+108	+93	+92
H_2S	nd	nd	nd			
DMS	88 b	89 b	112 a	+388	+394	+559
CS_2	2 a	3 a	2 a	−50	−40	−50

[a] Control, 100 mg/L YAN; 250, same must as the control but initial YAN increased until 250 mg/L by means of DAP addition; 400, same must as the control but initial YAN increased until 400 mg/L by means of DAP addition. For each compound, different letters denote significant differences between treatments at $p < 0.05$.

Evolution of Wine Volatile Composition during Model Aging. Under the conditions of the model aging experiment, significant variations occurred in the volatile composition of the wines. The compounds that changed in concentration over the 6 weeks of storage at 30 °C (concentration variation ≥ 10% in at least one treatment) are shown in **Table 5**. Esters were the group of compounds undergoing the most significant changes. Acetates generally decreased in concentration, which was quantitatively more significant in the 250 and 400 mg/L YAN wine samples, where the initial concentration of acetates was higher. A decrease was also observed for MCFA ethyl esters, which was larger in the 400 mg/L YAN samples. These trends seem to be consistent with the observation that, in this study, higher YAN availability stimulated ester production, thereby increasing the postfermentation wine concentration of these compounds and, consequently, their rate of hydrolysis during aging. Nevertheless, it has to be pointed out that, at the end of the aging experiment, the DAP-treatment wines were still characterized by significantly higher concentrations of acetates and MCFA ethyl esters compared to the controls. This result suggests that the effects of DAP on these fermentation-derived esters are likely to persist even after aging, to which red wines are typically subjected.

Branched-chain esters were the only group of esters showing an increase during aging. The extent of these increases was lower in the low YAN treatment wines, in agreement with the higher postfermentation concentration of branched acids observed in these same samples. This confirms recent observations indicating acid−ester equilibrium as the major pathway of formation of branched-chain ethyl ester during wine aging (*45*). In general, on the basis of their respective odor threshold and odor activity values (OAV) (*37*), none of the esters for which

concentration changes were observed was found to become sensorially active (OAV > 1) or to drop at sub-threshold concentrations as a consequence of aging. The only exception was ethyl-3-methylbutanoate, which was found at concentrations of 11 μg/L in the 400 YAN treatment (OAV 3.7), from an initial OAV of 0.3.

Total monoterpenes were found at slightly higher concentrations in the non-supplemented treatments. Individual compounds showed specific patterns, with linalool and α-terpineol increasing and geraniol and nerol decreasing. Differences however were generally quantitatively small, suggesting negligible sensory effects. These changes probably reflect the fact that acid-catalyzed hydrolysis of geraniol glucosides is slower than linalool glucosides, while free forms of geraniol can be converted into linalool and eventually to α-terpineol during aging (*46*). Other precursors, such as terpene diols, might also be involved (*46*). The increase observed for β-damascenone is consistent with the mechanism of acid-catalyzed formation of this compound described by other authors (*46*), and it did not appear to depend upon DAP supplementation of the must.

Among the sulfur volatiles, an extremely marked increase of DMS was observed, particularly in the 400 mg/L YAN wines, where concentrations of up to 559% higher than those detected in the young wines resulted. H_2S was not detected in any of the YAN treatment wine samples, and CS_2 was also found to decrease. DMS contribution to wine aroma, particularly in aged wines, has been described as enhancing the strawberry/raspberry character, with additional truffle, black olive, and cabbage notes, and this compound has been reported to play an important role in the sensory profile of Syrah wines (*47*). Enhanced formation of DMS during aging has also been observed in conjunction with higher concentrations of cysteine (*48*) and *S*-methylmethionine (*49*). It is possible, therefore, that the higher DMS found here after aging is linked to the higher residual YAN of the DAP-supplemented fermentations. DMS showed the most interesting aging-related change in terms of odor activity under the conditions of this experiment, because the final concentration was clearly affected by both aging and fermentation treatment. As a consequence, while initial OAVs (on the basis of the threshold of 10 μg/L reported in ref *37*) were all in the range of 1.7−1.8, a clear separation between treatments could be observed following aging, with values of 8.8−8.9 for the 100 and 250 YAN treatments and of 11.2 for the 400 YAN treatments. Given the complex role of DMS both as an enhancer of red wine fruitiness and responsible of cabbage-like off-flavors, the sensory implications of these changes will have to be specifically investigated.

In summary, DAP supplementation of must prior to alcoholic fermentation has a strong influence on the composition of constituents responsible for key quality parameters of red wines, such as color, aroma, and flavor. Increased DAP supplementation resulted in a higher postfermentation concentration of malvidin-3-glucoside and was associated with higher color intensity. Wine concentrations of glycerol increased, and acetic acid decreased in response to YAN supplementation, which is likely to enhance wine quality. DAP supplementation of must was also positively related to the formation of acetates and MCFA ethyl esters. At the same time, the formation of branched-chain acids and their corresponding ethyl esters was reduced under conditions of high DAP supplementation. The model aging experiment indicated that, during aging, some of the differences observed in young wines tend to disappear, particularly regarding MCFA ethyl esters, which decreased at a higher rate in DAP-treatment wines. However, the powerful odorant DMS, for which no difference was observed in the young wines, showed a positive relationship with prefermentative DAP supplementation in the aged wines. Further studies are now in progress to rationalize the effect of DAP supplementation of red grape must on Shiraz volatile and color composition and the evolution of volatile compounds undergoing dynamic changes during wine aging.

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LITERATURE CITED

(1) Bell, S. J.; Henschke, P. A. Implications of nitrogen nutrition for grapes, fermentation and wine. *Aust. J. Grape Wine Res.* **2005**, *11*, 242–295.

(2) Jiranek, V.; Langridge, P.; Henschke, P. A. Regulation of hydrogen sulfide liberation in wine-producing *Saccharomyces cerevisiae* strains by assimilable nitrogen. *Appl. Environ. Microbiol.* **1995**, *61*, 461–467.

(3) Ingledew, W. M.; Kunkee, R. E. Factors influencing sluggish fermentations of grape juice. *Am. J. Enol. Vitic.* **1985**, *36*, 65–76.

(4) Bely, M.; Sablayrolles, J. M.; Barre, P. Automatic detection of assimilable nitrogen deficiencies during alcoholic fermentation in enological conditions. *J. Ferment. Bioeng.* **1990**, *70*, 246–252.

(5) Stines, A. P.; Grubb, J.; Gockowiak, H.; Henschke, P. A.; Hoj, P. B.; van Heeswick, R. Proline and arginine accumulation in developing berries of *Vitis vinifera* L. in Australian vineyards: Influence of vine cultivar, berry maturity and tissue type. *Aust. J. Grape Wine Res.* **2000**, *6*, 150–158.

(6) Butzke, C. Survey of yeast assimilable nitrogen status in musts from California, Oregon, and Washington. *Am. J. Enol. Vitic.* **1998**, *49*, 220–224.

(7) Nicolini, G.; Larcher, R.; Versini, G. Status of yeast assimilable nitrogen in Italian grape musts and effect of variety, ripening and vintage. *Vitis* **2004**, *43*, 89–96.

(8) Äyräpää, T. Biosynthetic formation of higher alcohols by yeast. Dependence on the nitrogenous nutrient level of the medium. *J. Inst. Brew.* **1971**, *77*, 266–276.

(9) Hernández-Orte, P.; Bely, M.; Cacho, J.; Ferreira, V. Impact of ammonium additions on volatile acidity, ethanol, and aromatic compounds production by different *Saccharomyces cerevisiae* strains during fermentation in controlled synthetic media. *Aust. J. Grape Wine Res.* **2006**, *12*, 150–160.

(10) Vilanova, M.; Ugliano, M.; Siebert, T.; Pretorius, I. J.; Henschke, P. A. Assimilable nitrogen utilization and production of volatile and non-volatile compounds in chemically defined medium by *Saccharomyces cerevisiae* wine strains. *App. Microbiol. Biotechnol.* **2007**, *77*, 145–157.

(11) Hernández-Orte, P.; Ibaz, M. J.; Cacho, J.; Ferreira, V. Effect of the addition of ammonium and amino acids to must of Airen variety on aromatic composition and sensory properties of the obtained wine. *Food Chem.* **2005**, *89*, 163–174.

(12) Bakker, J.; Timberlake, C. F. Isolation, identification, and characterization of new color-stable anthocyanins occurring in some red wines. *J. Agric. Food Chem.* **1997**, *45*, 35–43.

(13) Fulcrand, H.; Benabdeljalil, C.; Rigaud, J.; Cheynier, V.; Moutounet, M. A new class of wine pigments generated by reaction between pyruvic acid and grape anthocyanins. *Phytochemistry* **1998**, *47*, 1401–1407.

(14) Ness, F.; Lavallee, F.; Dubourdieu, D.; Aigle, M.; Dulau, L. Identification of yeast stains using the polymerase chain reaction. *J. Sci. Food Agric.* **1993**, *62*, 89–94.

(15) Parker, M.; Smith, P. A.; Brise, M.; Francis, I. L.; Kwiatkowsky, M. J.; Lattey, K. A.; Liebich, B.; Herderich, M. J. The effect of pre- and post-ferment additions of grape derived tannin on Shiraz

wine sensory properties and phenolic composition. *Aust. J. Grape Wine Res.* **2007**, *13*, 30–37.
(16) Mercurio, M. D.; Dambergs, R. G.; Herderich, M. J.; Smith, P. A. High throughput analysis of red wine and grape phenolics—Adaptation and validation of methyl cellulose precipitatable tannin assay and modified Somers color assay to a rapid 96 well plate format. *J. Agric. Food Chem.* **2007**, *5*, 4651–4657.
(17) Siebert, T. E.; Smyth, H. E.; Capone, D. L.; Neuwöhner, C.; Pardon, K. H.; Skouroumounis, G. K.; Herderich, M. J.; Sefton, M. A.; Pollnitz, A. P. Stable isotope dilution analysis of wine fermentation products by HS−SPME−GC−MS. *Anal. Bioanal. Chem.* **2005**, *381*, 937–947.
(18) Ugliano, M.; Bartowsky, E. J.; McCarthy, J.; Moio, L.; Henschke, P. A. Hydrolysis and transformation of grape glycosidically bound volatile compounds during fermentation with three *Saccharomyces* yeast strains. *J. Agric. Food Chem.* **2006**, *54*, 6322–6331.
(19) Kosteridis, Y.; Baumes, R.; and Skouroumounis, G. K. M. A. Synthesis of labeled [$^2H_4$] β-damascenone, [$^2H_2$] 2-methoxy-3-isobutylpyrazine, [$^2H_3$] α-ionone and [$^2H_3$] β-ionone for quantification in grapes, juices and wines. *J. Chromatogr., A* **1998**, *824*, 71–78.
(20) Siebert, T. E.; Pollnitz, A. P. Analysis of low molecular weight sulfur compounds in wine. In *Proceedings of the 13th Australian Wine Industry Technical Conference*; Blair, R. J.; Williams, P. J.; Pretorius, I. S., Eds.; Australian Wine Industry Technical Conference, Inc.: Adelaide, Australia, 2008; pp 281−282.
(21) Ugliano, M.; Moio, L. The influence of malolactic fermentation and *Oenococcus oeni* strain on glycosidic aroma precursors and related volatile compounds of red wine. *J. Sci. Food Agric.* **2006**, *86*, 2468–2476.
(22) Correia, I.; Salgueiro, S. P.; Viegas, C. A.; Novais, J. M. Leakage induced by ethanol, octanoic and decanoic acids in *Saccharomyces cerevisiae*. *Yeast* **1989**, *5*, S123–S127.
(23) Bely, M.; Rinaldi, A.; Dubourdieu, D. Influence of assimilable nitrogen on volatile acidity production by *Saccharomyces cerevisiae* during high sugar fermentation. *J. Biosci. Bioeng.* **2003**, *96*, 507–512.
(24) Osbourne, J. P.; Edwards, C. G. Inhibition of malolactic fermentation by *Saccharomyces* during alcoholic fermentation under low- and high-nitrogen conditions. A study in synthetic media. *Aust. J. Grape Wine Sci.* **2006**, *12*, 69–78.
(25) Zimman, A.; Joslin, W. S.; Lyon, M. L.; Meier, J.; Waterhouse, A. L. Maceration variables affecting phenolic composition in commercial-scale Cabernet Sauvignon winemaking trials. *Am. J. Enol. Vitic.* **2002**, *53*, 93–98.
(26) Morata, A.; Gomez-Cordoves, M. C.; Suberviola, J.; Bartolomé, B.; Colomo, B.; Suárez, J. A. Adsorption of anthocyanins by cell walls during the fermentation of red wines. *J. Agric. Food Chem.* **2003**, *51*, 4084–4088.
(27) Manzanares, P.; Rojas, V.; Genoves, S.; Valles, S. A preliminary search for anthocyanin-β-D-glucocidases activity in non-*Saccharomyces* wine yeasts. *Int. J. Food Sci. Technol.* **2000**, *35*, 95–103.
(28) Romero, C.; Bakker, J. Interactions between grape anthocyanins and pyruvic acid, with effect of pH and acid concentration on anthocyanin composition and color in model systems. *J. Agric. Food Chem.* **1999**, *47*, 3130–3139.
(29) Bakker, J.; Bridle, P.; Bellworthy, S. J.; Garcia-Viguera, C.; Reader, H. P.; Watkins, S. J. Effect of sulphur dioxide and must extraction on color, phenolic composition and sensory quality of red table wine. *J. Sci. Food Agric.* **1998**, *78*, 297–307.
(30) Oshita, K.; Kubota, M.; Uchida, M.; Ono, M. Clarification of the relationship between fusel alcohol formation and amino acid assimilation by brewing yeast using ^{13}C-labeled amino acid. In Proceedings of the European Brewing Convention, Bruxelles, Belgium, 1995; pp 387−394.
(31) Rapp, A.; Versini, G. Influence of nitrogen compounds in grapes on aroma compounds of wine. In Proceedings of the International Symposium on Nitrogen in Grapes and Wine, American Society for Enology and Viticulture, Davis, CA, 1991; pp 156−164.
(32) Yoshimoto, H.; Fukushige, T.; Yonezawa, T.; Sone, H. Genetic and physiological analysis of branched-chain alcohols and isoamyl acetate production in *Saccharomyces cerevisiae*. *Appl. Microbiol. Biotechnol.* **2002**, *9*, 501–508.
(33) Crowell, E. A.; Guymon, J. F. Influence of aeration and suspended material on higher alcohols, acetoin, and diacetyl during fermentation. *Am. J. Enol. Vitic.* **1963**, *14*, 214–222.
(34) Mason, A. B.; Dufour, J.-P. Alcohol acetyltranferase and the significance of ester synthesis in yeast. *Yeast* **2000**, *16*, 1278–1298.
(35) Guth, H.; Sies, A. Flavor of wines. Towards an understanding by reconstitution experiments and an analysis of ethanol's effect on odour activity of key compounds. In *Proceedings of the 11th Australian Wine Industry Technical Conference*; Blair, R. J., Williams, P. J., Høj, P. B., Eds.; Australian Wine Industry Technical Conference, Inc.: Glen Osmond, Australia, 2002; pp 128−139.
(36) Large, P. J. Degradation of organic nitrogen compounds by yeasts. *Yeast* **1986**, *2*, 1–34.
(37) Francis, I. L.; Newton, J. L. Determining wine aroma from compositional data. *Aust. J. Grape Wine Res.* **2005**, *11*, 114–126.
(38) Carrau, F. M.; Medina, K.; Boido, E.; Farina, L.; Gaggero, C.; Dellacassa, E.; Versini, G.; Henschke, P. A. *De novo* synthesis of monoterpenes by *Saccharomyces cerevisiae* wine yeasts. *FEMS Microbiol. Lett.* **2005**, *243*, 107–115.
(39) Loscos, N.; Hernandez-Orte, P.; Cacho, J.; Ferreira, V. Release and formation of varietal aroma compounds during alcoholic fermentation from nonfloral grape odorless flavor precursors fractions. *J. Agric. Food Chem.* **2007**, *55*, 6674–6684.
(40) Rauhut, D.; Kürbel, H.; Dittrich, H. H.; Grossmann, M. Properties and differences of commercial yeast strains with respect to their formation of sulphur compounds. *Vitic. Enol. Sci.* **1996**, *51*, 187–192.
(41) Spiropoulos, A.; Tanaka, J.; Flerianos, I.; Bisson, L. Characterization of hydrogen sulphide formation in commercial and natural wine isolates of *Saccharomyces*. *Am. J. Enol. Vitic.* **2000**, *51*, 233–248.
(42) Rauhut, D. Yeast—Production of sulphur compounds. In *Wine. Microbiology and Biotechnology*; Fleet, G. H., Ed.; Harwood Academic Publishers: Chur, Switzerland, 1993; pp 77−164.
(43) de Mora, S. J.; Eschenbruch, R.; Knowles, S. J.; Spedding, D. J. The formation of dimethyl sulphide during fermentation using a wine yeast. *Food Microbiol.* **1986**, *3*, 27–32.
(44) Gibson, R. M.; Large, P. J. The influence of assimilable nitrogen compounds in wort on the ability of yeast to reduce dimethyl sulphoxyde. *J. Inst. Brew.* **1985**, *91*, 401–405.
(45) Diaz-Maroto, M. C.; Schneider, R.; Baumes, R. Formation pathways of ethyl esters of branched short-chain fatty acids during wine aging. *J. Agric. Food Chem.* **2005**, *53*, 3503–3509.
(46) Skouroumounis, G. K.; Sefton, M. A. Acid-catalyzed hydrolysis of alcohols and their β-D-glucopyranosides. *J. Agric. Food Chem.* **2000**, *48*, 2033–2039.
(47) Segurel, M. A.; Razungles, A. J.; Riou, C.; Salles, M.; Baumes, R. Contribution of dimethyl sulfide to the aroma of Syrah and Grenache noir wines and estimation of its potential in grapes of these varieties. *J. Agric. Food Chem.* **2004**, *52*, 7089–7093.
(48) De Mora, S. J.; Lee, P.; Shooter, D.; Eschenbruch, R. The analysis and importance of dimethylsulfoxyde in wine. *Am. J. Enol. Vitic.* **1993**, *44*, 327–332.
(49) Segurel, M. A.; Razungles, A. J.; Riou, C.; Triguiero, M. G. L.; Baumes, R. Ability of possible DMS precursors to release DMS during wine aging in the conditions of heat-alkaline treatment. *J. Agric. Food Chem.* **2005**, *53*, 2637–2645.

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