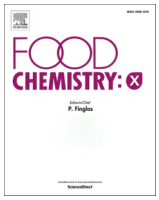
Food Chemistry: X 24 (2024) 101793

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Effect of different nitrogen source and *Saccharomyces cerevisiae* strain on volatile sulfur compounds and their sensory effects in chardonnay wine

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ARTICLE INFO

Keywords:
Nitrogen
Volatile sulfur compounds
Fermentation
Saccharomyces cerevisiae
Sensory analysis

ABSTRACT

Three commercial *Saccharomyces cerevisiae* strains with low, medium, and high H_2S-producing capacity were chosen to investigate the effect of yeast assimilable nitrogen (YAN) levels and composition on volatile compounds in a chemically defined medium, specifically high, medium, and low initial YAN levels with varying proportions of DAP or sulfur-containing amino acids (cysteine and methionine). The results revealed that the initial YAN containing a larger proportion of diammonium phosphate resulted in a higher YAN consumption rate during the early stages of fermentation. The yeast strain had a greater effect on the volatiles than the YAN level and composition. Keeping the total YAN constant, a higher proportion of sulfur-containing amino acids resulted in a considerably higher production of 3-methylthiopropanol. The sensory impact of three key volatile sulfur compounds was investigated in a Chardonnay wine matrix, indicating that 3-methylthiopropanol at subthreshold or greater concentrations was effective in enhancing the cantaloupe aroma.

1. Introduction

The volatile metabolites generated by yeast and other microorganisms are closely related with the wine sensory characteristics (Liu et al., 2017). The metabolism of yeast during wine fermentation is closely related to the nitrogen in the grape must. Insufficient yeast assimilable nitrogen (YAN) mainly result in fermentation failure, such as lag or even stuck of fermentation, microbial contamination and flavor deterioration caused by the accumulation of hydrogen sulfide (H_2S) (Ruiz et al., 2020). Nitrogen deficiencies in vineyards is common worldwide (Stephanie Rollero, Bloem, Ortiz-Julien, Camarasa, & Divol, 2018). Therefore, nitrogen supplementation is a common practice to avoid lag fermentation and off-flavor in winemaking (Ugliano, Siebert, Mercurio, Capone, & Henschke, 2008). YAN can be classified into three categories: ammonium nitrogen, α-amino acids, and small polypeptides. Most studies included ammonium salts as the principal supplementary YAN source. Nowadays, amino-acid addition to must has become increasingly common. However, the effect of nitrogen addition varies widely (Gobert, Tourdot-Maréchal, Sparrow, Morge, & Alexandre, 2019). In general, ammonium nitrogen, asparagine and glutamine are widely considered to be the preferred YAN sources. Glutamine and asparagine can be rapidly absorbed by yeast and have a promoting effect on the synthesis of other amino acids, nucleic acids and coenzymes (Beltran, Esteve-Zarzoso, Rozès, Mas, & Guillamón, 2005).

VSCs in wine are mainly produced by yeast during fermentation, for example methyl mercaptan and H_2S has undesirable cooked vegetable and rotten egg like odor, respectively (Smith, Bekker, Smith, & Wilkes, 2015). Other VSCs are produced by hydrolysis of some aroma precursors in grape juice, such as volatile thiols, commonly contribute to the tropical fruit odor. These VSCs are present in the grapes in the form of cysteine conjugates, which are hydrolyzed by the yeast during fermentation (Segurel, Razungles, Riou, Trigueiro, & Baumes, 2005). The regulation effect of nitrogen on H_2S in wine has been widely studied. H_2S is a product of the sulfate reduction sequence (SRS) pathway. In the SRS pathway, H_2S is derived from HS^-, which is a metabolic intermediate of sulfate or sulfite reduction required for the synthesis of organosulfur compounds (Englezos et al., 2021). If at the right level of nitrogen source, then HS^- is chelated by *O*-acetylserine and *O*-acetylhomoserine from nitrogen metabolism to form organosulfur compounds such as methionine and cysteine. However, when the nitrogen source is insufficient or inappropriate, free H_2S accumulates in the cells and diffuses into the grape juice (Ferreira, Franco-Luesma, Vela, López,

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https://doi.org/10.1016/j.fochx.2024.101793
Received 3 August 2024; Received in revised form 27 August 2024; Accepted 27 August 2024
Available online 31 August 2024

Table 1
The N composition of stock solutions as nitrogen source added to the fermentation.

Nitrogen source classification	Compounds	Stock solution A (Control)	Stock solution B (High DAP)	Stock solution C (High S-amino acids)
Organic source	Proline	46.8	46.8	46.8
	Alanine	11.1	11.1	11.1
	Arginine	28.6	28.6	28.6
	Aspartic acid	3.4	3.4	3.4
	Cysteine	**1**	**1**	**10**
	Glutamine	38.6	38.6	38.6
	Glutamic acid	1.4	1.4	1.4
	Glycine	1.4	1.4	1.4
	Histidine	2.5	2.5	2.5
	Isoleucine	2.5	2.5	2.5
	Leucine	3.7	3.7	3.7
	Lysine	1.3	1.3	1.3
	Methionine	**2.4**	**2.4**	**24**
	Phenylalanine	2.9	2.9	2.9
	Serine	6	6	6
	Threonine	5.8	5.8	5.8
	Tryptophan	13.7	13.7	13.7
	Tyrosine	1.4	1.4	1.4
	Valine	3.4	3.4	3.4
Inorganic source	**DAP**	**25**	**250**	**25**
Total		30.46	78.19	33.53

Note: The unit is g/L. The bold values are showing the differences between treatments.

Table 2
Nitrogen composition and final YAN level for the 27 fermentations in this study.

Treatment abbreviations	Strain	Nitrogen sources	Stock solution added	Final YAN (mg/L)
A-Con-110	AU	Control	3.61 mL A	110
A-Con-220			7.22 mL A	220
A-Con-330			10.83 mL A	330
A-DAP-110		High DAP	1.4 mL B	110
A-DAP-220			2.8 mL B	220
A-DAP-330			4.2 mL B	330
A-S-110		High S-amino acids	3.28 mL C	110
A-S-220			6.56 mL C	220
A-S-330			9.84 mL C	330
D-Con-110	DV-10	Control	3.61 mL A	110
D-Con-220			7.22 mL A	220
D-Con-330			10.83 mL A	330
D-DAP-110		High DAP	1.4 mL B	110
D-DAP-220			2.8 mL B	220
D-DAP-330			4.2 mL B	330
D-S-110		High S-amino acids	3.28 mL C	110
D-S-220			6.56 mL C	220
D-S-330			9.84 mL C	330
F-Con-110	LA-FR	Control	3.61 mL A	110
F-Con-220			7.22 mL A	220
F-Con-330			10.83 mL A	330
F-DAP-110		High DAP	1.4 mL B	110
F-DAP-220			2.8 mL B	220
F-DAP-330			4.2 mL B	330
F-S-110		High S-amino acids	3.28 mL C	110
F-S-220			6.56 mL C	220
F-S-330			9.84 mL C	330

Different amount of stock solution was added to each fermentation (100 mL of YNB without nitrogen) to achieve a final YAN of 110 mg/L, 220 mg/L and 330 mg/L, respectively. Then inoculated with different yeast strains.

& Hernández-Orte, 2018; Siebert, Solomon, Pollnitz, & Jeffery, 2010; Swiegers & Pretorius, 2007). Clearly, the concentration and composition of YAN can influence the production of H_2S during fermentation, but H_2S is a highly reactive compound and could react with other compounds in wine to form sulfides. For example, ethanethiols could be formed by the reaction of H_2S with ethanol or acetaldehyde (Kinzurik, Herbst-Johnstone, Gardner, & Fedrizzi, 2016; Mauricio et al., 1993). In wine, dimethyl disulfide, dimethyl trisulfide and dimethyl tetrasulfide are formed by oxidation of methyl mercaptan, and methyl mercaptan is formed by degradation of methionine (Swiegers & Pretorius, 2007). Although the impact of YAN on H_2S has received considerable study, and a number of studies have investigated the formation of VSCs during wine production (Kraft, Zhou, Qian, & Osborne, 2023), the underlying causes driving their formation are still not well elucidated, especially little is known about the impact of YAN on the formation of other VSCs or possible VSC precursors such as cysteine, methionine, and glutathione. Methionine and cysteine are amino acids found in grape juice in low amounts, often below 10 mg/L (Petrovic, Aleixandre, & Buica, 2019). However, given their catabolic network, these sulfur amino acids can be considered key precursors for VSC production (Jimenez-Lorenzo, Bloem, Farines, Sablayrolles, & Camarasa, 2021). Their contribution, which has previously been inadequately reported, warrants more exploration.

YAN status has consistently influenced volatile compounds production in wine. However, recent studies show that the correlation between YAN and volatile compounds production may be much weaker than previously thought, especially with the use of more complex nitrogen sources, the linear correlation between YAN levels and the concentration of volatile compounds disappears and the production of volatile compounds becomes unpredictable. In addition to nitrogen source, the influence of yeast metabolism on the volatile profile should also be considered, emphasizing the importance of selecting the right yeast in the wine fermentation process to balance the aroma profile of wine. Meanwhile, determining the effect of nitrogen on VSC production is complicated by the fact that the time of nitrogen addition, sources, the strain of *S. cerevisiae* used, the initial sugar concentration, temperature and the YAN already present in the must appear to influence the VSC profile. Enological nitrogen addition using amino acids is being increasingly performed, but the impact on different *S. cerevisiae* in terms of fermentation parameters and VSC production is not clear yet. Therefore, in this study, 3 yeast strains with different H_2S-producing capacities were selected to study the effect of nitrogen concentration and composition on yeast-produced VSCs as well as other volatiles. Through aroma addition and sensory analysis, the effects of important VSCs on the overall aroma of wine were also investigated. The results obtained in this work should be useful, e.g. to optimize VSC production of the given yeast upon N-source supplementation during wine fermentations and provide important insights into the sensory effects of VSCs on wine aroma.

2. Materials and methods

2.1. Yeast strains

Three different commercial *S. cerevisiae* strains, DV-10 (Lavlvin®, Germany), LA-FR (Selectys®, France), AU (Selectys®, France), were used for fermentation. The yeast strain was firstly hydrated in 10 mL of PBS buffer, and inoculated into YPD medium under aseptic conditions, followed by activation for 1–2 times and propagation in YPD at 28 °C for 48 h.

2.2. Determination of yeast H_2S-producing capability

Hydrogen sulfide production was evaluated using the bismuth method (Linderholm Angela, Findleton Carrie, Kumar, Hong, & Bisson Linda, 2008). Bismuth in BIGGY agar medium could be used as an indicator of H_2S production to form a black bismuth sulfide precipitate. So it is possible to differentiate the strains according to their H_2S production based on the color change (Bizaj et al., 2012). The BIGGY agar medium (not autoclavable) was sterilized in an oil bath at 100 °C for 10–15 min. The yeast was propagation in 5 mL of YPD liquid medium for 48 h, and 100 μL of broth was spread on BIGGY agar medium. The

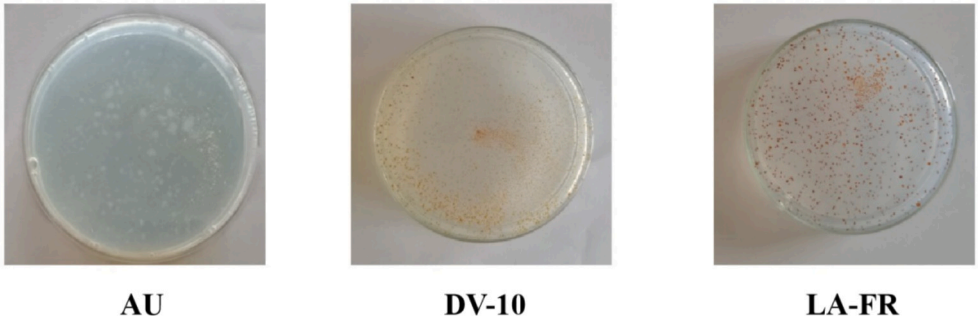

Fig. 1. The sulfur production capacity of three commercial *S. cerevisiae* strains. The yeasts were categorized into low, medium and high sulfur producing according to a white, medium brown and dark brown color on the BIGGY agar respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
The fermentation capacity of three selected commercial *Saccharomyces cerevisiae* strains.

Strain	8 h	16 h	24 h	48 h
AU	−	+	++	+++
DV-10	+	++	+++	++++
LA-FR	++	+++	++++	++++

fermentations were performed in triplicate, incubated at 28 °C and monitored over 3 days and the color of the colony were recorded.

2.3. Determination of yeast gas-producing capability

Durham tubes were used to detect gas production. The test tubes (6 by 50 mm) were inserted upside down inside larger (13 by 100 mm) test tubes. After sterilization, Durham tubes become filled with the media. The activated yeasts (0.1 mL of broth culture) were aseptically inoculated in each test tube. The tubes were incubated at 37 °C for 48 h, and the air bubble trapped inside the Durham tube were recorded every 8 h. The absence of gas was recorded as '−', the slight presence of gas was recorded as '+', the gas accounted for 1/3 of the volume was recorded as '++', and the gas accounted for 2/3 of the volume was recorded as '++++'. ', and full was recorded as '++++'.

2.4. Fermentation conditions

Commercial yeast strains LA-FR, DV-10 and AU were hydrated in 100 mL of water at 35 °C for 20 min. Stock solution A, B and C with different nitrogen source concentration and composition were prepared (Table 1). Three mL of activated yeast were cultured in 100 mL YNB broth (glucose, 65 g/L; fructose, 65 g/L) with different amounts of stock solution to determine the effects of inorganic nitrogen (DAP) and organic nitrogen (sulfur-containing amino acid) in a constant temperature incubator at 22 °C (Table 2).

Samples were collected from fermentation flask daily to monitor the changes in volatile compounds and chemicals. Fermentation were carried out in triplicate and collected samples were stored at −20 °C until analysis.

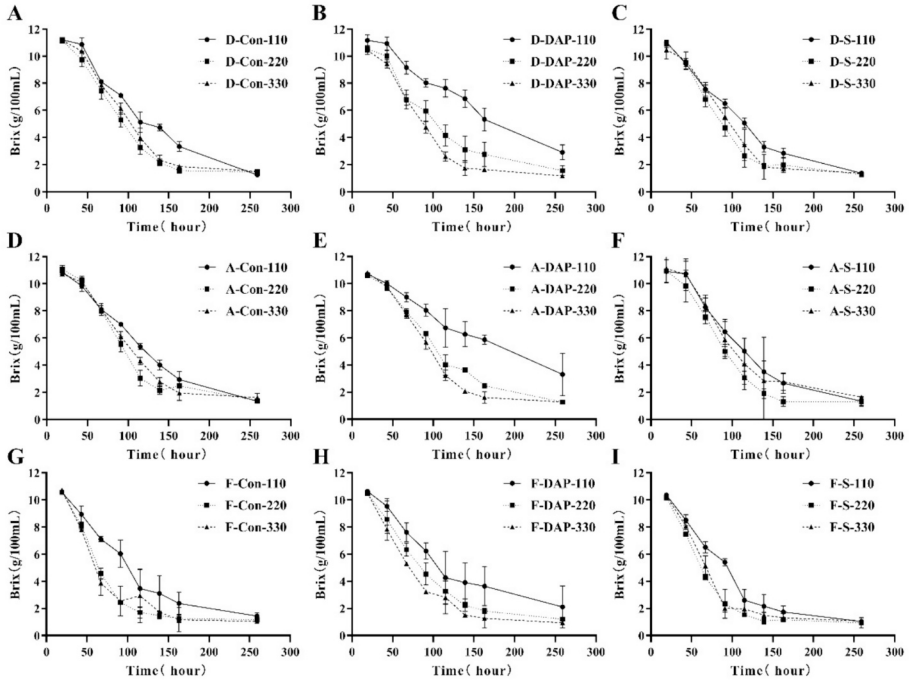

Fig. 2. Glucose consumption after 6 days of fermentation of *S. cerevisiae* DV-10, AU and LA-FR from fermentations in YNB supplemented with different N-sources at 22 °C. The treatment codes can be found in Table 2.

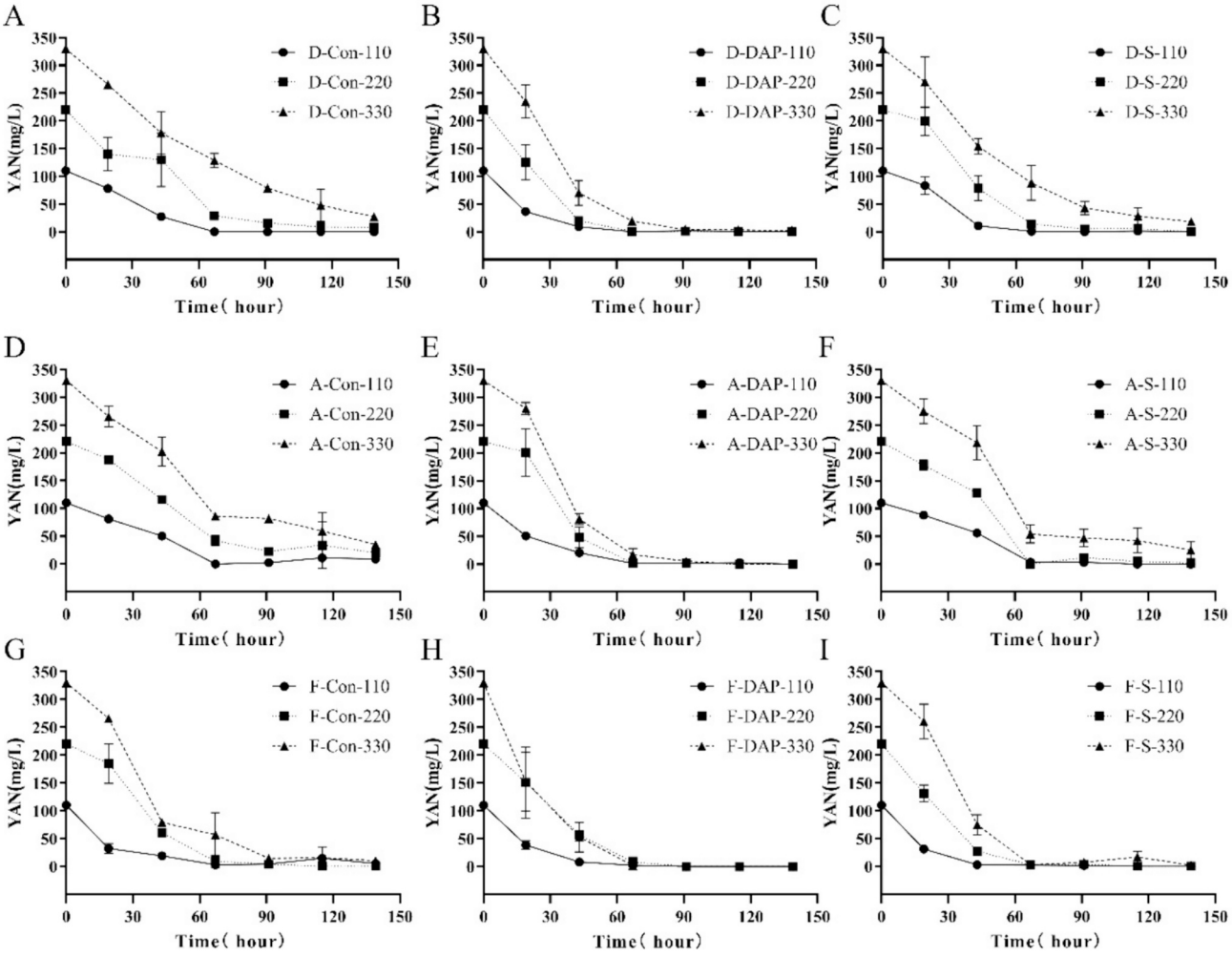

Fig. 3. The effect of different N-sources on YAN utilization by different yeast strains. The treatment codes can be found in Table 2.

Table 4
The effect of different nitrogen source compositions and levels on the average H_2S production of different yeasts during fermentation.

Strain	N level			N composition			level*composition
	110 mg/L	220 mg/L	330 mg/L	Con	High DAP	High S-amino acids	
AU	1.72 ± 2.31a	1.14 ± 1.74a	0.27 ± 0.65b	0.15 ± 0.20b	1.14 ± 1.74a	1.83 ± 2.31a	<0.001
DV-10	1.03 ± 1.56a	0.15 ± 0.20b	0.05 ± 0.001b	0.05 ± 0.001b	0.15 ± 0.20b	1.03 ± 1.56a	<0.001
LA-FR	1.94 ± 0.85b	2.35 ± 2.52b	4.92 ± 4.04a	1.07 ± 0.96c	3.13 ± 3.68b	5.01 ± 2.46a	<0.001

The H_2S production is presented as mg of H_2S in 100 mL fermentation. Different letters indicate statistical differences between treatments by ANOVA (Duncan, $p < 0.05$).

2.5. YAN, sugar and H_2S analysis during fermentation

YAN consists of amino nitrogen and ammonia nitrogen. The amino acid nitrogen was determined by the o-phthalaldehyde (OPA) fluorescence method. OPA solution was prepared by dissolving 0.1678 g OPA in 25 mL 95 % ethanol/water (*v/v*), which was combined with 0.9593 g NaOH, 2.117 g boric acid and 0.204 g *N*-acetyl-L-cysteine (NAC) and then fixed with distilled water into a 250 mL volumetric flask. The stock solution was prepared in deionized water and was then diluted to a series of concentrations to obtain the working standard solutions. Fifty μL of the working standard solution was mixed with 3 mL of OPA solution in a colorimetric tube and left to stand for 10 min, and the standard curve was plotted by measuring the absorbance of isoleucine at 335 nm using a UV spectrophotometer. Free ammonium in samples were determined using an Ammonium Assay Kit (Megazyme, Cat. No. 4001534) following the manufacture's instruction. (Yuan, Schreiner, Osborne, & Qian, 2018).

The residual sugar was determined by a glucose meter (Sinocare Inc. Changsha, China). H_2S production was monitored by a lead acetate H_2S test tube. The detection tube was connected to the fermentation bottle through a silicone stopper, which was observed periodically to record the H_2S produced during fermentation(Ugliano, Kolouchova, & Henschke, 2011). The calibration was conducted following the procedure described by Ugliano and Henschke (Ugliano & Henschke, 2010).

2.6. Analysis of major volatile compounds by SPE-GC–MS

Volatile compounds produced during fermentation were extracted by solid-phase extraction and analyzed by GC–MS, according to the method described by Gracia-Moreno (Gracia-Moreno, Lopez, & Ferreira,

Table 5
The effect of different nitrogen compositions and YAN levels on the volatile compounds production of AU.

Compound	YAN level			N composition			level* composition
	110 mg/L	220 mg/L	330 mg/L	Control	High DAP	High S-amino acids	
Volatile sulfur compounds							
1-propanol,3-(methylthio)-	2744 ± 4032c	6967 ± 10351b	13,499 ± 20176a	65.78 ± 30.86b	60.2 ± 37.6b	23,085 ± 14108a	<0.001
dimethyl disulfide	3.61 ± 3.28	2.44 ± 1.87	3 ± 2	3.1 ± 2.5b	4.42 ± 2.81a	1.52 ± 1.25c	<0.001
diethyl sulfide	0.32 ± 0.36	0.2 ± 0.1	0.26 ± 0.21	0.35 ± 0.17	0.19 ± 0.20	0.23 ± 0.36	ns
2-methyltetrahydrothiophen-3-one	394.8 ± 277.0	361.2 ± 214.7	345.5 ± 216.9	205.1 ± 26.3b	225.7 ± 29.0b	670.6 ± 119.1a	ns
Dimethyl trisulfide	0.42 ± 0.23	0.3 ± 0.2	0.29 ± 0.22	0.32 ± 0.15	0.42 ± 0.25	0.27 ± 0.23	ns
Esters							
ethyl butyrate	2.89 ± 3.14b	1 ± 1b	16.24 ± 17.47a	4.26 ± 4.43b	14.34 ± 18.61a	1.53 ± 1.93b	<0.001
isobutyl acetate	1158 ± 276a	1216 ± 417a	931.6 ± 195.2b	1299 ± 486a	1047 ± 76b	959.8 ± 166.4b	<0.001
ethyl valerate	71.59 ± 18.20b	121.1 ± 17.5a	137.45 ± 22.60a	114.1 ± 37.3	103.2 ± 38.0	112.8 ± 29.4	ns
ethyl lactate	2.33 ± 1.16	1.98 ± 2.27	4.02 ± 3.28	2.24 ± 1.00	3.09 ± 2.88	3 ± 3	ns
ethyl caprylate	27.74 ± 29.13b	130.8 ± 194.3ab	254.6 ± 236.4a	135.5 ± 177.5	60.03 ± 119.10	217.6 ± 252.8	ns
ethyl hexanoate	169.8 ± 238.0a	55.78 ± 17.99b	67.67 ± 47.04b	69.86 ± 49.29b	177.4 ± 232.5a	45.95 ± 22.31b	0.001
ethyl acetate	69.65 ± 32.74b	122.0 ± 49.3a	131.6 ± 83.2a	149.7 ± 91.4a	93.91 ± 33.85b	79.68 ± 18.83b	<0.001
methyl dodecanoate	64.41 ± 32.17b	100.0 ± 30.5a	91.41 ± 24.74a	93.92 ± 37.17a	72.74 ± 35.41b	89.17 ± 21.11a	<0.001
Alcohols							
isoamyl alcohol*	50.95 ± 6.75a	42.68 ± 10.59b	38.23 ± 8.43c	43.5 ± 12.7b	48.88 ± 4.41a	39.48 ± 9.56c	<0.001
phenethyl alcohol*	28.96 ± 8.33a	20.15 ± 9.72b	12.76 ± 4.92c	19.46 ± 12.40	22.17 ± 8.24	20.23 ± 10.49	<0.001
β-hydroxyphenethylalcohol	1466 ± 1242	1466 ± 1226	1644 ± 1161	695.4 ± 532.3b	2069 ± 1138a	1812 ± 1281	0.003
Acids							
acetic acid*	8.30 ± 1.61b	10.32 ± 1.54b	18.5 ± 13.2a	17.18 ± 13.90a	9.88 ± 3.12b	10.06 ± 1.85b	ns
butyric acid	51.48 ± 14.75c	75.47 ± 36.53b	105.4 ± 60.8a	82.49 ± 35.61b	49.59 ± 11.37c	100.3 ± 63.3a	<0.001
hexanoic acid	107.5 ± 43.5b	131.9 ± 28.8b	218.9 ± 124.8a	139.4 ± 34.6	171.5 ± 152.2	147.4 ± 31.6	0.002
decanoic acid	154.6 ± 296.3	48.31 ± 32.08	32.29 ± 28.39	20.93 ± 18.48	163.4 ± 289.6	50.9 ± 51.4	ns
octanoic acid	209.3 ± 46.5	225.4 ± 86.7	247.3 ± 43.7	225.3 ± 60.2	207.9 ± 84.5	248.9 ± 26.5	ns
Others							
4-heptenal	19.28 ± 6.43	18.52 ± 3.66	20.05 ± 3.48	20.09 ± 3.80a	15.55 ± 2.13b	22.22 ± 4.80a	0.016
acetoin	157.9 ± 247.7a	38.53 ± 19.05b	53.85 ± 38.88b	46.92 ± 43.39b	164.6 ± 243.6a	38.77 ± 18.18b	0.002

*The units of these compound concentration are mg/L. The units of all the other volatile compound's concentration are μg/L. Different letters indicate statistical differences between treatments by ANOVA (Duncan, $p < 0.05$).

2015). Twenty mL of wine containing 30.4 mg/L of 4-octanol (IS 1) and 5.06 mg/L of naphthalene (IS 2) were injected into a LiChrolut EN cartridge. After the sample was completely passed through the cartridge, 5 mL of dichloromethane was used to elute the volatile compounds. The collected eluent was placed at −20 °C to further remove the water and then transferred to sample vial for GC-QTOF-MS analysis.

GC-QTOF-MS analysis were conducted using a 7890 gas chromatograph equipped with a 7200 A MS detector and a PAL autosampler (Agilent Technologies, Santa Clara, CA, USA). The sample injection volume was 1 μL with a split ratio of 20:1. The column was a DB-WAX column (30 m × 0.25 mm × 0.25 μm, J&W Scientific, Santa Clara, CA). The oven temperature program was 50 °C for 5 min, increased by 4 °C/min to 160 °C for 5 min, and finally the temperature was increased at 10 °C/min to 230 °C for 5 min. The carrier gas was helium with a constant flow rate of 1 mL/min. The electron ionization mode (EI) with an ionization energy of 70 eV was used. The temperature of the ion source and the interface temperature was 230 °C and 280 °C, respectively. The mass scan range was set from 35 to 400 amu.

The volatile compounds were qualified by comparing the retention time and mass spectrum to authentic standards or by searching the NIST 20 library. The compounds were quantified by calibration curves if authentic standards were available. The compounds without authentic standards were semi-quantified as relative to the concentration of internal standard. Each sample was analyzed three times. The chromatograph and compound quantification information were showed in Table S1 and Fig. S1.

2.7. Analysis of higher alcohol and ethyl acetate by SPME-GC-FID

One mL of synthetic wine was mixed with 99 mg/L of n-hexane as internal standard. Higher alcohols and ethyl acetate were sampled using a 2 cm, 50/30 μm DVB/CAR/PDMS fiber (Supelco Inc., Bellefonte, PA, USA). Samples were equilibrated at 55 °C for 10 min, then extracted at the same temperature for 10 min and desorbed for 7 min in the injection port.

Analyses were quantified using an 8850 GC (RuiHong Technology,

Table 6
The effect of different nitrogen compositions and YAN levels on the volatile compounds production of DV-10.

Compound	YAN level			N composition			level* composition
	110 mg/L	220 mg/L	330 mg/L	Control	High DAP	High S-amino acids	
Volatile sulfur compounds							
1-propanol,3-(methylthio)-	1781 ± 2583c	5314 ± 7867b	10,491 ± 15671a	48.63 ± 32.29b	73.3 ± 42.1b	17,465 ± 11408a	<0.001
dimethyl disulfide	4.22 ± 1.64b	2.56 ± 1.87a	2.41 ± 1.35a	3.83 ± 0.94	2.5 ± 2.0	2.85 ± 2.04	ns
diethyl sulfide	1.00 ± 0.87a	0.47 ± 0.20b	0.42 ± 0.10b	1.09 ± 0.81a	0.33 ± 0.08b	0.47 ± 0.04b	<0.001
2-methyltetrahydrothiophen-3-one	299.2 ± 174.0b	343.0 ± 249.9a	328.3 ± 218.0ab	179.8 ± 9.9b	185.5 ± 18.0b	605.1 ± 84.2a	0.024
Dimethyl trisulfide	0.48 ± 0.27a	0.34 ± 0.12b	0.24 ± 0.07b	0.47 ± 0.28a	0.28 ± 0.12b	0.3 ± 0.1b	0.013
Esters							
ethyl butyrate	3.78 ± 5.76	4.81 ± 5.40	4.33 ± 6.06	4.6 ± 6.9	4.06 ± 4.41	4.26 ± 5.69	ns
isobutyl acetate	775.9 ± 143.0b	936.6 ± 164.2a	944.2 ± 151.9a	985.5 ± 119.3a	858.2 ± 212.7b	813.1 ± 115.7b	ns
ethyl valerate	85.18 ± 27.14c	123.0 ± 35.0b	151.7 ± 14.6a	149.0 ± 29.4a	95.34 ± 37.19c	115.5 ± 28.0b	0.006
ethyl lactate	2.28 ± 2.39	3.07 ± 4.19	1.98 ± 3.26	2.07 ± 2.94ab	4.47 ± 4.14a	0.79 ± 0.97b	ns
ethyl caprylate	95.39 ± 65.02b	170.8 ± 102.5b	1011 ± 1548a	894.8 ± 1577.2	140.7 ± 92.2	242.0 ± 349.4	ns
ethyl hexanoate	217.5 ± 260.9a	26.39 ± 4.08b	30.27 ± 8.04b	43.97 ± 16.42b	205.2 ± 269.6a	25.05 ± 3.83b	<0.001
ethyl acetate	65.27 ± 16.38a	49.08 ± 17.07b	42.31 ± 15.33c	46.15 ± 13.21b	44.34 ± 22.65b	66.18 ± 9.48a	<0.001
methyl dodecanoate	333.3 ± 157.1a	342.3 ± 47.5a	278.6 ± 32.2b	362.3 ± 94.8a	260.3 ± 113.9b	331.6 ± 50.2a	<0.001
Alcohols							
isoamyl alcohol*	48.39 ± 5.09b	52.6 ± 5.8a	49.98 ± 5.73ab	54.08 ± 5.21a	50.91 ± 5.76a	45.98 ± 2.26b	0.011
phenethyl alcohol*	22.17 ± 5.43a	19.57 ± 5.57a	11.82 ± 6.49b	15.81 ± 7.83b	19.69 ± 7.10a	18.05 ± 6.91ab	<0.001
β-hydroxyphenethylalcohol	1676 ± 1824	1328 ± 1359	1152 ± 1084	1776 ± 1659	1584 ± 1613	797.1 ± 742.1	ns
Acids							
acetic acid*	9.77 ± 3.05	9.66 ± 2.92	9.89 ± 2.66	12.17 ± 1.43a	10.72 ± 1.26b	6.43 ± 1.10c	0.007
butyric acid	98.35 ± 14.19	100.8 ± 17.8	111.4 ± 38.3	105.2 ± 11.5b	82.06 ± 11.29c	123.2 ± 29.7a	ns
hexanoic acid	324.8 ± 130.4b	326.6 ± 75.5b	430.2 ± 80.5a	442.3 ± 74.5a	255.8 ± 80.0c	383.5 ± 70.5b	ns
decanoic acid	118.6 ± 46.7a	52.94 ± 32.42b	78.12 ± 43.24b	84.08 ± 46.09	78.19 ± 66.10	87.43 ± 32.12	0.005
octanoic acid	419.4 ± 154.4b	445.7 ± 86.6b	553.4 ± 82.0a	559.5 ± 75.2a	354.9 ± 115.2b	504.0 ± 72.7a	ns
Others							
4-heptenal	44.33 ± 19.45	38.27 ± 7.42	44.96 ± 10.80	54.96 ± 14.81a	35.64 ± 6.71b	36.96 ± 7.37b	0.032
acetoin	156.8 ± 191.0a	21.71 ± 6.09b	21.84 ± 8.40b	32.98 ± 8.64b	149.4 ± 196.2a	17.91 ± 6.21b	<0.001

*The units of these compound concentration are mg/L. The units of all the other volatile compound's concentration are μg/L. Different letters indicate statistical differences between treatments by ANOVA (Duncan, $p < 0.05$).

Shandong, China), equipped with a flame ionization detector (FID) and DB-WAX column (30 m × 0.25 mm × 0.25 μm, J&W Scientific, Santa Clara, CA). The GC oven initial temperature was 50 °C for 10 min, increased at 10 °C/min to 230 °C, held for 5 min. The carrier gas used was nitrogen at flow rate of 1 mL/min.

The compounds were qualified by comparing the retention time with authentic standards, and quantified by calibration curves that plotted the response ratio of the target compound and the internal standard versus the concentration ratio. Each sample was analyzed three times. The chromatograph was showed in Fig. S2.

2.8. Analysis of volatile sulfur compounds by SPME-GC–MS/MS

The detection method for VSCs was referred to the previous literature (Dziekońska-Kubczak, Pielech-Przybylska, Patelski, & Balcerek, 2020). Two mL of wine was diluted with 8 mL of deionized water, and 2 g NaCl and 0.1 g EDTA were added to each sample. Ten μl of methyl ethyl sulfide at 1 mg/L was added as an internal standard. All operations were performed at 4 °C. Volatile sulfur compounds were sampled headspace using a 1 cm, 85 μm CAR/PDMS fiber (Supelco Inc., Bellefonte, PA, USA). Samples were equilibrated at 35 °C for 15 min, then extracted at the same temperature for 20 min and desorbed at 250 °C for 5 min.

VSCs was analyzed using 8890 gas chromatograph equipped with a 7000D MS detector and a HP-5MS column (30 m × 0.25 mm × 0.25 μm, Agilent Technologies, Santa Clara, CA, USA). The sample was injected in splitless mode. The heating procedure of the oven temperature is 40 °C for 7 min, increased by 3 °C/min to 50 °C and increased by 10 °C/min to 130 °C for 1 min. The temperature was finally increased at 20 °C/min to 220 °C, and held for 5 min. The transfer line and ion source temperatures were set at 280 °C and 250 °C, respectively. Data collection was performed using a multi-response monitoring (MRM) mode as previously described by (Zhu et al., 2023). Briefly, fragment ions from VSCs were detected in full scan mode. The mass range was set from 35 to 400 amu. Then, the selected precursor ions were subjected to collision-induced dissociation (CID) in the collision energies (CEs) which range from 5 to 25 eV. Finally, based on the optimized collision energies, VSCs in wine were characterized by retention time and collision fragment ions.

The compounds were qualified by comparing the retention time with authentic standards, and quantified by calibration curves. Each sample was analyzed three times. The chromatograph and compound quantification information were showed in Table S2 and Fig. S3.

Table 7
The effect of different nitrogen compositions and YAN levels on the volatile compounds production of LA-FR.

Compound	YAN level			N composition			level* composition
	110 mg/L	220 mg/L	330 mg/L	Control	High DAP	High S-amino acids	
Volatile sulfur compounds							
1-propanol,3-(methylthio)-	3464 ± 4814c	8694 ± 12850b	16,730 ± 22229a	229.1 ± 175.4b	1450 ± 3741b	27,209 ± 15701a	<0.001
dimethyl disulfide	2.03 ± 1.61	1.12 ± 1.33	1.78 ± 1.02	1.23 ± 1.11	2.39 ± 1.51	1.32 ± 1.20	ns
diethyl sulfide	0.57 ± 0.34	0.47 ± 0.38	0.62 ± 0.31	0.61 ± 0.34	0.55 ± 0.40	0.5 ± 0.3	0.002
2-methyltetrahydrothiophen-3-one	455.8 ± 305.2	551.7 ± 460.6	587.7 ± 421.0	253.7 ± 26.0b	312.6 ± 202.9b	1028 ± 179a	ns
Dimethyl trisulfide	0.32 ± 0.13a	0.19 ± 0.06b	0.24 ± 0.12ab	0.3 ± 0.1a	0.3 ± 0.1a	0.15 ± 0.03b	ns
Esters							
ethyl butyrate	3.65 ± 5.20	4.79 ± 4.26	15.67 ± 41.30	5.3 ± 5.2	15.76 ± 41.30	3.05 ± 3.67	ns
isobutyl acetate	2191 ± 609b	1847 ± 413c	2716 ± 328a	1801 ± 498b	2346 ± 522a	2606 ± 425a	ns
ethyl valerate	121.1 ± 23.2	130.7 ± 21.0	143.2 ± 29.9	115.0 ± 21.2b	145.7 ± 16.4a	134.3 ± 29.8ab	ns
ethyl lactate	4.15 ± 1.40a	4.08 ± 3.00a	2.75 ± 1.71b	1.55 ± 1.15b	4.65 ± 2.28a	4.79 ± 1.10a	<0.001
ethyl caprylate	51.14 ± 28.40a	101.9 ± 81.5b	95.92 ± 67.13b	132.2 ± 88.2a	71.77 ± 23.03b	45.06 ± 29.85b	<0.001
ethyl hexanoate	36.9 ± 41.9	16.79 ± 16.00	31.92 ± 19.98	25.74 ± 15.19ab	45.73 ± 42.46a	14.19 ± 7.19b	ns
ethyl acetate	80.52 ± 44.80b	124.4 ± 73.7a	65.21 ± 38.39c	111.1 ± 79.3a	118.46 ± 22.90a	40.55 ± 8.50b	<0.001
methyl dodecanoate	49.43 ± 15.69	50.62 ± 28.41	43.65 ± 25.61	40.18 ± 18.93b	63.2 ± 27.2a	40.33 ± 15.70b	0.006
Alcohols							
isoamyl alcohol*	78.16 ± 8.77ab	71.63 ± 12.20b	83.13 ± 15.94a	72.65 ± 8.66b	79.78 ± 18.00a	80.49 ± 10.66a	<0.001
phenethyl alcohol*	33.38 ± 9.52	38.68 ± 9.03	34.04 ± 9.60	34.93 ± 7.93	35.12 ± 10.75	36.05 ± 10.18	<0.001
β-hydroxyphenethylalcohol	7681 ± 4112a	2655 ± 1036b	2299 ± 1274b	3105 ± 3548	5146 ± 4669	4384 ± 1790	ns
Acids							
acetic acid*	9.91 ± 2.01a	7.39 ± 1.20b	9.65 ± 3.39a	8.91 ± 2.16ab	10.24 ± 3.05a	7.8 ± 2.0b	ns
butyric acid	50.12 ± 7.73b	67.40 ± 18.52a	75.22 ± 26.56a	57.75 ± 4.42b	53.51 ± 13.20b	81.48 ± 27.83a	0.031
hexanoic acid	264.0 ± 97.0	257.7 ± 107.4	276.2 ± 80.5	334.1 ± 54.7a	227.1 ± 101.1b	236.9 ± 80.9b	ns
decanoic acid	7.08 ± 11.32	4.25 ± 2.29	2.81 ± 1.13	7.23 ± 11.09	2.62 ± 2.42	4.29 ± 2.02	0.023
octanoic acid	389.2 ± 73.1	400.0 ± 76.3	384.9 ± 118.9	442.6 ± 59.39a	418.3 ± 77.1a	313.2 ± 73.2b	0.015
Others							
4-heptenal	28.06 ± 6.35b	23.9 ± 6.7b	34.21 ± 9.95a	34.1 ± 6.6a	29.05 ± 10.79b	23.03 ± 4.02c	<0.001
acetoin	45.47 ± 47.80a	18.55 ± 11.87c	26.41 ± 11.11b	25.98 ± 11.46b	52.02 ± 43.55a	12.43 ± 6.41c	<0.001

*The unit of these compound concentration are mg/L. The units of all the other volatile compound's concentration are μg/L. Different letters indicate statistical differences between treatments by ANOVA (Duncan, $p < 0.05$).

2.9. Standard addition of VSCs and sensory analysis

A commercial Chardonnay wine with low VSC content was selected as the matrix for the standard addition and sensory analysis. The volatile composition of Chardonnay wine was analyzed using the same methods as described above (Table S3). 3-Methylthiopropanol (3-MTP), dimethyl disulfide (DMDS), and 2-methyltetrahydrothiophen-3-one (2-MO) were added to the Chardonnay wine at sub-threshold, threshold, and double threshold concentrations for sensory analysis. Specifically, the addition amounts of 3-MTP were 0.5, 1 and 2 mg/L. Addition amounts of DMDS were 0.0001, 0.0002 and 0.0004 mg/L. Addition amounts of 2-MO were 0.00025, 0.0005 and 0.001 mg/L.

For quantitative descriptive analysis (QDA), the sensory panel was consisted of 6 judges (1 male and 5 females) recruited from Huazhong Agricultural University. Panelists were selected based on their olfactory sensitivity and the ability to describe the odor and were trained before the formal descriptive analysis. The panelists were trained for basic aroma detection and scale use. During the training sessions (6 sessions, 1 h for each session), the assessors were presented with samples of commercial wines that represented a wide range of sensory characteristics. They were asked to generate their individual descriptors. The panelists discussed about the best descriptors, their definitions, and how to assess them. To increase its homogeneity, the panel was trained to correctly use the selected terms.

At the end of the training phase, the samples were evaluated using a 5-point scale with 1 being “weak” and 5 being “strong”. The samples were coded using 3-digit random numbers and presented at a random order. Two repeat evaluations were performed by each panelist for each sample. All sensory experiments were conducted in the sensory laboratory of Huazhong Agricultural University that was designed according to ISO 8589, under artificial daylight and temperature control (22 °C).

A total of 29 consumers (9 males and 20 females) were recruit for a Check-All-That-Apply (CATA) analysis. They were asked to evaluate each sample and answer a CATA questionnaire with 16 descriptors. The descriptors were selected based on previous consumer studies (Ares et al., 2015). Terms were presented in different order for each product and each panelist according to the recommendations (Ares et al., 2014), following a randomized block design. The consumers were asked to rate over 16 attributes. Data was recorded in a binary format (0: attribute not checked; 1: attribute checked).

Participants were told they were being recruited for a study on the wine flavor. We conducted the collection of this data at Huazhong

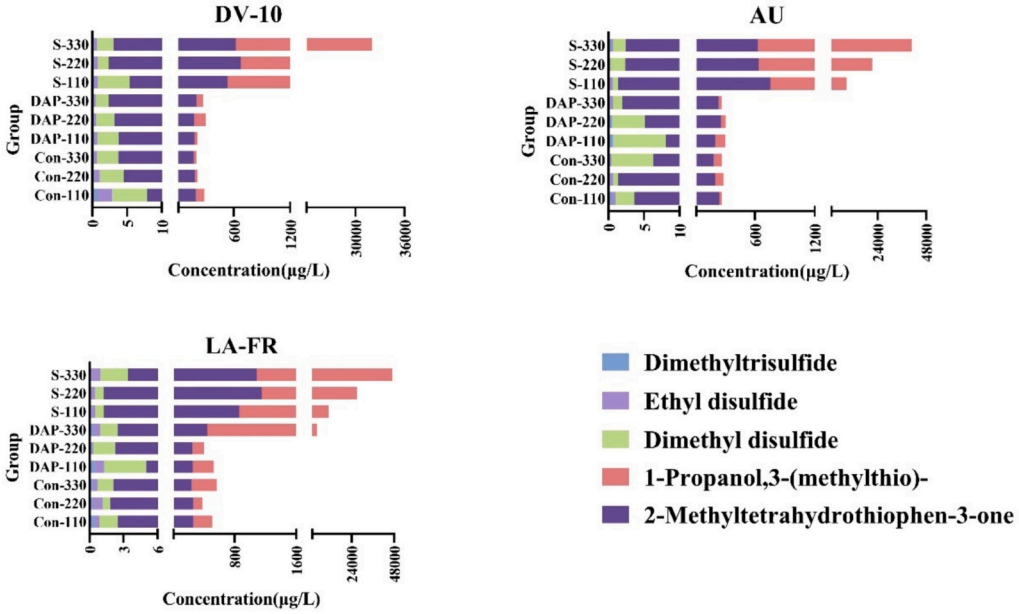

Fig. 4. The effect of different nitrogen source compositions and levels on the volatile sulfur compounds production of different yeasts.

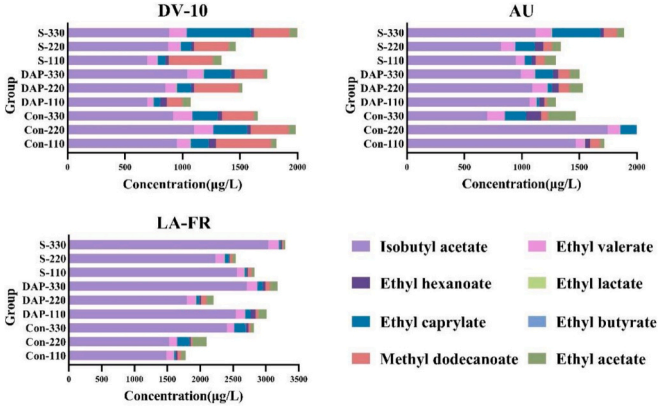

Fig. 5. The effect of different nitrogen source compositions and levels on the esters production of different yeasts.

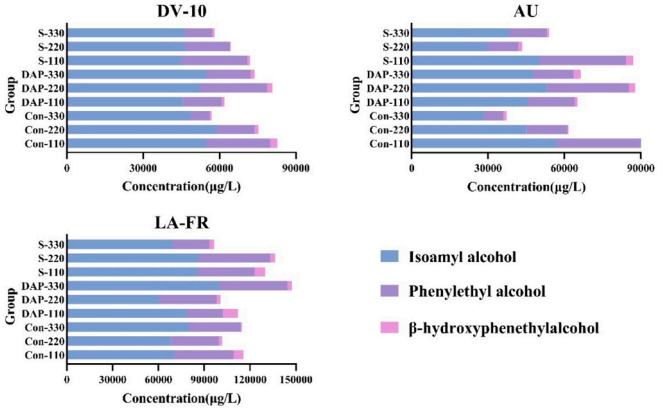

Fig. 6. The effect of different nitrogen source compositions and levels on the higher alcohols production of different yeasts.

Agricultural University. The study was approved by the HZAU Institutional Review Board (IRB), as the protocol was deemed to be of no potential harm. Each and all subjects read, agreed to, and signed a written consent form, which was also reviewed and approved by the HZAU IRB.

2.10. Statistical analysis

Analysis of variance (ANOVA) was performed using SPSS version 27.0 (SPSS Inc., Chicago, IL, USA) for the volatile compound data. The effects of YAN compositions and levels were investigated using two-way ANOVA. Results were considered significantly different if the associated p-value <0.05. Principal component analysis (PCA) was performed using SIMCA software (Umetrics, Malmo, Sverige). Graphs was made using GraphPad Prism 9 (GraphPad Software, CA, USA) and Origin 2017 software (Origin Lab Co., Northampton, MA). The frequency of descriptors obtained by CATA was analyzed by Cochran's Q test and corresponding analysis (CA) by SPSS version 27.0 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Analysis of yeast H_2S-producing and gas-producing capacity

Since yeast strain is a very important factor that affect the wine volatile composition, we firstly selected three commercial *S. cerevisiae* strains with different H_2S-producing capacity. The AU strain were white colonies on the medium, the DV-10 was light brown, and LA-FR was dark brown, which represented a low, medium and high H_2S-producing strain, respectively (Fig. 1).

The gas producing capacity of three strains were also analyzed (Table 3). The results showed that the gas producing capacity of the three yeast strains were highly consistent with their H_2S-producing capacity. Among the three strains of yeast, LA-FR was the earliest one to produce gas, and produced highest amount of gas after 48 h. While DV-10 produced gas slightly slower compared to the LA-FR, and AU had the lowest gas-producing capacity.

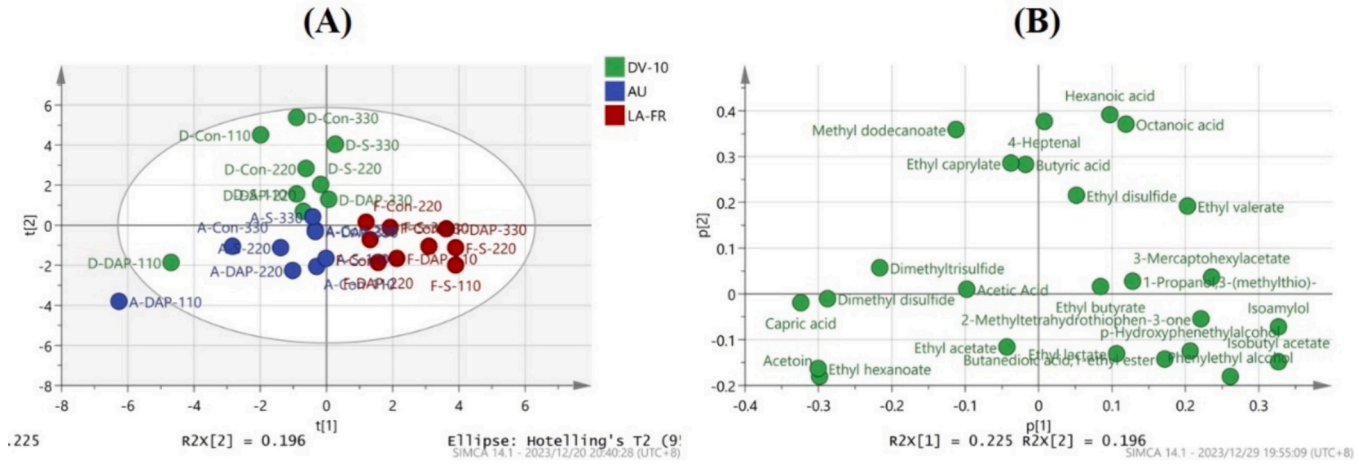

Fig. 7. Principal component analysis: scores plot (A) and loading plot (B) for volatile compounds under 27 fermentation conditions. Mean concentrations of volatiles for each treatment ($n = 3$) were used for PCA. The treatment codes can be found in Table 2.

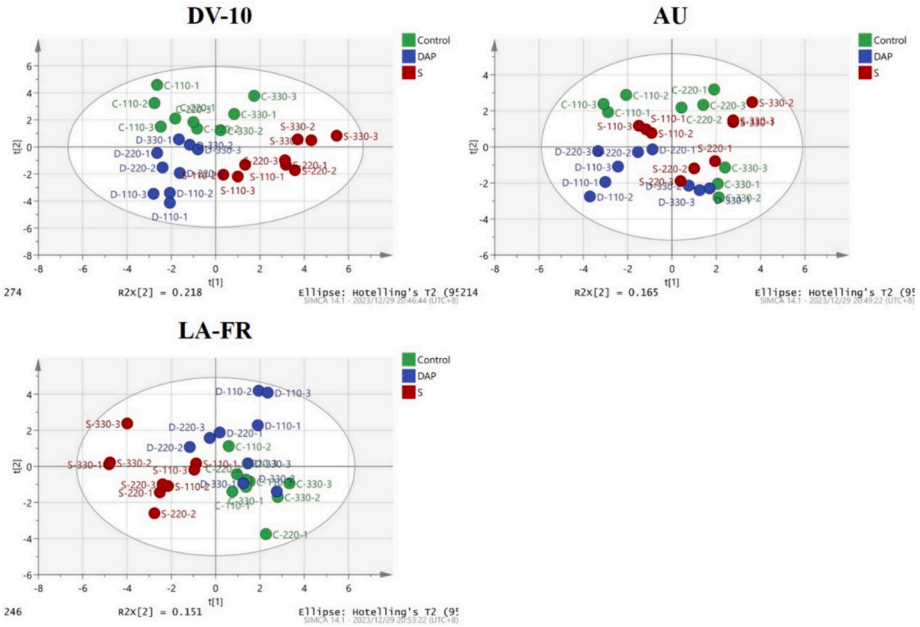

Fig. 8. Scores plot of principal component analysis for volatile flavor compounds in wine fermented by different strains.

Table 8
Quantitative descriptive analysis (QDA) results for a standard addition test of 3-MTP, DMDS and 2-MO in a commercial Chardonnay wine.

		3-MTP			DMDS			2-MO		
	Control	Sub threshold	Threshold	Double threshold	Sub threshold	Threshold	Double threshold	Sub threshold	Threshold	Double threshold
yeasty	2.83a	1.50b	1.33b	1.33b	3.33	3.67	3.00	3.67	3.33	3.50
carlic	1.33	1.83	2.00	1.17	1.00	1.17	1.00	1.00	1.33	1.33
refreshing	2.17	2.00	2.00	1.33	2.17	2.00	2.17	2.00	1.67	2.33
floral	1.00	1.50	1.00	1.50	1.00	1.50	0.83	1.33	1.50	1.17
honey	1.33	2.17	1.00	2.50	1.17	1.83	1.17	1.17	1.50	1.50
cantaloupe	1.33b	2.67ab	3.00ab	3.33a	1.17	1.50	1.33	1.50	1.83	1.67
green apple	1.83	3.00	3.00	3.17	1.33	1.50	1.83	2.33	2.00	1.67
cooked vegetable	1.67b	2.50ab	3.00ab	3.50a	1.33	1.33	0.83	2.17	2.17	2.33

Different letters indicate statistical differences between treatments by ANOVA (Duncan, $p < 0.05$).

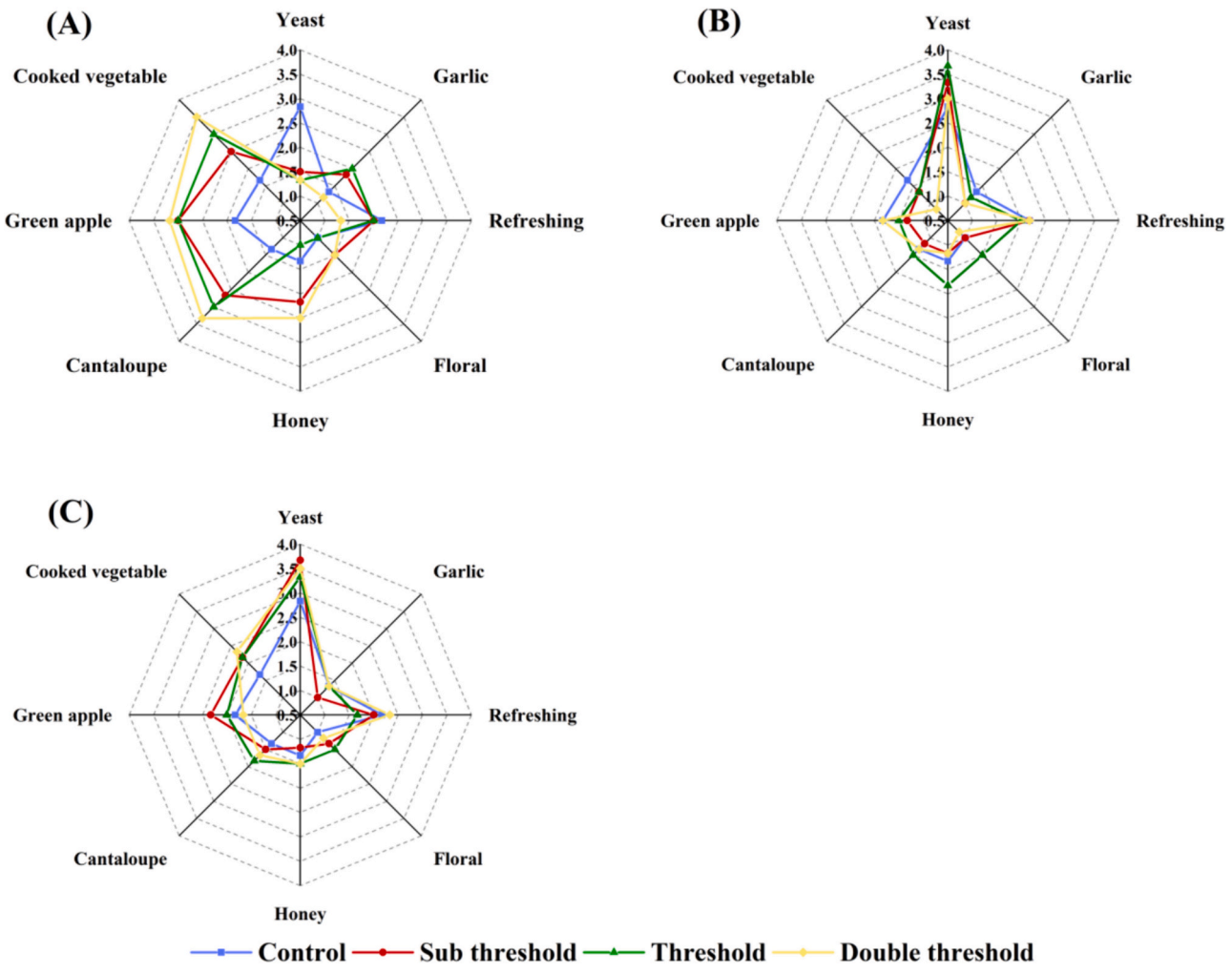

Fig. 9. Odor profile of wines with different volatile sulfur compound additions. (A) 3-MTP. (B) DMDS. (C) 2-MO.

3.2. Effect of nitrogen source on sugar and N consumption for different strains during fermentation

It has been suggested that fermentation performance may be influenced by numerous parameters, including the type of nitrogen supply, the initial YAN level of the medium, and the timing of nitrogen addition (Lola et al., 2023). The nitrogen source ratio designed in this study was similar to that in natural grape juice (Jimenez-Lorenzo et al., 2021; Rollero et al., 2014). The changes of residual sugar and YAN were monitored during the fermentation to explore the effects of different nitrogen source on sugar and N consumption for DV-10, LA-FR and AU strains. The results showed that for all the three strains, the sugar consumption were obviously slower when the initial YAN level was low (110 mg/L) (Fig. 2), indicating a slow metabolism of yeast (Beaudeau et al., 2023). The initial YAN level at 220 mg/L or 330 mg/L had little impact on the sugar consumption rate for all three strains.

The initial YAN composition also had an impact on sugar consumption of the yeasts. For all the three strains, low YAN with high DAP proportion resulted in a sluggish fermentation (Fig. 2B, E and H). However, low YAN with a high proportion of cysteine and methionine supported the fermentation to be finished, with the same residual sugar level (Fig. 2C, F and I) as the control groups (Fig. 2A, D and G).

When it came to N consumption behavior, three yeast strains differed significantly. Compared to DV-10 and AU, LA-FR's N consumption rate was noticeably higher and the N consumption rate for LA-FR was not significantly affected by the YAN composition (Fig. 3G, H and I). Despite the initial YAN level, a higher proportion of DAP resulted in quicker N consumption for DV-10 and AU. High DAP improved N utilization during fermentation when fermented with a high initial YAN (330 mg/L) possibly because DAP was the preferred nitrogen source for yeast (Gobert et al., 2019), which could support the rapid growth of yeast at beginning of fermentation (Godard et al., 2007). However, our findings also revealed that when the initial YAN concentration was insufficient, a higher proportion of DAP could result in an excess of biomass at the start of fermentation, leading to fermentation failure at a later stage.

3.3. Effect of nitrogen source on volatile sulfur compound production

3.3.1. Hydrogen sulfide

Hydrogen sulfide (H_2S) yield was recorded during the fermentation to evaluate the effects of different treatments (Table 4). Large variations were observed within the treatment groups probably because the H_2S was a highly reactive compound. However, the results showed that for the three strains, the interaction between N composition and level had significant impact on its H_2S production. Generally, the LA-FR strain produced more H_2S than AU and DV-10 during fermentation, which was consistent to the BIGGY test results. For LA-FR, higher initial YAN resulted in a higher H_2S production. For DV-10 and AU, initial YAN at low (110 mg/L) and medium (220 mg/L) levels resulted in a higher H_2S production compared to the high (330 mg/L) level. Song, Gibney, Cheng, Liu, and Peck (2020) reported that in apple juice, a high-H_2S production strain UCD522 produced up to 0.228 mg/100 mL H_2S, which was lower compared to the low and medium H_2S-producing strains AU and DV-10 in our study (up to 1.72 and 1.03 mg/100 mL), which could

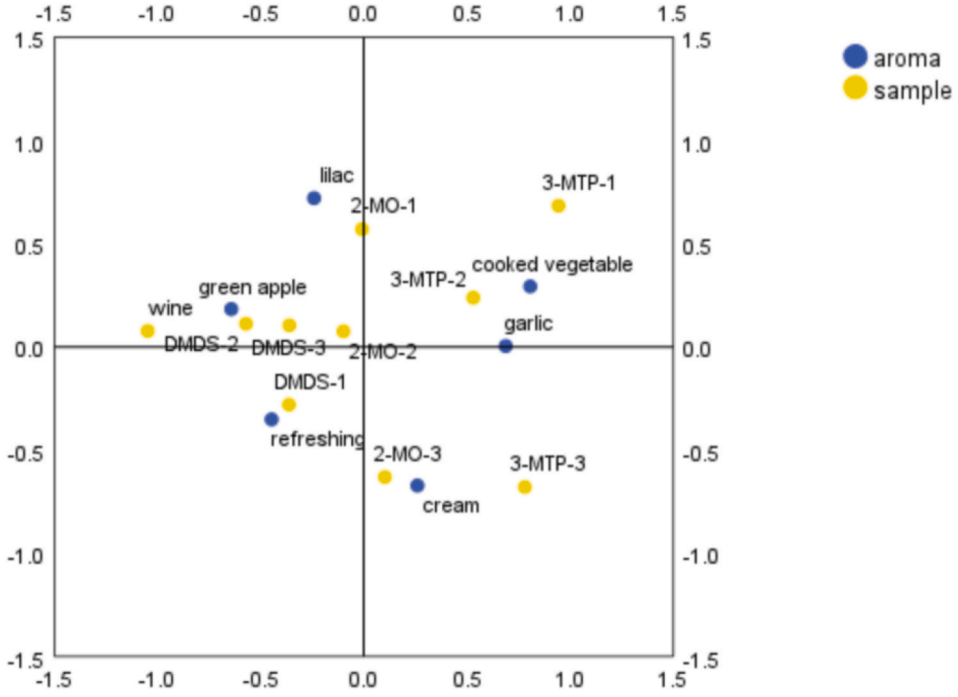

Fig. 10. A bi-plot by correspondence analysis of the 13 samples in association with the 6 sensory attributes. 3-MTP-1, 3-MTP-2, and 3-MTP-3 represent the addition of 3-MTP to Chardonnay wine at sub-threshold, threshold, and double-threshold concentrations, respectively; the same applies for the other experimental groups.

Table 9
Check-all-that-apply (CATA) results for a standard addition test of 3-MTP, DMDS and 2-MO in a commercial Chardonnay wine.

		3-MTP			DMDS			2-MO		
Sensory attribute	Control	Sub threshold	Threshold	Double threshold	Sub threshold	Threshold	Double threshold	Sub threshold	Threshold	Double threshold
grassy	6	5	6	4	3	5	3	4	5	2
minty	5	3	2	5	8	3	5	2	2	2
green apple***	14	5	5	4	7	13	10	7	11	9
garlic*	2	7	8	7	3	3	2	4	5	7
cooked vegetable***	2	11	10	10	3	3	6	4	7	5
cantaloupe	2	2	2	2	4	5	4	3	1	3
almond	3	6	5	6	2	7	4	4	0	3
refreshing**	10	2	7	7	9	8	9	3	9	8
grapefruit	8	4	3	3	5	6	6	9	4	4
yeasty	19	18	14	13	17	16	17	17	23	16
sweaty	4	5	4	3	1	1	2	3	5	5
lilac*	2	3	6	0	4	6	4	3	8	2
creamy*	1	3	6	8	5	6	4	2	9	9
grape	7	7	3	5	6	9	7	9	8	6
floral	4	4	2	1	7	6	5	7	3	3
honey	3	8	2	4	5	2	5	5	1	5

Selection frequencies of sensory attributes presented ($N = 29$). For a given attribute, Cochran's Q test allows to test the effect of an explanatory variable (Products) on whether the consumers feel the attribute or not. A low p-value beyond a significance threshold indicates that products significantly differ from each other. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

be associated with the strain characteristic or the matrix differences. However, the effect of DAP on UCD522 was similar with AU and DV-10 in our study that the high DAP treatment produced the least amount of H_2S throughout the fermentation. These results indicated that the effect of YAN on yeast H_2S production might vary depending on the strain. For strains with low sulfur production capacity, insufficient N would lead to more H_2S production. However, for strains with strong sulfur production capacity, they would still produce a large amount of H_2S even when N was sufficient.

When comparing the effect of different N composition on H_2S production, the results showed that the production of H_2S was lower when the strain was cultured in the medium with a balanced N composition (Spiropoulos, Tanaka, Flerianos, & Bisson, 2000). When the total YAN level kept the same, more S-amino acids led to a significant increase of total H_2S production. It has been widely reported that S-amino acids are potential precursors of VSCs in synthetic medium. However, the ability of cysteine and methionine to act as a precursor to H_2S is yet to be confirmed in real wine. Only Smith et al. (2015) reported that cysteine and glutathione were associated with small increases in H_2S concentrations in wine, with a maximum yield of 0.18 % and 1.3 %, respectively, but the H_2S concentration also affected by many other factors and treatments such as copper and pH.

3.3.2. Other volatile sulfur compounds

The sulfides in wine were partly produced by yeast metabolism, such as H_2S and lower mercaptans such as methyl mercaptan, ethanethiol, which gave wine an unpleasant odor (Coetzee & du Toit, 2012; Darriet, Tominaga, Lavigne, Boidron, & Dubourdieu, 1995; Tominaga, Furrer, Henry, & Dubourdieu, 1998). Except for H_2S, five VSCs were detected by SPME-GC–MS/MS after fermentation, namely dimethyl trisulfide (DMTS), dimethyl disulfide (DMDS) and diethyl sulfide (DES), 3-(methylthio)-1-propanol (3-MTP) and 2-methyltetrahydrothiophen-3-one (2-MO) (Tables 5, 6 and 7).

Among them, 2-MO is a VSC that was less commonly reported in wine but has important aroma contribution (usually as off-flavor), which has chlorine and wet notes (Pino & Queris, 2011). In this study, for all three strains, high S-amino acid treatment significantly increased the concentration of 2-MO. Our findings provide credence to the theory that methionine serves as a precursor in yeast metabolism to 2-methyltetrahydrothiophen-3-one (Moreira, Guedes de Pinho, Santos, & Vasconcelos, 2010), but the mechanism of the transformation is still unknown.

It could be found that for all the three strains, nitrogen level and nitrogen composition had a significant effect on the yield of 3-MTP. Higher proportion of S-amino acids increased the 3-MTP significantly (Fig. 4), which was consistent with previous reports (Huang et al., 2023; Pinu et al., 2014; Rollero et al., 2021). 3-MTP was deemed to be the main compound responsible for yeast-induced reduction faults in wine, with a threshold of 500 μg/L in wine and a concentration of up to 5 mg/L in some wines with sulfur odor (Swiegers, Bartowsky, Henschke, & Pretorius, 2005). In our investigation, the level of YAN also significantly affected the 3-MTP production for all three yeast strains, with higher YAN levels yielding more 3-MTP (Tables 5, 6 and 7). It was consistent with several investigations that methionine supplementation before fermentation resulted in an increase in 3-MTP and its corresponding acid (Bell & Henschke, 2005; Moreira et al., 2002). However, some investigations have shown different results, probably due to differences in fermentation conditions and yeast strains. For example, Hernández-Orte, Ibarz, Cacho, and Ferreira (2005) reported that the 3-MTP content decreased in wines obtained from nitrogen-supplemented fermentations (DAP and free amino acid). However Moreira, de Pinho, Santos, and Vasconcelos (2011) reported that the content of 3-MTP was not affected by the amount of ammonium sulfate and grape variety. There were variations in the three yeast strains' capability to produce VSC as well. For example, high YAN level (330 mg/L) with high proportion of DAP led to a significant increase of 3-MTP for LA-FR strain, but same phenomenon was not observed for DV-10 and AU.

DES, DMDS, and DMTS were present at lower concentrations after fermentation and were less influenced by the treatments. Low YAN (110 mg/L) enhanced DMTS levels in DV-10 and LA-FR fermented samples, but no other consistent pattern was detected for these compounds.

3.4. Effect of nitrogen source on other volatile compounds

3.4.1. Esters

A total of eight volatile ester compounds were detected in the fermented wines (Fig. 5). Isobutyl acetate in DV-10 samples and ethyl acetate in AU samples increased with increasing amounts of the initial YAN (Tables 5 and 6). Although acetate esters were derived directly from the corresponding higher alcohols by condensation with acetyl CoA, in this study, the production of acetate esters was not related to the production of higher alcohols. The increased production of acetate ester in response to nitrogen supplementation might be caused by increased expression of the gene encoding alcohol acyl transferase enzymes in yeasts (Verstrepen et al., 2003). In general, moderate YAN level resulted in more acetate esters.

Similar to acetate esters, ethyl esters are yeast-derived metabolite that contribute to the fruity flavor of wines (Swiegers et al., 2005). A total of five ethyl esters were detected in this study. It was found that the concentration of ethyl valerate was positively correlated with the level of YAN, regardless of strain and nitrogen composition. For DV-10 and AU, ethyl caprylate was positively correlated with the level of YAN. The positive relationship between YAN level and ethyl esters has been reported in many other studies (Bloem, Sanchez, Dequin, & Camarasa, 2016; Hu et al., 2019; Lola et al., 2023; Saerens et al., 2008).

N composition also had some effect on the ester production but was also depend on the yeast strain. For AU and DV-10, high YAN (330 mg/L) with a large proportion of S-amino acid, and medium YAN (220 mg/L) with balanced N composition appeared to be the best conditions for total ester synthesis (Fig. 5), which was consistent with the general viewpoint of winemakers. However, for LA-FR, it was interesting that high YAN (330 mg/L) with unbalanced N composition (S-330 and DAP-330, in Fig. 5) resulted in more ester production. Our findings suggested that, in addition to nitrogen, the properties of the yeast employed in the winemaking process should be completely studied in order to achieve a higher wine aroma quality.

3.4.2. Higher alcohols

Higher alcohols are one of the main metabolic byproducts produced by *S. cerevisiae* during winemaking. A total of three higher alcohols, namely isoamyl alcohol, β-hydroxyphenethyl alcohol and phenylethyl alcohol, were detected in wine after fermentation (Fig. 6). It was also found that the higher alcohols produced by the fermentation of the LA-FR strain were much higher than those of the other two strains. The amount of higher alcohols in wine usually depends on the strain of yeast used for fermentation, and various *S. cerevisiae* strains produce different levels of higher alcohols (Furdikova, Makysova, & Spanik, 2017; Molina et al., 2009; Ut et al., 2022). Our results also indicated a large variation of alcohol produce capabilities among the three strains. Generally, sufficient and balanced composition of nitrogen source could reduce the total yield of higher alcohols in wine, and it was more obvious for AU and DV-10.

Phenylethyl alcohol has a sweet rose-like floral aroma, and its content trends in the S treatment of the DV-10 strain was negatively correlated with the YAN level, which was possibly because low YAN levels inhibited amino acid biosynthesis pathways and generated excess ketoacids, which were decarboxylated and reduced to generate new higher alcohols. With sufficient YAN level, there was a sufficient source of N for the biosynthesis of amino acids, so the α-keto acids and higher alcohols would be reduced (Bell & Henschke, 2005). In addition, it was found that in the high S-amino acids group of AU strains, the content of higher alcohols appeared to decrease and then increase with increasing nitrogen concentration, which was consistent with the observation by Vilanova et al. (2007).

3.4.3. Other volatile compounds

Generally, high initial YAN led to higher total volatile acids for all the three strains, but the impact was not consistent for the individual compound. Our results were similar with previous studies that DAP-supplementation had increased concentrations of acetates, straight chain fatty acids, and straight chain fatty acid ethyl esters but lower concentrations of branched-chain fatty acids and their ethyl esters in the Shiraz wines (Ugliano et al., 2008). We also observed an inverse relationship between the initial YAN and acetoin concentration. It is not surprising because the synthesis of carbonyl compounds (diacetyl, 2,3-butanediol and acetoin) is regulated by the availability of nitrogen. When the nitrogen content is low, the synthesis of these compounds is activated and it is suppressed when the availability of nitrogen is sufficient (Bell & Henschke, 2005).

3.5. Principal component analysis

PCA analysis was applied to obtain diagrams of simplified relationship between groups and compounds by processing quantitative results of volatile flavor compounds from different treatment groups (Fig. 7A).

AU strains was negatively distributed in the first and second principal components, with principal components 1 and 2 explaining 42.1 % of variability in the contents of volatile flavor compounds among treatments. The overall variance contribution rate was not high, indicating that PC1 and PC2 cannot explain the differences between different treatments well. But scores plot still can revealed that samples fermented by the LA-FR strain were better differentiated from the AU and DV-10 strains, whereas the volatile profile of DV-10 and AU samples were very similar.

Combining the two plots for analysis could visualize the relationship between different treatment groups and flavor compounds. It could be observed that strain was the main factor to distinguish the samples (Fig. 7), so the PCA was further conducted for each strains (Fig. 8). For DV-10, it was clear that the differentiation of volatile profile was mainly driven by the nitrogen composition. For AU and LA-FR strain, there were more interactions between the factor of N level and composition, so that the samples were not clearly separated on the PCA plot.

3.6. Sensory analysis

Volatile sulfides play an important role in the overall flavor profile of wine, with lower thresholds and significant differences in aroma properties at different concentrations. In the above experiments, we detected five volatile sulfides in the fermentation broth, DMDS, DES, 2-MO, DMTS and 3-MTP. Among them, the concentration of DES was below its odor threshold (6 μg/L in 12 % ethanol) (Davis & Qian, 2019). Therefore, three volatile sulfides, 3-MTP, DMDS, and 2-MO were selected to be conducted sensory analysis. From of the QDA (Table 8 and Fig. 9), it can be found that when 3-MTP was added to the wine, it had a bigger impact on the overall profile, altering the scent qualities of yeasty, cooked vegetables, apples, melon, and honey, with slight increases in all four olfactory attributes except the yeasty one. In the PCA plot (Fig. 10), the fruity flavor scores of both cantaloupe and green apple were also positively correlated with the amount of 3-MTP added. The intensity of cantaloupe aroma increased significantly when 3-MTP was added at a subthreshold concentration compared to the original, and the intensity of cantaloupe aroma reached three times that of the control when it was added at double threshold level. In addition, there was a significant drop in yeasty intensity, which might be due to the rise of cantaloupe aroma. Thiols could be further oxidized into disulfide or trisulfide compounds with a “rubbery” or “garlicky” odor, which in turn affect the flavor of the wine (Kinzurik et al., 2016). DMDS has a cooked vegetables smell, but in the wine matrices, addition of DMDS did not lead to an enhancement of cooked vegetable or garlic aromas, but rather to a positive effect on the floral and honey-like aromas, indicating that DMDS has a positive effect on the floral and honey-like aromas of the wines at certain concentration. 2-MO was a sulfuric compound with a metallic and chlorine-like flavor (Moreira et al., 2010). But in wine it significantly increased the aroma intensity of cooked vegetables and yeasty at subthreshold or threshold level.

The *p*-values derived by Cochran's Q test suggest that 6 sensory qualities were evaluated to be significantly different among the 13 samples (Table 9). In order to see the relationship that exists between the three sulfides and the significantly different odor descriptors, sensory profiles were created using CA analysis (Table. 10). The first two dimensions in the graph account for 78.5 % of the descriptor variance, with 62 % (F1) and 16.5 % (F2), respectively. Sample 3-MTP-2 showed a strong link with garlic and cooked vegetables, whereas sample 3-MTP-1 was considerably closer to the attribute cooked vegetables, and the two characteristics garlic and cooked vegetables were more closely associated. Sample 2-MO-1 was extremely similar to the property of lilac, whereas sample 2-MO-3 was closer to the property of cream, indicating that the concentration of VSCs added can result in various sensory qualities. Meanwhile, sample DMDS-1 was greatly connected with cool, but samples DMDS-2, DMDS-3, 2-MO-2, and wine were strongly correlated with green apples. QDA results on some of the attributes differed significantly from CATA. This may be related to the fact that QDA involves a trained panel of experts, whereas CATA is a consumer-oriented sensory analysis method, where consumers may disagree or be uncertain about individual sensory descriptors, eventually leading to discrepancies between the two sensory methods, as demonstrated in other studies (Francis & Williamson, 2015).

4. Conclusion

This study revealed how nitrogen sources (levels and compositions) and *S. cerevisiae* affect the fermentation kinetics and aroma profile of wines. The rate of nitrogen consumption was faster when the proportion of DAP was higher, however, when the total YAN level was low, the high proportion of DAP lead to a risk of sluggish fermentation. This work demonstrated the importance of yeast strain on the production of VSCs, which should be discussed together with nitrogen status. Our showed that due to the different nitrogen requirements and genetic background of *S. cerevisiae* strains, a simple linear relationship could not be established between the nitrogen source and volatile compounds. For practical wine production, the addition of DAP was a common practice to improve the aroma, but it also changed the ratio between inorganic and organic N sources, which might increase the risk of off-flavors in wine. Overall, this study found that the aroma compounds generated during wine fermentation differed depending on the nitrogen source and *S. cerevisiae*, and that the aroma of wine can be optimized in by selecting the optimum YAN level and balanced N composition.

CRediT authorship contribution statement

Yihong Wang: Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis. **Hangxin Zhu:** Writing – original draft, Methodology, Investigation, Formal analysis. **Siyi Pan:** Resources, Conceptualization. **Xiaoyun Xu:** Resources, Conceptualization. **Fang Yuan:** Writing – review & editing, Supervision, Project administration.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Fang Yuan reports financial support was provided by National Natural Science Foundation of China. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by the National Natural Science Foundation of China [grant number 31701561].

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101793.

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