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Volatile Composition and Sensory Properties of Shiraz Wines As Affected by Nitrogen Supplementation and Yeast Species: Rationalizing Nitrogen Modulation of Wine Aroma

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The effects of yeast assimilable nitrogen (YAN) supplementation on Shiraz volatile composition and sensory properties have been investigated. A low YAN Shiraz must (YAN 100 mg/L) was supplemented with nitrogen in the form of diammonium phosphate (DAP) to a final YAN of either 250 or 400 mg/L. Fermentation was carried out with either *Saccharomyces cerevisiae* or *Saccharomyces bayanus*, with maceration on skins. For both yeast strains, high DAP additions increased the ratings of positive sensory attributes such as "red fruit" and "dark fruit" and decreased the "yeast/cheese", "vegetal", and "earth/dirty" attributes. For the *S. cerevisiae* yeast moderate DAP addition resulted in higher "reduced" attribute scores. DAP supplementation had a strong influence on formation of acetates, fatty acid ethyl esters, higher alcohols, hydrogen sulfide, ethyl mercaptan, methyl mercaptan, DMS, and DES. Partial least-squares regression analysis of chemical and sensory data indicated that esters, sulfides, and mercaptans were associated with fruit-related descriptors, whereas hydrogen sulfide was associated with the "reduced" attribute. Nitrogen-related variations in the concentration of other yeast metabolites such as ethanol and 2- and 3-methylbutanoic acids also affected perceived fruitiness. Depending on yeast species DAP supplementation to a low nitrogen must can result in increased reduction off-odor.

KEYWORDS: Wine aroma; esters; hydrogen sulfide; mercaptans; nitrogen; *Saccharomyces*

INTRODUCTION

Wine aroma is one of the main attributes determining consumers' preference (*1*). In the case of red wines, perceived aroma quality depends on complex interactions between a relatively large number of aroma compounds that are predominantly derived from grapes, yeast and bacterial metabolism, and post-fermentation technological operations, when applied (*2*). From a sensory point of view, berry fruit aromas are of primary importance for consumers preference of red wines from different grape varieties and origin (*3*−*6*). In contrast, leaving aside the obvious quality depreciation associated with the occurrence of cork, microbial, or oxidative off-flavors, the so-called "reduction" character, often described as rotten egg or burnt rubber, can result in low consumer preference and is considered to be a primary cause of loss of perceived quality (*7*).

Volatile aroma-active metabolites produced by the yeast during fermentation provide the backbone of wine aroma composition (*8*). Among these, esters have often been proposed as the group of compounds more directly responsible for wine fruity attributes (*5*, *9*), and specific ester profiles have been shown to determine whether red berry or black berry characters will be perceived in red wines (*6*). However, a specific investigation on the role of esters in the aroma of young wines did not find any clear relationship between fruity esters and red wine fruity aromas (*10*). This might depend on the fact that the contribution of esters to wine perceived fruitiness can be modulated by other aroma compounds simultaneously present in the wine, including other yeast-derived metabolites such as higher alcohols (*11*), and the sulfur compounds dimethyl sulfide (DMS) and methionol (*2*,*5*,*12*). Additionally, yeast metabolism can generate significant amounts of the low molecular weight sulfur compounds hydrogen sulfide (H_2S), methanethiol, and ethanethiol. Whereas these compounds are considered the main agents of "reduction" faults, recent data suggest that low concentrations of H_2S can in some cases add complexity to wine aroma (*13*). Due to this complex picture, the achievement of specific red wine styles displaying adequate levels of clean fruity aromas with no perceivable aroma fault still represents a major challenge in red wine production.

Choice of yeast strain is often used in the modern winemaking as a tool to achieve specific aroma profiles (*14*). Different strains of the winemaking yeast *Saccharomyces cerevisiae* have been long known to generate a variety of aroma profiles, due to their unique genetic traits (*15*). In addition, increased aroma diversity is achievable by the use of non-*Saccharomyces* strains (*16*). However, strains with a volatile fingerprint having potentially "desirable" traits often possess other less desirable characters such as suboptimal fermentation performance or production of off-flavors, which

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limit their effectiveness in producing wines with optimized aroma profiles. More recently, the importance of available nitrogen to the ability of yeast to synthesize key wine aroma compounds such as esters has been shown (*17*−*20*), and it has been suggested that nitrogen management of wine fermentation can be an effective tool to modulate wine aroma composition and style (*21*). In the case of red wine fermentations, Ugliano et al. (*20*, *22*) have shown that nitrogen supplementation can significantly alter the pattern of esters and sulfur compounds produced by yeast during fermentation of red wine. As esters are likely to positively influence wine aroma and sulfur compounds are associated with the so-called "reduced" off-flavors, the actual benefits of nitrogen supplementation to red wine aroma profiles remain therefore to be established.

In this study, we have investigated the effect of nitrogen supplementation on the aroma chemical composition and sensory properties of Shiraz wines obtained by fermentation with two different yeast species. By using multivariate analysis, the correlations between chemical and sensory changes induced by nitrogen supplementation and yeast strain have been explored to ascertain the effects of the chemical changes induced by nitrogen additions on the perceived aroma of wine.

MATERIALS AND METHODS

Winemaking. Low yeast assimilable nitrogen (YAN) Shiraz grapes were obtained from the Langhorne Creek winemaking district in South Australia during the 2007 vintage. The grapes were hand-picked and collected in 15 kg plastic bins. Once in the winery, fruit from different bins was pooled together to obtain a homogeneous mass. Individual lots of 30 kg were destemmed and crushed, and the must was collected in 50 L stainless steel fermentation vessels. The analytical parameters of the must were as follows: total soluble solids, 24.0 °Brix; titratable acidity, 7.2 g/L as tartaric acid; pH, 3.30; YAN, 101 mg/L. Potassium metabisulfite was added at 100 mg/kg to each must lot approximately 2 h before inoculation. Diammonium phosphate (DAP) additions were performed according to an experimental design consisting of three YAN concentrations, each fermented in triplicate, for a total of nine fermentations for each yeast. A control that did not receive any DAP addition represented the lowest nitrogen concentration (101 mg/L YAN), whereas in the two other treatments DAP was added to a final YAN concentration of 250 or 400 mg/L YAN. All DAP additions were performed prior to inoculation. Following DAP additions, the pH of the musts was measured and readjusted to 3.30 by means of 1 M HCl. The musts were inoculated with either of two yeasts, *Saccharomyces cerevisiae* D254 (Lallemand, Montreal, Canada) or *Saccharomyces bayanus* AWRI 1176 (Lallemand), 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 ± 1.5 °C, with the cap submerged three times per day. Fermentation progress was monitored by enzymatic analysis of the sugars. Dominance of the inoculated strain in all of the treatments was confirmed by transposon PCR analysis. 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; the wines were collected in 20 L stainless steel containers and placed at 4 °C under a headspace of inert gas 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 in 375 mL bottles with roll-on tamper-evident screw-cap closures fitted with a Saranin wad. The wines were stored at 14−16 °C until analyzed, with all chemical and sensory analyses carried out after 3 months of storage. One replicate of the D254 250 mg/L YAN, which had shown anomalous behavior during fermentation, proved to be an outlier. Therefore, it was decided to leave this out of the study. In all cases, ammonia was not detected in any of the wines at the end of fermentation. Data on fermentation performance and nitrogen uptake during fermentation have been previously reported (*22*).

Grape Must Analyses. Titratable acidity, free amino nitrogen (FAN), and ammonia were measured as previously described (*20*). YAN was calculated as the sum of ammonia-derived nitrogen and FAN and, therefore, did not include proline. Fermentation progress was monitored by daily analysis of residual sugar by means of a glucose and fructose enzymatic kit (*20*).

Table 1. Composition of Aroma Reference Standards

aroma attribute	recipes used for standards[a]
reduced	1/4 teaspoon of ash/charcoal
bruised apple	15 μL of acetaldehyde (>99% pure)
dark fruit	5 mL of Ribena black currant cordial + 1 frozen blackberry, squashed + 1/2 canned dark plum (SPC) squashed + 1 tinned black cherry squashed
red fruit	1 frozen raspberry, 1 canned Morello cherry
confectionary	1 raspberry lolly (Black and Gold)
vegetal	1 teaspoon of canned corn juice + 1 teaspoon of canned bean juice
savory	1 small pinch of beef stock cube
yeast	1/4 teaspoon of dried yeast + 1/4 teaspoon of Vegemite
cheese/sweat	1/8 teaspoon of Roquefort cheese
earth/dirty	1/4 teaspoon of fresh compost, 10 μL of geosmin (62 μg/L), 10 μL of octen-3-one (1 mg/mL)
floral	80 μL of β-ionone (1 g/L)
nail polish remover	10 μL of ethyl acetate (1000 g/L)
coffee/chocolate	1 chocolate-coated coffee bean, crushed

[a] In 25 mL of base wine (Yalumba Shiraz, 2007, 2 L cask wine 11% alcohol/vol).

Analysis of Volatile Compounds in Wines. Acetaldehyde was analyzed by means of an enzymatic kit (Megazyme, Wicklow, Ireland). Cuvettes were sealed during incubation to avoid volatilization of the compound. Analysis of fermentation-derived esters, alcohols, and acids was carried out by gas chromatography−mass spectrometry (GC-MS) using a stable isotope dilution assay (SIDA), as described previously (*20*). The low molecular weight sulfur compounds H_2S and methyl mercaptan were analyzed by GC coupled with atomic emission detection (AED), using static headspace sampling and cool on-column injection, as described by Siebert et al. (*23*). Wine bottles were cooled to 4 °C prior to opening, and all sample handling was completed in a temperature-controlled room at 4 °C. An aliquot of wine (10 mL) was added to a 20 mL amber glass headspace vial containing 2 g of NaCl and a 3 mm magnetic stir bar. Ethylmethyl sulfide and propylthio acetate (Sigma-Aldrich, Castle Hill, NSW, Australia) were added as internal standards to a final concentration of 50 or 125 μg/L, respectively. The vials were tightly sealed with a white PTFE/blue silicone-lined screw cap (Grace Davison Discovery Sciences, Baulkham Hills, NSW, Australia). Analytical conditions for the other volatile sulfur compounds have been described previously (*22*). All analyses were performed in duplicate. The data on volatile sulfur compounds have been previously published (*22*).

Sensory Descriptive Analysis. A panel of 11 assessors (7 female, 4 male) were convened for this study. Assessors were selected on the basis of their performance in previous studies carried out in our laboratories (discrimination, reliability, and degree of agreement with the panel mean) with all but one of the assessors having had previous experience in wine sensory descriptive analysis.

A list of aroma and flavor attributes for the experimental wines was generated by the panel during three preliminary discussion sessions. Two practice rating sessions were held under the same conditions as the subsequent formal sessions, except that the same sample presentation order was used for each assessor. Samples were assessed in duplicate in six formal sessions with six samples presented per session. The samples were presented in groups according to their fermentation replicate (i.e., all fermentation replicate one samples were presented in session one, fermentation replicate two samples were presented in session two, and fermentation replicate three samples were presented in session three) and were presented in a random order across tasters. For the second presentation replicate, the order of the fermentation replicates was changed so that session four had fermentation replicate three, session five had fermentation replicate one, and session six had fermentation replicate two. Using this design one example of each treatment was presented per tasting session. All samples for sensory evaluation were assessed in isolated tasting

Table 2. Probability Values (p) from ANOVA for Each Attribute Rated for the Three Nitrogen Treatments, the Two Different Yeast Strains, and Their Interaction

attribute	yeast	nitrogen	yeast × nitrogen
reduced	0.2059	**0.0023**	**0.0008**
bruised apple	**<0.0001**	**0.0003**	**0.0047**
dark fruit	0.4372	**0.0036**	0.1801
red fruit	0.0508	**<0.0001**	0.0557
confectionary	0.2845	**<0.0001**	0.1388
vegetal	0.0684	**0.0019**	**0.0469**
coffee/choc	0.3758	0.6111	0.3864
yeast	0.2322	**<0.0001**	**0.0213**
cheese/sweat	0.1105	**<0.0001**	**0.0017**
earth/dirty	0.3001	**0.0011**	**0.0474**
floral	0.2121	**0.0001**	**0.0364**
savory	0.2027	**<0.0001**	0.1556
nail polish remover	0.8453	0.3325	0.1683
degrees of freedom	1	2	2

booths. Samples were presented in coded, covered ISO tasting glasses at 22−23 °C (30 mL aliquots) under daylight-type lighting. The samples were rated for 14 aroma attributes (13 defined terms and "other") on unstructured line scales, with anchors of "low" and "high" placed at 10 and 90% of the line, respectively. All data were collected using FIZZ software (version 2.30B, Biosystemes, Couternon, France). Reference standards (**Table 1**) were available to the panelists in the booths for the aroma attributes.

Statistical Analysis. For the data on volatile compounds, analysis of variance and least significant difference (LSD) test were used to interpret the differences in means at the 95% significance level. The data were processed using JMP 5.0.1 (SAS, Cary, NC). For the sensory analysis data, analysis of variance (ANOVA) was carried out for the effects of judge, yeast, treatment, yeast × treatment interaction, presentation replicate nested within yeast and treatment, fermentation replicate nested within yeast and treatment, and fermentation replicate nested within yeast and treatment, with judge treated as a fixed effect. Panelist performance was assessed using FIZZ and Senstools 3.3.1 (O&P Product Research BV, The Netherlands). Principal component analysis (PCA) and partial least-squares regression (PLSR) were carried out using Unscrambler 9.5 (CAMO Technologies Inc., Woodbridge, NJ). All PLSR analyses were carried out using a PLSR2 algorithm with full cross-validation, with the y data set being the sensory aroma attribute scores and the x data being the chemical compositional data. All data were standardized.

RESULTS AND DISCUSSION

Sensory Descriptive Analysis. ANOVA carried out on the results obtained from sensory descriptive analysis showed that both yeast strain and nitrogen supplementation had a strong influence on the sensory characteristics of the experimental wines (**Table 2**). For the attributes "dark fruit", "red fruit", "confectionary", "savory", there was a significant effect of the YAN treatment, with no yeast by nitrogen interaction effect, showing that the YAN effect was independent of the yeast strain used. For the attributes "reduced", "bruised apple", "vegetal", "yeast", "cheese/sweat", "earth/dirty", and "floral", there was a significant effect of yeast by nitrogen interactions. No significant effect of yeast, nitrogen, or yeast by nitrogen interactions was observed for the attribute "nail polish". In no case was there a significant difference between fermentation replicates within treatment for any attribute.

The results of the sensory descriptive analysis are shown in **Figure 1**. For yeast D254, addition of nitrogen at 250 mg/L resulted in a sensory profile similar to that of the wine made with no addition, although there was a slight but not significantly different increase of "dark fruit", "red fruit", and "confectionary" attributes, as well as a decrease of the "cheese/sweaty" and "earth/dirty" attributes. The main effect of the moderate nitrogen supplementation was a substantial and significant increase of

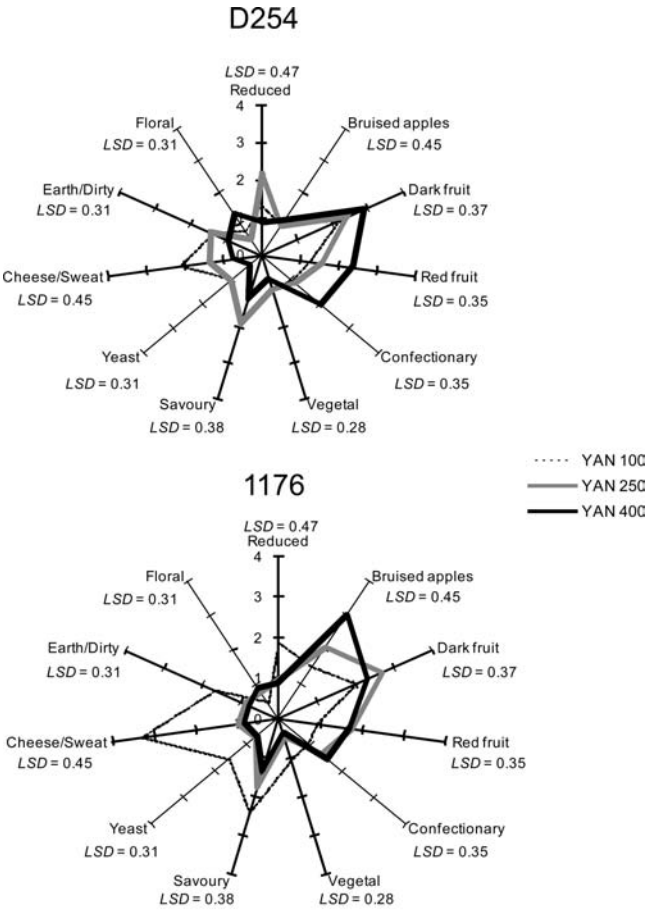

Figure 1. Sensory profiles of the experimental wines. 100 mg/L YAN has no nitrogen addition; 250, same must as the 100 but initial YAN increased until 250 mg/L by means of DAP addition; 400, same must as 100 but initial YAN increased until 400 mg/L by means of DAP addition.

the "reduced" attribute. In contrast, the 400 mg/L treatment was rated as significantly lower than the control wine in the "reduced", "savory", "yeast", and "cheese/sweaty" attributes and significantly higher in "confectionary", "floral", "dark fruit", and "red fruit". For the AWRI 1176 yeast, the attributes "reduced", "savory", "vegetal", "yeast", "cheese/sweat", and "earth/dirty" were rated most highly in the control wine. Both YAN addition treatments were rated similarly, being higher in "dark fruit", "red fruit", "confectionary", and "floral" than the control, with lower scores in the other attributes. The major difference between the two nitrogen addition treatments was in the "bruised apple" attribute, which increased with increasing nitrogen.

Globally, 12 descriptors were significantly affected by the addition of DAP, many of which can be considered of high importance to red wine aroma quality, including dark fruit, red fruit, confectionary, and reduced (**Table 2** and **Figure 1**). To our knowledge, this is the first time that the effects of nitrogen supplementation on the fruity aromas of red wine are described. Previous work carried out in our laboratory on Chardonnay indicated that supplementation of a low-nitrogen juice (YAN 160 mg/L) with DAP to achieve a YAN of 320 mg/L resulted in an increase in fruit-related descriptors, although higher concentrations of initial nitrogen resulted in excessive nail polish remover aromas, probably due to the very high concentrations of ethyl acetate and other acetate esters (*24*). Other studies, however, have observed that nitrogen supplementation induces only relatively

Table 3. Primary Composition of the Experimental Wines

	D254			1176		
	100 YAN	250 YAN	400 YAN	100 YAN	250 YAN	400 YAN
alcohol (%)	14.2 a[a]	13.5 b	13.3 c	14.3 a	13.9 b	13.5 c
pH	3.53 a	3.42 b	3.07 c	3.53 a	3.35 b	3.11 c
volatile acidity (expressed as g/L of acetic acid)	0.49 a	0.45 b	0.45 b	0.47 a	0.45 a	0.45 a
titratable acidity (expressed as g/L of tartaric acid)	6.8 a	6.6 a	7.2 a	8 a	7.4 b	7.1 c
free SO_2	39 b	41 b	43 a	41 b	42 b	45 a
total SO_2	67 a	67 a	70 a	70 c	82 b	90 a
glycerol (g/L)	8.9 c	9.3 b	9.8 a	13.2 c	14.1 b	14.5 a
glucose + fructose (g/L)	nd	nd	nd	0.3 a	0.6 a	0.3 a

[a] Within each yeast, different letters denote statistically significant differences at $p < 0.05$. nd, not detected.

small changes in wine fruity attributes (*19*), possibly due to a smaller range of nitrogen concentrations (175−375 mg/L) studied. In addition, our results indicate that, depending on the yeast strain, moderate nitrogen addition can increase reduced attributes, as observed here for D254. This observation is surprising, as addition of nitrogen is commonly carried out to prevent the occurrence of reduction off-odors in the wines. This effect, however, was observed for only one of the two strains used, highlighting the complex relationship between yeast selection, nitrogen management, and wine sensory characteristics. In this sense, the observation that, overall, seven sensory attributes were significantly affected by yeast by nitrogen interactions indicates that modulation of wine aroma can be achieved by the use of different yeasts at specific nitrogen concentrations. It has to be mentioned that, in this study, the range of nitrogen additions tested was quite broad, which might not be allowed in certain countries.

Chemical Composition. Primary compositional parameters of the wines are given in **Table 3**. A decrease in ethanol concentration with increasing nitrogen was observed, with an overall reduction in ethanol content of up to nearly 1%. Glycerol content increased with increasing nitrogen in both yeasts, whereas pH decreased. Metabolically, it is possible that increased nitrogen availability determined a shift in carbon utilization within the yeast cell, resulting in lower final ethanol concentrations (*25*). Glycerol data support this hypothesis, and biomass was also increased at increasing nitrogen (*22*). Titratable acidity increased with increasing nitrogen for the D254 yeast and decreased with nitrogen addition for the AWRI 1176 yeast. The pH decreased with nitrogen addition for both yeasts.

Table 4 shows the results of the analysis of the major yeast-derived aroma compounds. Both yeast and nitrogen had a strong influence on the volatile composition of the wines. In the case of higher alcohols and branched-chain acids and esters, a decrease with increasing nitrogen was observed, consistent with findings from other studies (*17*, *18*, *20*). This trend was common to both yeasts, although final concentrations were strongly affected by yeast, with *S. cerevisiae* D254 generally showing higher concentrations of branched-chain acids and *S. bayanus* 1176 being characterized by very high concentrations of 2-phenylethanol, a property that seems to be characteristic of this species (*26*, *27*). Production of acetates and MCFA ethyl esters emerged as one major difference among the experimental wines. Generally, yeast D254 had a higher production of esters than AWRI 1176, and addition of nitrogen strongly increased formation of these compounds for this strain. Biosynthesis of acetate esters is regulated by expression of genes encoding for alcohol acetyltransferases (*28*), which in *S. cerevisiae* is higher under conditions of increased available nitrogen (*29*). As for MCFA ethyl esters, precursor availability rather than expression of esterification pathway has been suggested to control their formation (*30*, *31*), which is in agreement with the trends observed here for the hexanoic and octanoic acids, precursors to ethyl hexanoate and ethyl octanoate, respectively. As for acetaldehyde, in the case of *S. bayanus* AWRI 1176 increasing nitrogen corresponded to increased acetaldehyde concentrations, whereas the opposite was found for D254. Acetaldehyde has been shown to respond to different fermentation variables, including yeast strain (*32*), although the relationship between nitrogen supplementation and formation of acetaldehyde has not been explored extensively. Additionally, acetaldehyde can bind to SO_2, which in this study was found to increase with increasing nitrogen. Among the various sulfur compounds measured, for H_2S the response to nitrogen depended on the yeast strain. Whereas no H_2S was detected in the AWRI 1176 wines, the final concentrations of H_2S in the wines obtained with D254 was maximum for moderate nitrogen additions, whereas minimum concentrations were observed when no addition was performed. A more detailed discussion on the metabolic factors involved in the sulfur compounds profile of these experimental wines has been published previously (*22*). More recently, in a study using a broader range of commercial wine yeasts, we have provided further evidence of a nonlinear relationship between DAP addition and wine H_2S content, with moderate nitrogen supplementation, can result in increased concentration of H_2S in the wine at the end of fermentation (*33*).

Relating Volatile Composition and Aroma Sensory Data. Partial least-squares regressions were used to explore the relationships between aroma composition and sensory properties of the experimental wines. This type of chemometric approach has been previously used to develop mathematical models to describe complex aroma interaction in different wines (*34*, *35*). The results of the PLSR2 analysis are shown in **Figure 2**. Only sensory data related to aroma properties of the wines were used in the analysis. The model obtained by using the first two optimum components accounted for 68 and 67% of the explained variance in the chemical and sensory data, respectively.

The scores for the descriptors "confectionary", "red fruit", and "dark fruit", which were higher for the high-nitrogen fermentations, were strongly positively associated with acetate esters and MCFA ethyl esters. This is in agreement with the hypothesis that esters are major contributors to the berry character of red wines (*5*, *6*). However, a closer look at the data presented here reveals that, although esters and fruit-related descriptors correlated when the whole nitrogen range studied was considered, this was not true when the intermediate nitrogen point was considered. That is, when moderate nitrogen additions were compared with the other two nitrogen treatments, changes in esters did not translate into changes in fruity attributes. For example, in the wines made with the yeast D254, comparison between the control and the nitrogen-supplemented samples revealed a significant increase in fruit-related descriptors, particularly when fermentations were started at 400 mg/L YAN. Nevertheless, the largest changes in acetate esters were observed between the control and

Table 4. Concentrations (Micrograms per Liter) of the Volatile Compounds Measured in the Experimental Wines

		D254			1176		
compound		100 YAN	250 YAN	400 YAN	100 YAN	250 YAN	400 YAN
acetaldehyde		49326 a[a]	36148 b	37289 b	52953 c	57657 b	69841 a
ethyl acetate	A[b]	58440 c	88813 b	99217 a	50591 a	36726 b	37666 b
2-methylpropyl acetate	A	154 c	279 b	296 a	44 a	28 b	17 c
2-methylbutyl acetate	A	79 c	350 b	393 a	49 b	106 a	32 c
3-methylbutyl acetate	A	571 c	2970 b	3880 a	571 b	795 a	583 b
hexyl acetate	A	26 c	87 b	152 a	15 c	37 b	54 a
2-phenylethyl acetate	A	102 b	107 b	167 a	155 c	287 a	220 b
ethyl propanoate	A	169 b	163 b	332 a	202 c	234 b	343 a
ethyl butanoate	A	154 b	279 a	296 a	132 a	119 b	81 a
ethyl hexanoate	A	541 b	679 a	643 ab	413 a	243 b	193 c
ethyl octanoate	A	525 c	661 b	795 a	501 a	320 b	173 c
ethyl 2-methylpropanoate	A	179 a	123 b	132 b	226 a	81 b	65 c
ethyl 2-methylbutanoate	A	21 a	8 b	10 b	22 a	9 b	4 c
hexanol	A	310 a	329 a	298 a	396 a	410 a	401 a
2-methylbutanol	A	73153 a	72420 a	54195 b	77041 a	57986 b	36400 c
3-methylbutanol	A	190722 a	174772 b	155715 c	195728 a	128814 b	83575 c
2-phenylethanol	A	71879 a	48244 b	32002 c	403885 a	242289 b	110870 c
2-methylbutanoic acid	B	6527 a	1935 b	584 c	2263 a	682 b	191 c
3-methylbutanoic acid	A	2797 a	1024 b	449 c	2205 a	687 b	432 c
hexanoic acid	A	2007 c	2124 b	2409 a	1379 a	1082 b	418 c
octanoic acid	A	834 c	1489 b	1709 a	848 a	573 b	253 c
hydrogen sulfide	C	nd c	2.3 a	0.5 b	nd	nd	nd
methanethiol	C	nd c	0.7 b	0.8 a	nd b	nd b	0.8 a
ethanethiol	A	1.1 a	2.2 b	2.8 a	1.2	1.9	1.8
diethyl sulfide	A	2 c	8.6 b	10.8 a	1.8 c	5.2 b	8.7 a
dimethyl sulfide	A	2.5 c	8.6 b	10.8 c	2 c	4 b	5.9 a
diethyl disulfide	A	2.6 c	4.5 b	6.1 a	1.9 c	3.3 b	4.3 a
dimethyl disulfide	A	nd c	2.3 b	3.8 a	nd c	0.7 b	1 a
methyl thioacetate	A	5 c	7.3 b	8.5 a	3.9 c	4.7 b	6.4 a
ethyl thioacetate	A	1.1 b	2.2 a	2.8 a	1.2 b	1.9 a	1.8 a
2-mercaptoethanol	A	38 a	39 a	40 a	40 a	40 a	41 a
benzothiazole	A	16 a	16 a	15 a	16 a	15 a	17 a
methionol	A	2900 a	3130 a	3003 a	3054 a	2994 a	3005 a

[a] Within each yeast, different letters denote statistically significant differences at $p < 0.05$. [b] Identification during GC analysis: A, identification confirmed by comparing mass spectra and linear retention indices with those of commercially available pure reference compounds; B, identification confirmed by comparing mass spectra and linear retention indices with those of pure reference compound synthesized in the laboratory; C, compound identified by comparing retention times and linear retention indices with those of commercially available pure reference standards. nd: not detected.

the first (250 mg/L) nitrogen addition (usually >4-fold increases), whereas other compounds with fruity aromas, including the ethyl fatty acid esters ethyl butanoate, ethyl hexanoate, and ethyl octanoate, showed similar increases across the two nitrogen concentrations tested. Furthermore, in the AWRI 1176 wines, certain esters such as ethyl octanoate decreased with increasing nitrogen, but this was not reflected in the rating of the fruit-related descriptors for these wines. The only compound that showed a trend consistent with these observations was ethyl propanoate, in agreement with the suggestion of Pineau et al. (*6*). It is noteworthy, in partial contrast with previously published data (*5*, *6*), that the branched-chain fatty acid esters ethyl 2-methylpropanoate and ethyl 2-methylbutanoate were not found to correlate well with berry-related descriptors. The apparent discrepancy between the concentration of fruit-smelling odorants and changes in intensity of fruit-related descriptors in the wines can be explained by looking at the effect of nitrogen supplementation on other aroma compounds. **Figure 3**, panels **a** and **b**, shows the regression coefficients for the various compounds for the attributes "red fruit" and "dark fruit", respectively. As can be seen, ethanol was among the compounds showing the highest negative correlation with these descriptors. According to Escudero et al. (*5*) and Guth and Sies (*36*), higher ethanol concentrations reduce the intensity of berry attributes. The reduced ethanol content of the wines from high-nitrogen fermentations (**Table 3**) might have contributed to their increased fruitiness.

In addition, the role of sulfur compounds has to be considered. H_2S was most strongly positively associated with "reduced", "vegetal", and "earth/dirty" and occurred at maximum concentrations in the wines obtained with the yeast D254 at moderate nitrogen supplementation, at which the highest increase in esters was also observed. In these wines, the descriptor "reduced" had the highest ratings, and this appeared to be mostly associated with H_2S (**Figure 3c**). The increase in H_2S is likely to have resulted in a suppression of the fruity attributes. A correlation was also observed between some of the sulfur compounds measured and the descriptors "red fruit" and "dark fruit". Previous studies have indicated that increased concentration of DMS can result in higher rating for "red fruits" and "berry fruits" descriptors (*12*), probably due to the synergy existing between DMS and some esters (*5*). Our results seem to point in the same direction, although in this study we were not able to separate the contribution of DMS from esters, which also increased at high nitrogen concentrations. Similarly, the apparent positive correlation between fruit-related attributes and methanethiol, known for its rotten egg-like aromas, might indeed be the result of the concomitant presence of high concentrations of different esters resulting from high nitrogen supplementation. It is possible that, at increasing nitrogen, the negative contribution of methanethiol is masked by the concomitant increase in the perception of fruit-related aromas.

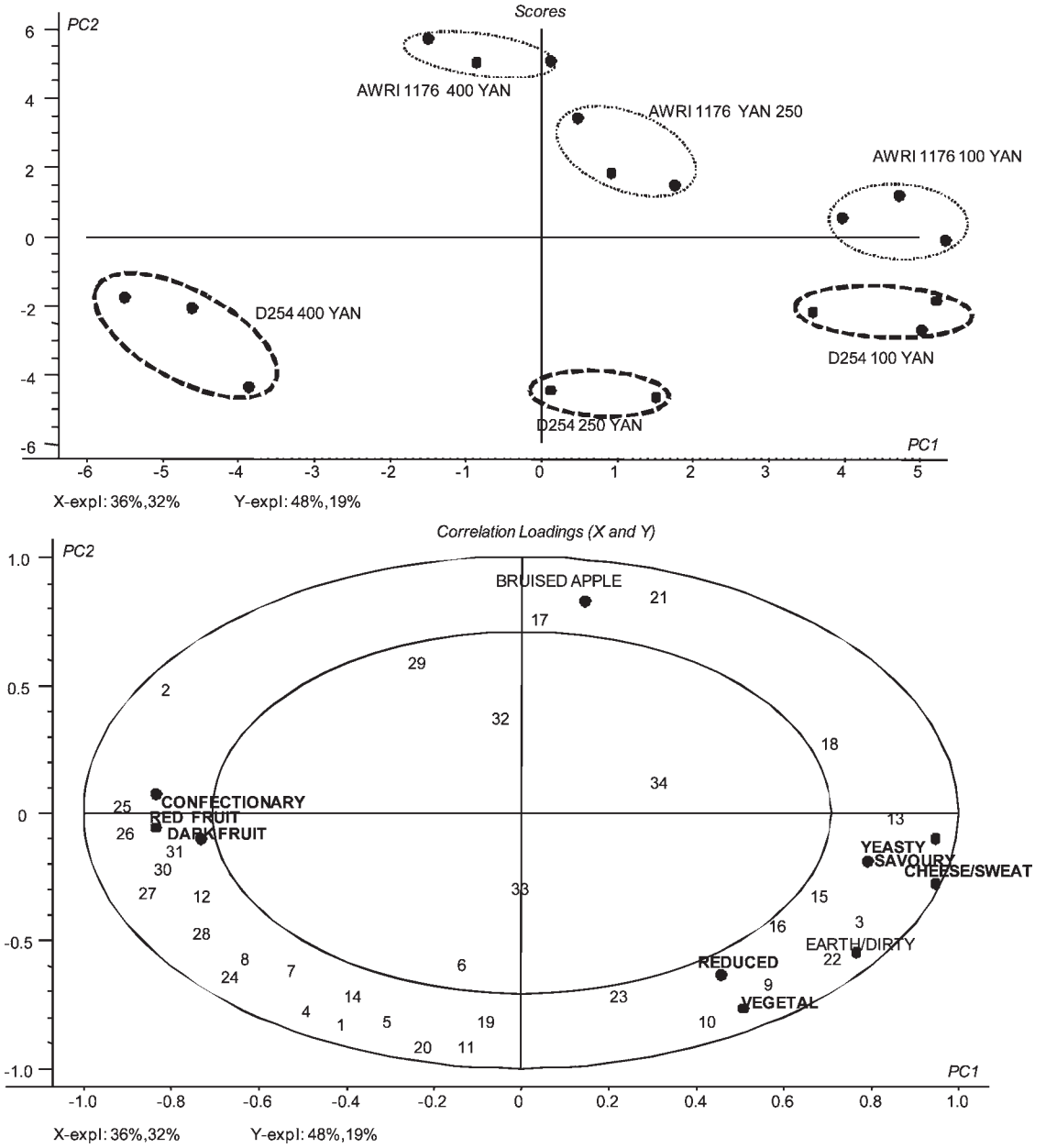

Figure 2. Partial least-squares regression analysis of volatile compounds and sensory attributes: scores and loadings for the first two principal components. 100 mg/L YAN has no nitrogen addition; 250, same must as the 100 but initial YAN increased until 250 mg/L by means of DAP addition; 400, same must as 100 but initial YAN increased until 400 mg/L by means of DAP addition. 1, ethyl acetate; 2, ethyl propanoate; 3, ethyl 2-methylpropanoate; 4, 2-methylpropyl acetate; 5, ethyl butanoate; 6, ethyl 2-methylbutanoate; 7, 2-methylbutyl acetate; 8, 3-methylbutyl acetate; 9, 2-methylbutanol; 10, 3-methylbutanol; 11, ethyl hexanoate; 12, hexyl acetate; 13, hexanol; 14, ethyl octanoate; 15, 2-methylbutanoic acid; 16, 3-methylbutanoic acid; 17, 2-phenylethyl acetate; 18, 2-phenylethanol; 19, hexanoic acid; 20, octanoic acid; 21, acetaldehyde; 22, ethanol (%); 23, H_2S; 24, methanethiol; 25, diethyl sulfide; 26, diethyl disulfide; 27, DMS; 28, dimethyl disulfide; 29, ethanethiol; 30, methyl thioacetate; 31, ethyl thioacetate; 32, 2-mercaptoethanol; 33, methionol; 34, benzothiazole.

Along with the clear influence on perceived fruitiness, the other major effect of nitrogen addition was a suppression of the perceived intensity of descriptors such as "cheese", "earth", and "yeast". PLSR indicated the existence of a strong correlation between these descriptors and the compounds 2- and 3-methylbutanoic acids, as well as with various higher alcohols. On the basis of odor activity values, 3-methylbutanoic acid, characterized by a rancid cheese aroma, was one of the most discriminant compound measured (data not shown). This observation suggests that the strong decrease of this compound associated with nitrogen supplementation might be responsible for the decrease in attributes such as "cheese". 2- and 3-methylbutanoic acids have also been reported among the most powerful odorants in dry yeast (*37*), suggesting an involvement of these compounds in the decrease of the "yeast" attribute. The decrease in concentration of these acids at high nitrogen might have further increased perceived fruitiness at increasing nitrogen, due to acids masking fruity attributes (*38*). This could explain why the intensity of fruit-related descriptors increased in the AWRI 1176 wines with increasing nitrogen, although increases in esters concentrations were marginal. It is worth observing that, in the AWRI 1176 wines, in which no H_2S was detected, the "reduced" attribute was found to decrease with increasing nitrogen, although the mercaptans were increased. This suggests the existence of complex interactions between wine "cheese", "earth", and "yeast" attributes and the perception of other attributes, including "fruity"

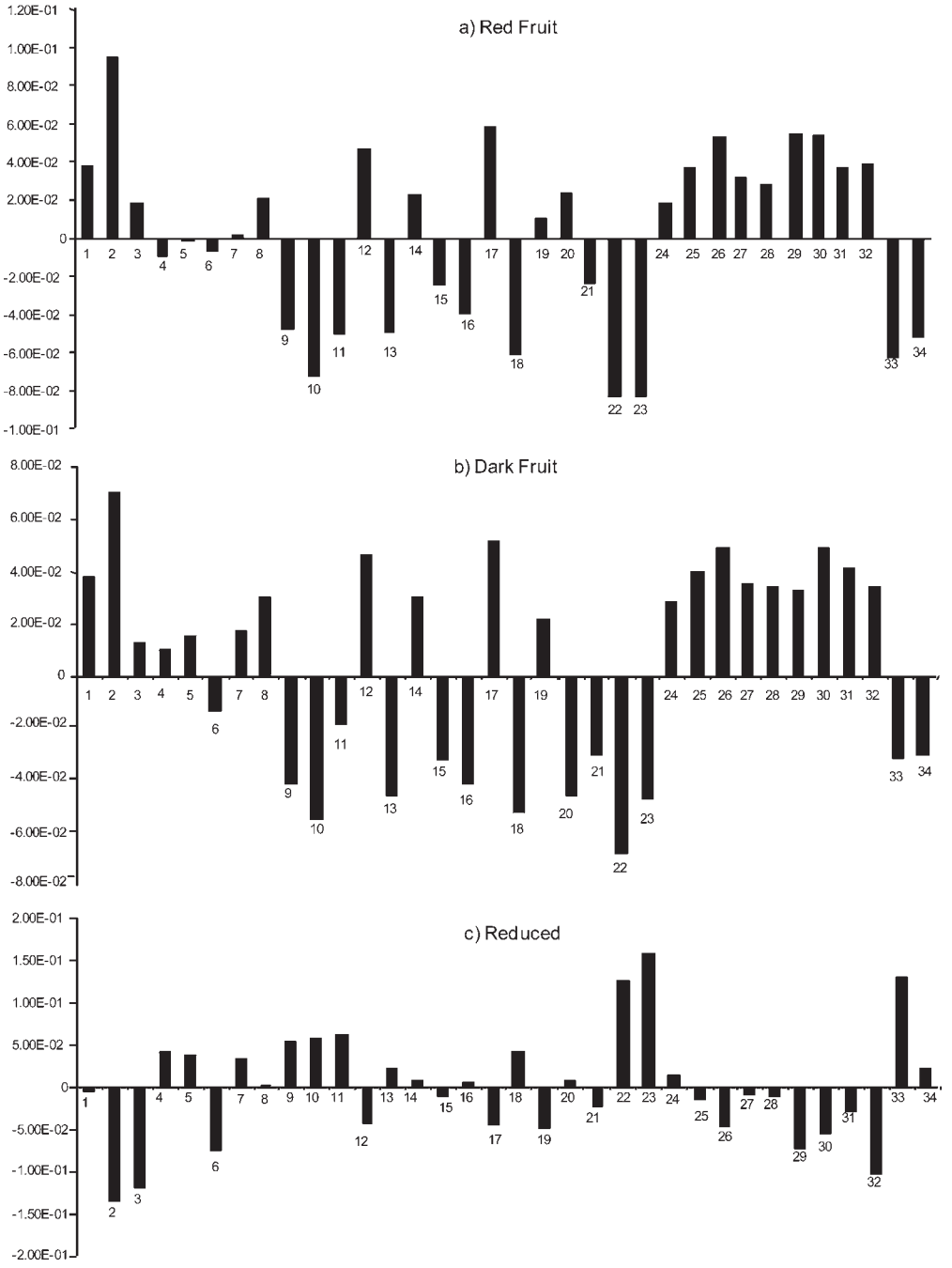

Figure 3. Regression coefficients for the "red fruit", "dark fruit", and "reduced" attributes of the experimental wines. 1, ethyl acetate; 2, ethyl propanoate; 3, ethyl 2-methylpropanoate; 4, 2-methylpropyl acetate; 5, ethyl butanoate; 6, ethyl 2-methylbutanoate; 7, 2-methylbutyl acetate; 8, 3-methylbutyl acetate; 9, 2-methylbutanol; 10, 3-methylbutanol; 11, ethyl hexanoate; 12, hexyl acetate; 13, hexanol; 14, ethyl octanoate; 15, 2-methylbutanoic acid; 16, 3-methylbutanoic acid; 17, 2-phenylethyl acetate; 18, 2-phenylethanol; 19, hexanoic acid; 20, octanoic acid; 21, acetaldehyde; 22, ethanol (%); 23, H_2S; 24, methanethiol; 25, diethyl sulfide; 26, diethyl disulfide; 27, DMS; 28, dimethyl disulfide; 29, ethanethiol; 30, methyl thioacetate; 31, ethyl thioacetate; 32, 2-mercaptoethanol; 33, methionol; 34, benzothiazole.

and "reduced", regardless of the concentration of fruity and reductive metabolites such as esters and mercaptans.

In conclusion, the results of this study demonstrate that management of yeast nitrogen, in conjunction with selection of yeast strain, provides a powerful tool to modulate the aroma characteristics of Shiraz wines. Red berry and black berry characters were among the attributes substantially affected by nitrogen addition. Increases in esters concentration were likely to contribute to this effect, with ethyl propanoate most strongly associated. However, a nonlinear dose response was observed between nitrogen additions and increase in fruity attribute, due to the concomitant effect of nitrogen on other yeast sensorially active metabolites, in particular ethanol, branched-chain fatty acids, and hydrogen sulfide, with the latter being associated with reductive aromas. The higher sensory ratings observed for the "reduced" descriptor for some of the wines at moderate nitrogen supplementation are of great interest, considering that elimination of reductive off-flavors is one of the main reasons for nitrogen supplementation in the winery. The fact that the effect of nitrogen addition on the perceived "reduced" character was strain dependent raises the question as to whether nitrogen supplementation can be used indiscriminately to prevent the

formation of reductive off-flavors. Different yeasts appear to respond to nitrogen supplementation in different ways, in some cases resulting in unwanted sensory effects.

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