

An indigenous *Saccharomyces cerevisiae* yeast strain isolated from Paarl regional Shiraz grapes to enhance Shiraz wine typicity.

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ABSTRACT

Wine yeast starter cultures differ in their ability to release aroma-enhancing metabolites associated with typical varietal wines. Therefore, this study investigated an indigenous *Saccharomyces cerevisiae* isolated from Paarl regional Shiraz grapes for the release of, amongst

others, volatile thiols (aroma compounds traditionally associated with white cultivars, especially Sauvignon blanc) during the 2016 and 2017 vintages using Shiraz grape must. Chemical analyses of final wines showed that the indigenous strain *i.e.* NI6 produced Shiraz wines lower volatile acidity (VA) and acetic acid concentrations than wines produced with reference strains *i.e.* WE372 and MERIT, respectively. This was further supported by descriptive sensory evaluations of wines, as NI6 wines had typical Shiraz varietal aromas and flavours, *i.e.* ‘berry’, ‘jammy’, ‘smoky’ and ‘spicy, and peppery’. This yeast strain also produced wines with more 3-mercapto-1-hexanol (3MH), a volatile thiol that imparts black currant aromas in red wines, than both red wine reference strains in 2016. Both red wine reference strains, however, produced red wines with higher ester compounds (imparts ‘fruity’ aroma) concentrations than strain NI6. Nonetheless, the ability of NI6 to consistently release volatile thiols during both vintages is advantageous Shiraz wine typicity. Overall, this study showed that wines with a positive correlation with black and/or fruits aromas and flavours also had volatile thiol levels above its sensory detection thresholds, which indicates that ester compounds are not solely responsible for Shiraz wine fruity aromas and flavours as was traditionally reported.

Keywords: *Saccharomyces cerevisiae*, esters, red wine, volatile thiols, volatile acidity.

1. Introduction

Red wines are said to have many health benefits such as the reduced risk of heart disease, depression some cancers (Shukla and Singh, 2011), hence moderate consumption of no more than two glasses per day were previously recommended (Arranz *et al.*, 2012; O’Keefe *et al.*,

2007). Besides health benefits, the consumption of wines should still be enjoyable, which implies that the wine should be characterised by typical varietal aroma and flavour. The wine yeast *Saccharomyces cerevisiae* was reported to be an efficient tool to modulate and enhance wine varietal aromas, viz. 'strawberry', 'raspberry', 'blackcurrant', 'plum', 'caramel', 'herbaceous and/or vegetative', to 'spicy' even 'peppery' (Du Plessis *et al.*, 2017; Van Breda *et al.*, 2013; Walsh *et al.*, 2006). Grape berries and juice contain free volatile and bound non-volatile compounds (metabolites) which are responsible for the primary sensory attributes of the cultivar (Robinson *et al.*, 2014; Swiegers *et al.*, 2005). The wine yeast strain (starter culture) used for alcoholic fermentation contributes to varietal aroma and flavour by converting the non-volatile bound compounds *i.e.* thiols namely 4-mercapto-4-methyl-2-pentanone (4MMP), 3-mercapto-1-hexanol (3MH) 3-mercapto-hexyl acetate (3MHA) present in the grape berries and juice to aromatic volatile thiols during fermentation. Aforesaid, volatile thiols are considered varietal impact aroma compounds of, especially Sauvignon blanc due to the characteristic tropical fruit aroma and flavours it brings about in this cultivar (Coetzee and Du Toit, 2012; Tominaga *et al.*, 2003). However, volatile thiols were shown to be present in other white cultivars *e.g.* Chenin Blanc (Wilson *et al.*, 2019), as well as red cultivars *i.e.* Cabernet Sauvignon and Merlot (Coetzee and Du Toit, 2012). Rigou *et al.* (2014) reported that volatile thiols present in red wines elicit black and/or fruits aromas and flavours, like blackcurrant as opposed to the tropical fruit aromas and flavours. Also, wine yeasts synthesise other aroma active metabolites, like esters which impart fruity aroma nuances, often referred to as the "fermentation bouquet" (Coetzee and du Toit, 2012; Van der Merwe and Van Wyk, 1981). Thus, wine sensory characteristics originate from grape-derived metabolites (Ebeler and Thorngate, 2009; González-Barreiro *et al.*, 2015), yeast-synthesised, and yeast-released metabolites (Bartowsky and Pretorius 2009; Hart *et al.*, 2017).

As winemaking methodologies and styles continuously change, mainly due to climate change which was reported to result in grapes with sub-optimal levels of aroma compound precursors (Jones *et al.*, 2005; Rienth *et al.*, 2016), higher sugar levels, etc. (Van Leeuwen and Darriet, 2016). Grape juice originating from high °Balling (sugar) grapes are known to induce volatile acidity (VA) production by the yeast starter culture (De Orduna, 2010). Acetic acid is the main contributor of VA, which imparts undesirable vinegar-like off-odour, that masks sought-after varietal aromas. Subsequently, final wine sensory characteristics will be affected, as the ability of yeast starter culture to produce typical varietal wines is dependent on the presence of these non-volatile aroma compound precursors. Moreover, climate change was shown to affect red wine sensory quality and varietal aromas (Drappier *et al.*, 2019). It is, therefore, important for oenologists to use yeast strains that still has the ability to enhance red wine typical varietal aromas despite changes in the chemical composition of grape berries and juice, as previous studies reported a positive correlation between varietal typicity and wine preference and/or liking (Jiang *et al.*, 2013; Varela *et al.*, 2017). Subsequently, an *S. cerevisiae* naturally isolated wine yeast strain from Shiraz grapes cultivated in the Paarl region (Western Cape, South Africa), renowned for varietal Shiraz, were evaluated for volatile thiol release and the enhancement of varietal typicity of Shiraz wines.

2. Materials and methods

2.1 Yeast strains

An experimental *S. cerevisiae* active dried wine yeast (ADWY), *i.e.* NI6 (isolated from Paarl Shiraz grapes), was used in this study. Two reference red wine yeasts *i.e.* WE372 (Anchor Yeast, Cape Town, South Africa) and MERIT (Chr. Hansen, Hørsholm, Denmark) were included as references.

2.2 Contour clamped homogeneous electric field (CHEF) DNA karyotyping

DNA karyotyping of yeast strains was conducted according to the embedded agarose procedure described by Hart *et al.* (2016). However, a few modifications were incorporated. Chromosomal DNA was separated in 0.5X TBE diluted from 10X TBE buffer (121.1 g/L Tris, 51.53 g/L boric acid 3.27 g/L EDTA [Sigma-Aldrich, St. Louis, USA]) at 14 °C with pulse-times of 60 s for 15 h and 90 s for 11 h using clamped homogenous electric field (CHEF) gel electrophoresis (CHEF-DR II, Bio-Rad Laboratories, Richmond, USA). Chromosomal banding patterns were visualised on a Bio-Rad image analyser, following staining with 0.01% (v/v) ethidium bromide. Subsequently, DNA karyotypes at the beginning and end of fermentation could be visually analysed to confirm that the respective yeast inoculums completed the fermentation and that the wine sensory profile could be attributed to the relevant yeast strain.

2.3 Small-scale winemaking

Shiraz grapes were harvested from vineyards situated on the ARC Nietvoorbij Research farm (Stellenbosch, South Africa, -33.9168 S 18.85988 E), and small-scale wines were made in triplicate according to the standard cellar method included in the ARC Infruitec-Nietvoorbij harvest programme 2016 and 2017 as described by Hart *et al.* (2017). Briefly, grapes were destemmed and crushed followed by skin contact for 1 h. Thereafter, fermentations were

conducted in 50 kg batches in plastic fermentation vessels. All ADWY were re-hydrated separately in sterile distilled water (30 g/300 mL) in a water bath at 37 °C, and inoculated into the crushed grapes (must) at a dosage of 150 mL/50 kg. All fermentations were conducted at an ambient temperature of ca. 24 °C the fermentation 'cap' formed by skins were punched down three times a day to allow carbon dioxide (CO₂) to escape. Fermentations proceeded until the residual sugar was 50 g/L, whereafter the fermenting skins and juice were pressed at 1 bar. Eighteen litres of juice per treatment were siphoned into clean stainless steel fermentation canisters, sealed with a fermentation lock, and further fermented to dryness (residual sugar > 5 g/L). Wines were then racked of the less and total-SO₂ was adjusted to 85 mg/L. Bentonite was added and wines were subjected to cold stabilisation at 0 °C for 2 weeks. Upon completion of cold stabilisation, wines were filtered and aseptically bottled and allowed to undergo bottle maturation for 5 months. Subsequently, standard chemical parameters of bottled wines, *i.e.* alcohol (%), pH, volatile acidity (VA) (g/L), total acidity (g/L), and residual glucose/fructose concentration (g/L) of all samples were measured using an OenoFoss™ Fourier Transform Infrared (FTIR) spectrometer (FOSS Analytical A/S, Denmark).

2.4 Descriptive sensory evaluation

The wines were subjected to descriptive sensory evaluation following three months of bottle stabilisation by a panel of seven trained wine tasters (judges) as described in Hart *et al.* (2016). The judges were requested to highlight the most prominent aromas and flavours perceived, *i.e.* 'blackcurrant', 'black cherry', 'blackberry', 'green pepper', 'herbs', and 'smoky', as well as intensity of each. All wine samples were coded (approx. 50 mL serving per wine) and served coded in a randomised order using standard wine glasses.

2.5 Aroma compound analyses

Aroma compounds *i.e.* esters, higher alcohols total fatty acids were quantified at the accredited Gas chromatography-mass spectrometry (GC-MS) laboratory, Mass spectroscopy unit, Central Analytical Facility (CAF), University of Stellenbosch (US) as described in Louw *et al.* (2010), with a slight adjustment. Liquid-liquid extraction was employed by sonicating the mixture of diethyl ether (2 mL) and wine (10 mL) for 30 min. As an internal standard, 100 μ L of 4-methyl-2-pentanol diluted to 0.5 mg/L in a model wine solution (12% (v/v) ethanol, 2.5 g/L tartaric acid in deionised water at a pH of 3.5 adjusted with 0.1 M NaOH) were used. Extracts were injected into a Thermo Scientific TRACE 1300 gas chromatograph (Thermo Scientific, Waltham, Massachusetts, USA) equipped with an autosampler split/splitless injector (CTC Analytics AG, Zwingen, Switzerland) coupled to a flame ionisation detector. Aroma compounds were separated with a polar J&W 122-3263 DB-FFAP (60 m length \times 320 μ m internal diameter \times 0.5 μ m) capillary column (Agilent Technologies, Wilmington, USA).

Volatile thiols, *i.e.* 3MH, 3MHA 4MMP, were extracted and quantified from Shiraz wines at the accredited Gas chromatography-mass spectrometry (GC-MS) laboratory, Mass spectroscopy unit, Central Analytical Facility (CAF), University of Stellenbosch (US) as described in Herbst-Johnstone *et al.* (2013) with a slight adjustment. The compound 4-methoxy-2-methyl-2-mercaptobutane (4M2M2MB) served as an internal standard using a Thermo Scientific TRACE 1300 gas chromatograph (Thermo Scientific, Waltham, MA) coupled to a Thermo Scientific TSQ 8000 triple quadrupole mass spectrometer detector (MSD). Separation of compounds was performed with a polar Zebron ZB-FFAP (30 m \times 0.25 mm \times 0.25 μ m) capillary column (Phenomenex; Torrance, CA, USA).

2.6 Statistical analyses

Chemical and sensory data were recorded and subjected to principal component analysis (PCA) using XLSTAT software (ver. 2015.1.03.15485, Addinsoft, 2013) and analysis of variance (ANOVA) using the SAS General Linear Models Procedure (PROC GLM) software (Version 9.2; SAS Institute Inc, Cary, USA. Fisher's least significant difference was calculated at the 5% level to compare treatment means (Ott and Longnecker, 2001). A probability level of 5% was considered significant for all significance tests.

3. Results

3.1 Contour clamped homogeneous electric field (CHEF) DNA karyotyping

The DNA karyotypes of all ADWY, namely WE372, MERIT, and NI6 matched those of the respective cultures isolated at the end of fermentation during both vintages (Fig. 1).



Figure 1. Contour clamped homogeneous electric field (CHEF) DNA karyotypes of two commercial red wine yeasts, i) WE372 (Anchor Yeast, South Africa) and ii) MERIT (Chr. Hansen, Denmark), and iii) NI6 (yeast previously isolated from Paarl Shiraz grapes), respectively.

*Start= Yeast starter culture; End = Yeast colonies randomly isolated at end of fermentation.

3.2 Small-scale winemaking trials

All yeast strains completed the fermentation within five days during the 2016 (Fig. 2) vintage and within nine days during the 2017 (Fig. 3) vintage. Duration of fermentation for both vintages is normal for a red wine harvested at this ripeness and fermented at an ambient temperature of ca. 24 °C.

3.3 Chemical parameter analyses

Standard chemical parameters of Shiraz wines produced during the 2016 and 2017 vintages are shown in Tables 1 and 2. All strains fermented the Shiraz grape must to dryness (residual sugar < 2g/L) during both vintages. Reference strain MERIT produced 2016 Shiraz wines with significantly lower levels of VA, of which acetic acid is the main contributor that imparts

undesirable vinegar-like off-odours, than both NI6 and WE372, respectively. Strain NI6 on the other hand produced 2017 Shiraz wines with significantly lower levels of VA than that produced with the respective references. This observation was complemented by the significantly lower total acidity (TA) levels measured in the NI6-produced wines, especially during the 2017 vintage. In terms of pH, all yeast strains produced wines with values ranging from pH 3.66 to 3.86, which is acceptable for locally produced red wines (Britz and Tracey, 1990). However, NI6-produced wines had marginally higher pH values than wines produced with references during both vintages. All Shiraz wines produced with strain NI6, irrespective of the vintage had marginally higher alcohol concentrations than wines produced with the respective references. Wines produced with NI6 during both vintages also had significantly lower malic acid levels than wines produced with the respective commercial reference strains.

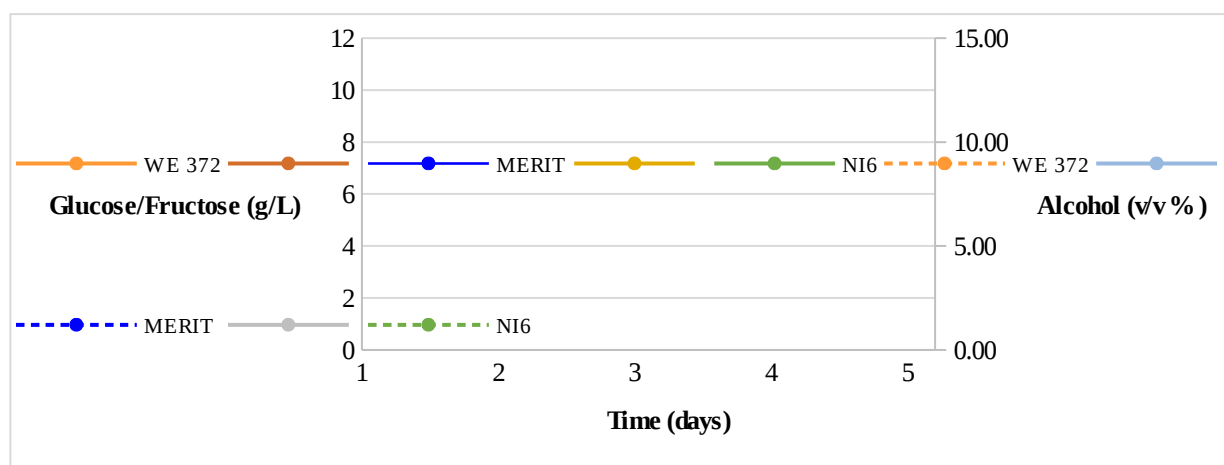


Figure 2. Graph of sugar utilisation (primary vertical axis) versus alcohol production (secondary vertical axis) during alcoholic fermentation of 2016 Shiraz grape must using two commercial red wine yeasts *i.e.* WE372 (Anchor Yeast, South Africa) and MERIT (Chr. Hansen, Denmark); and NI6 (yeast previously isolated from Paarl Shiraz grapes), respectively.

Average values of triplicate fermentations (standard deviation shown as error bars).

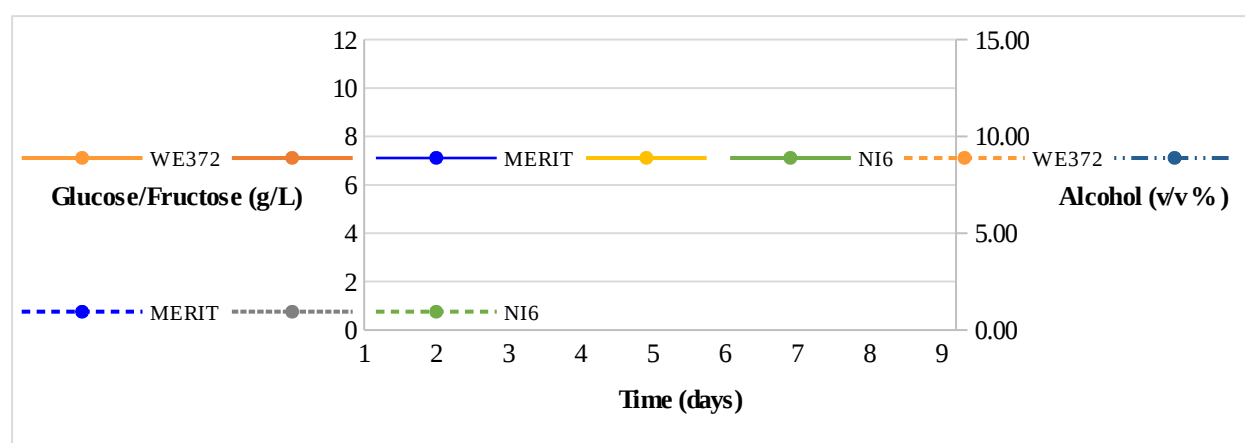


Figure 3. Graph of sugar utilisation (primary vertical axis) versus alcohol production (secondary vertical axis) during alcoholic fermentation (AF) of 2017 Shiraz grape must using two commercial red wine yeasts *i.e.* WE372 (Anchor Yeast, South Africa) and MERIT (Chr. Hansen, Denmark); and NI6 (yeast previously isolated from Paarl Shiraz grapes), respectively.

Average values of triplicate fermentations (standard deviation shown as error bars).

Table 1 Chemical parameters of small-scale 2016 Shiraz wines¹ following fermentation by yeasts selected for the production of Paarl regional Shiraz.

	Glucose/Fructose (g/L)	Total acidity (g/L)	pH	Alcohol (%)	Volatile acidity (g/L)	Malic acid (g/L)
WE372 ³	0,00 ± 0,00 ^a	5,99 ± 0,19 ^a	3,79 ± 0,05 ^b	15,29 ± 0,16 ^b	0,92 ± 0,18 ^a	0,30 ± 0,01 ^a
MERIT ⁴	0,00 ± 0,00 ^a	5,44 ± 0,14 ^b	3,85 ± 0,03 ^a	15,99 ± 0,28 ^a	0,63 ± 0,14 ^b	0,30 ± 0,02 ^a
NI6 ⁵	0,00 ± 0,00 ^a	5,33 ± 0,07 ^b	3,86 ± 0,05 ^a	16,11 ± 0,18 ^a	0,82 ± 0,10 ^a	0,25 ± 0,03 ^b

¹ Original 2016 crushed Shiraz grapes (26.2°B, pH 4.01, total acidity = 4.2 g/L and Free-SO₂ = 30 mg/L) enriched with 50g/100kg DAP. Fermented at an ambient temperature of ca. 24 °C.

² Average values of triplicate fermentations. Total acidity, pH, ethanol, volatile acidity, and malic acid analyses by OenoFoss™ FTIR (ARC Infruitec-Nietvoorbij).

³ Commercial *Saccharomyces cerevisiae* wine yeast (Anchor Yeast, South Africa).

⁴ Commercial *Saccharomyces cerevisiae* wine yeast (Chr. Hansen, Denmark).

⁵ *Saccharomyces cerevisiae* wine yeast (yeast isolated yeast from Paarl Shiraz grapes).

*Mean totals with the same letter in the same column are not significantly different as calculated by Fisher's least significant difference (LSD) at P ≤ 0.05

Table 2 Chemical parameters of small-scale 2017 Shiraz wines¹ following fermentation by yeasts selected for the production of Paarl regional Shiraz.

	Glucose/Fructose (g/L)	Total acidity (g/L)	pH	Alcohol (%)	Volatile acidity (g/L)	Malic acid (g/L)
WE372 ³	0,00 ± 0,00 ^a	6,66 ± 0,01 ^a	3,66 ± 0,03 ^b	14,85 ± 1,04 ^a	1,76 ± 0,13 ^a	0,28 ± 0,02 ^a
MERIT ⁴	0,00 ± 0,00 ^a	5,95 ± 0,15 ^b	3,79 ± 0,05 ^a	14,41 ± 3,64 ^a	1,37 ± 0,05 ^b	0,27 ± 0,05 ^a
NI6 ⁵	0,00 ± 0,00 ^a	5,22 ± 0,50 ^c	3,83 ± 0,09 ^a	15,56 ± 1,09 ^a	1,07 ± 0,20 ^c	0,22 ± 0,01 ^b

¹ Original 2017 crushed Shiraz grapes (26.8°B, pH 3.91, total acidity = 3.88 g/L and Free-SO₂ = 30 mg/L) enriched with 50g/100kg DAP. Fermented at an ambient temperature of ca. 24 °C.

² Average values of triplicate fermentations. Total acidity, pH, ethanol, volatile acidity, and malic acid analyses by OenoFoss™ FTIR (ARC Infruitec-Nietvoorbij).

³ Commercial *Saccharomyces cerevisiae* wine yeast (Anchor Yeast, South Africa).

⁴ Commercial *Saccharomyces cerevisiae* wine yeast (Chr. Hansen, Denmark).

⁵ *Saccharomyces cerevisiae* wine yeast (yeast isolated yeast from Paarl Shiraz grapes).

*Mean totals with the same letter in the same column are not significantly different as calculated by Fisher's least significant difference (LSD) at P ≤ 0.05

3.4 Descriptive sensory evaluation

The PCA (Fig. 4) shows that all yeast strains, namely WE372, MERIT and NI6 produced Shiraz wines during the 2016 vintage with a positive association with typical varietal aromas viz. ‘berry’, ‘jammy’, ‘smokey’ and ‘spicy and peppery’ (Herderich *et al.*, 2012; Frost *et al.*, 2021). Overall, the WE372-produced 2016 wines had the best association with ‘jammy’, ‘smokey’, and ‘spicy and peppery’ aroma and flavour. However, MERIT and NI6 had a similar and better association with ‘colour intensity’, ‘body’ or ‘mouthfeel, as well as ‘finish’ or ‘aftertaste’ than the WE372-produced wines. In terms of ‘overall quality’, the NI6-produced 2016 wines had a closer association than both reference strains.

The WE372-produced 2017 wines equally to the 2016 wines had the best association with ‘berry’ and ‘jammy’ aromas, compared to wines produced with NI6 and MERIT, respectively (Fig. 5). The NI6-produced 2017 wines had the best association with ‘body’ or mouthfeel, as well as ‘smokey’ and ‘spicy and peppery’ aromas. Contrariwise, the MERIT-produced 2017 wines had a negative association with ‘berry’, ‘jammy’, ‘smokey’ and ‘spicy and peppery’ aromas (Fig. 5). However, these wines had a positive association with vegetative (herbaceous) aromas, which include Shiraz varietal aromas like olive (Moran *et al.*, 2021) and herbs (Albanese *et al.*, 2013).

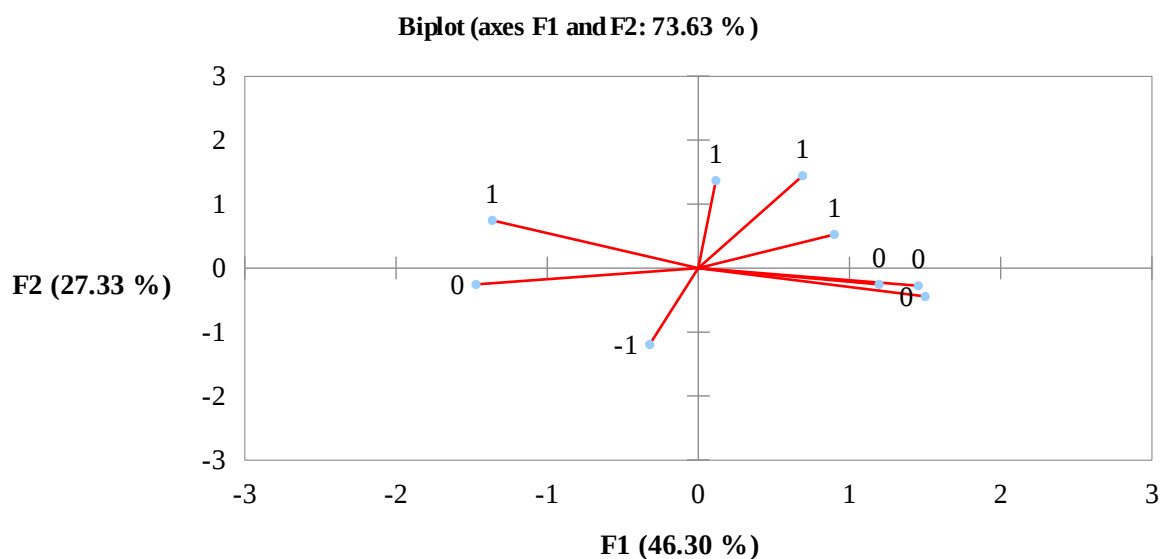
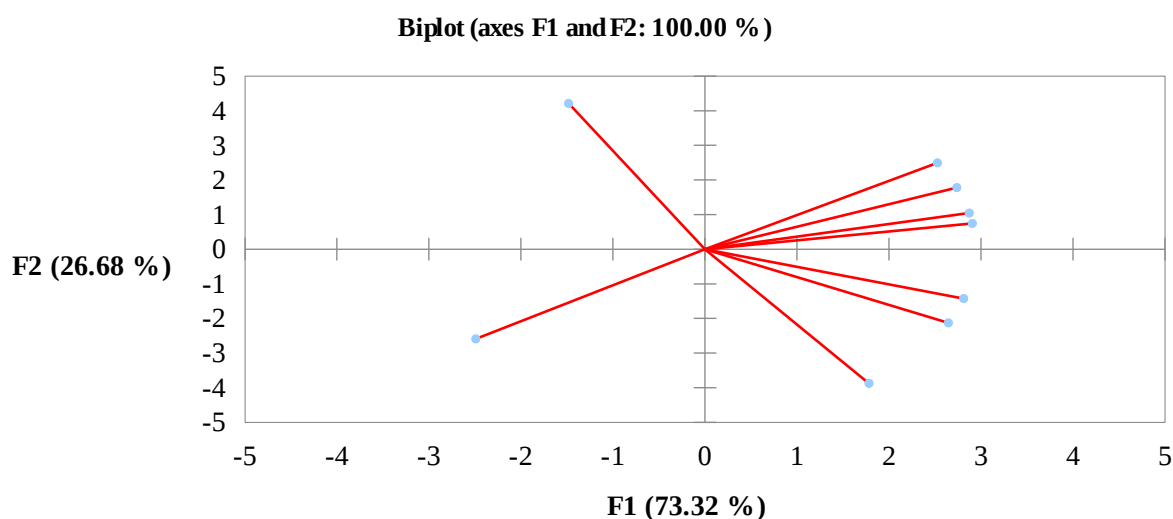


Figure 4. Principal component analysis (PCA) biplots illustrating the descriptive sensory analysis of small-scale 2016 Shiraz wines following fermentation by two commercial red wine yeasts *i.e.* WE372 (Anchor Yeast, South Africa) and MERIT (Chr. Hansen, Denmark); and NI6 (previously isolated yeast from Paarl Shiraz grapes), respectively.



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figure 5. Principal component analysis (PCA) biplots illustrating the descriptive sensory analysis of small-scale 2017 Shiraz wines following fermentation by two commercial red wine yeasts *i.e.* WE372 (Anchor Yeast, South Africa) and MERIT (Chr. Hansen, Denmark); and NI6 (yeast previously isolated from Paarl Shiraz grapes), respectively.

3.5 Aroma compound analyses

Volatile aroma compounds, namely esters, higher alcohols, and fatty acids detected in 2016 and 2017 Shiraz wines are shown in Tables 3 and 4, respectively. The concentration of unwanted acetic acid, a major contributor to fatty acids and volatile acidity in 2016 wines produced with NI6 (270.46 ± 22.03 mg/L) was profoundly lower than in wines produced by both references, namely WE372 (295.95 ± 11.72 mg/L) and MERIT (364.09 ± 87.52 mg/L)(Table 3). The difference in concentrations of the remaining fatty acids *viz.* butyric acid, hexanoic acid, isobutyric acid, isovaleric acid, octanoic acid, and propionic acid was insignificant, except for valeric acid that was significantly less in wines produced with MERIT (0.68 ± 0.03 mg/L) than wines produced with NI6 (0.83 ± 0.04 mg/L) and WE372 (0.93 ± 0.05 mg/L), respectively.

Different acetate and ethyl esters, which generally imparts fruity aroma and flavour, were detected in all 2016 Shiraz wines (Table 3). Overall, the reference strain, namely WE372 produced 2016 Shiraz wines with the highest total ester and higher alcohol (fusel oil) levels, whilst the reference strain, namely MERIT produced wines with the highest fatty acid levels. With reference to acetate esters, significantly more 2-phenylethyl acetate, which imparts Shiraz varietal aromas *i.e.* ‘rose’, ‘honey’ and ‘fruity’ aromas, was measured in wines produced with WE372 (0.73 ± 0.09 mg/L), than wines produced with MERIT (0.55 ± 0.06 mg/L) and NI6 (0.54 ± 0.06 mg/L), respectively (Table 3). A similar observation was made with regard to the remaining acetate esters *viz.* ethyl acetate, hexyl acetate, isoamyl acetate and isobutyl acetate, which are associated with *i.a.* ‘cherry’, ‘floral’ and ‘banana’ aromas. With reference to ethyl esters, an important ethyl ester *i.e.* ethyl lactate, which imparts another varietal note *i.e.* ‘strawberry’, concentration was significantly higher in wines produced with WE372 (12.74 ± 0.30 mg/L) compared to wines produced with MERIT (5.76 ± 0.12 mg/L) and NI6 (7.29 ± 3.00 mg/L), respectively. The concentration of ethyl butyrate which also imparts a varietal note *i.e.*

'fruit', was higher in wines produced with WE372 (0.29 ± 0.03 mg/L) than wines produced with NI6 (0.22 ± 0.02 mg/L) and MERIT (0.17 ± 0.03 mg/L), respectively. The concentration of another important ethyl ester *i.e.* ethyl-3-hydroxybutanoate which impart the varietal note 'berry', was higher in wines produced with WE372 (0.63 ± 0.09 mg/L) than wines produced with NI6 (0.37 ± 0.11 mg/L) and MERIT (0.36 ± 0.01 mg/L), respectively. The yeast WE372 also produced 2017 wines with a higher concentration of diethyl succinate (7.46 ± 0.09 mg/L) which imparts a 'fruity' aroma, than wines produced with MERIT (6.91 ± 0.33 mg/L) and NI6 (5.71 ± 3.51 mg/L). The remaining ethyl esters *viz.* ethyl caprate, ethyl caprylate, ethyl hexanoate, and ethyl phenylacetate, which are associated with *i.a.* 'fruity', 'floral' and 'sweet-associated' aromas showed the same trend.

With reference to higher alcohols, both reference strains, namely WE372 (46.14 ± 3.08 mg/L) and MERIT (38.75 ± 3.61 mg/L) produced 2016 wines with noticeably more 2-phenyl ethanol, which impart 'rose' aromas than wine produced with NI6 (32.84 ± 5.27 mg/L) (Table 3). Strain WE372 (2.56 ± 0.06 mg/L) produced 2016 wines with significantly higher concentration of 3-methyl-1-pentanol, which impart 'fruity' aromas than wines produced with NI6 (1.47 ± 0.60 mg/L) and MERIT (1.16 ± 0.02 mg/L), respectively. The same observation was made regarding butanol, which is associated with 'alcohol' aromas (Table 3). Strains WE372 (90.18 ± 10.81 mg/L) and NI6 (77.41 ± 5.52 mg/L) produced 2016 wines with significantly higher concentrations of n-propanol, which impart 'fruity' aromas than wine produced with MERIT (50.25 ± 22.08 mg/L), respectively. The concentrations of remaining higher alcohols *viz.* 3-ethoxy-1-propanol, 4-methyl-1-pentanol, hexanol, isoamyl alcohol, isobutanol and pentanol which are associated with *i.a.* 'fruity', 'tropical fruit', 'buttery', 'grassy', 'alcohol' and 'banana' aromas were insignificant.

As was observed during 2016 strain NI6 once more produced 2017 wines with noticeably lower total fatty acid concentrations, than wines produced with both references (Table 4). The acetic acid concentration in 2017 wines produced with NI6 (285.22 ± 40.60 mg/L) was significantly lower than in wines produced by both references, namely WE372 (357.48 ± 6.88 mg/L) and MERIT (412.66 ± 8.79 mg/L). The difference in concentrations of the remaining fatty acids viz. butyric acid, hexanoic acid, isobutyric acid, isovaleric acid, octanoic acid, and valeric acid was insignificant, except for propionic acid that was significantly higher in wines produced with WE372 (6.16 ± 0.54 mg/L) than wines produced with NI6 (3.62 ± 1.12 mg/L) and MERIT (1.96 ± 0.09 mg/L), respectively.

The same acetate and ethyl esters detected in 2016 Shiraz wines were detected in 2017 wines (Table 4). Overall, both reference strains, namely WE372 and MERIT produced 2017 Shiraz wines with marginally higher total ester concentrations than wines produced with NI6. However, the latter produced 2017 wines with significantly higher alcohol (fusel oil) concentrations than both references. With reference to ethyl esters, no significant differences in concentrations of 2-phenylethyl acetate, diethyl succinate, ethyl acetate, hexyl acetate, isoamyl acetate and isobutyl acetate were measured in all 2017 produced by the respective strains included in this study (Table 4). With reference to ethyl esters, as was observed during 2016, ethyl lactate concentration was higher in wines produced with WE372 (33.55 ± 1.41 mg/L) compared to wines produced with MERIT (27.19 ± 1.84 mg/L) and NI6 (24.82 ± 7.27 mg/L), respectively. The remaining ethyl esters viz. ethyl butyrate, ethyl caprate, ethyl caprylate, ethyl hexanoate, and ethyl phenylacetate had insignificant differences in concentrations in wines produced with all strains included in the study.

With reference to higher alcohols, strain WE372 (46.86 ± 3.61 mg/L) produced 2017 wines with significantly more 2-phenyl ethanol than wine produced with MERIT (30.64 ± 2.31 mg/L) and

NI6 (18.93 ± 1.08 mg/L), respectively (Table 4). On the hand, MERIT (65.97 ± 1.58 mg/L) produced 2017 wines with significantly more isobutanol than wine produced with NI6 (41.63 ± 9.16 mg/L) and WE372 (37.78 ± 4.42 mg/L), respectively. Strains WE372 (229.17 ± 10.36 mg/L) and NI6 (196.07 ± 25.85 mg/L) as was observed during 2016, produced 2017 wines with significantly higher concentrations of n-propanol than wine produced with MERIT (82.26 ± 1.31 mg/L), respectively. The differences in concentrations of remaining higher alcohols viz. 3-methyl-1-pentanol, 4-methyl-1-pentanol, acetoin, butanol, hexanol, isoamyl alcohol and pentanol which are associated with *i.a.* 'fruity', 'tropical fruit', 'buttery', 'grassy', 'alcohol' and 'banana' aromas were insignificant.

The sulphur-containing volatile thiols, namely 4MMP, 3MH and 3MHA detected in 2016 and 2017 Shiraz wines are shown in Figures 6 and 7, respectively. It was observed that reference strain, namely MERIT released the most 4MMP (396.83 ± 21.90 ng/L) (imparts 'passion fruit' 'berry' [blackcurrant] aromas) followed by NI6 (292.57 ± 20.08 ng/L) and WE372 (125.83 ± 6.09 ng/L) in the 2016 wines (Fig. 6). With reference to 3MHA, nothing was detected in any of the 2016 wines (Fig. 6). However, strain NI6 (290.53 ± 76.40 ng/L) produced wines with a higher 3MH (imparts 'passion fruit' and 'citrus fruit' aromas) concentration than wines produced with MERIT (235.94 ± 31.66 ng/L) and WE372 (220.21 ± 59.02 ng/L), respectively.

As was observed during the 2016 vintage, MERIT released the most 4MMP (2720.92 ± 82.12 ng/L) (imparts 'passion fruit' 'berry' [blackcurrant] aromas) followed by NI6 (2067.92 ± 82.12 ng/L) and WE372 (1220.23 ± 137.31 ng/L) in the 2017 wines (Fig. 7). Once again the WE372-produced 2017 had no detectable 3MHA concentrations (Fig. 7). Both, MERIT (54.05 ± 15.20 ng/L) and NI6 (22.60 ± 3.36 ng/L) produced wines during this vintage with 3MHA concentrations above the compound's sensory detection threshold. In terms of 3MH, MERIT (566.19 ± 14.51 ng/L) produced wines with higher concentrations during 2017 compared to NI6 (510.84 ± 75.98 ng/L) and WE372 (231.66 ± 20.45 ng/L), respectively.

Table 3. Major wine volatile aroma compounds analysed in small-scale Shiraz wines produced in 2016 at the Nietvoorbij research cellar, following fermentation by two commercial red wine yeasts *i.e.* WE372 (Anchor Yeast, South Africa) and MERIT (Chr. Hansen, Denmark); and NI6 (yeast previously isolated from Paarl Shiraz grapes), respectively.

Aroma compounds (mg/L)	Aroma descriptor**	WE372	MERIT	NI6
		Mean \pm Std Dev*	Mean \pm Std Dev*	Mean \pm Std Dev*
Esters				
2-Phenylethyl acetate	Rose, honey, fruity	0.73 \pm 0.09 ^a	0.55 \pm 0.06 ^b	0.54 \pm 0.06 ^b
Diethyl succinate	Fruity	1.19 \pm 0.19 ^a	1.04 \pm 0.14 ^{ab}	0.70 \pm 0.11 ^b
Ethyl acetate	Cherry, floral	40.49 \pm 6.22 ^a	32.59 \pm 2.12 ^a	32.20 \pm 4.09 ^a
Ethyl butyrate	Fruity	0.29 \pm 0.03 ^a	0.17 \pm 0.03 ^b	0.22 \pm 0.02 ^b
Ethyl caprate	Fruity	0.13 \pm 0.02 ^a	0.07 \pm 0.01 ^a	0.09 \pm 0.01 ^a
Ethyl caprylate	Fruity, floral	0.19 \pm 0.03 ^a	0.14 \pm 0.03 ^a	0.17 \pm 0.03 ^a
Ethyl hexanoate	Fruity	0.02 \pm 0.03 ^a	0.00 \pm 0.00 ^a	0.00 \pm 0.00 ^a
Ethyl-3-hydroxybutanoate	Sweet-associated, berry	0.63 \pm 0.09 ^a	0.36 \pm 0.01 ^b	0.37 \pm 0.11 ^b
Ethyl lactate	Strawberry, buttery	12.74 \pm 0.30 ^a	5.76 \pm 0.12 ^b	7.29 \pm 3.00 ^b
Ethyl phenylacetate	Sweet-associated	2.24 \pm 0.07 ^a	1.91 \pm 0.30 ^a	1.87 \pm 0.21 ^a
Hexyl acetate	Cherry, floral	0.21 \pm 0.05 ^a	0.15 \pm 0.00 ^a	0.18 \pm 0.02 ^a
Isoamyl acetate	Banana, tropical fruit	4.59 \pm 0.97 ^a	2.90 \pm 0.32 ^a	3.04 \pm 0.92 ^a
Isobutyl acetate	Banana, tropical fruit	0.07 \pm 0.02 ^a	0.10 \pm 0.02 ^a	0.05 \pm 0.04 ^a
Alcohols				
2-Phenyl ethanol	Roses, honey-like	46.14 \pm 3.08 ^a	38.75 \pm 3.61 ^{ab}	32.84 \pm 5.27 ^b
3-Ethoxy-1-propanol	Fruity	2.17 \pm 0.30 ^a	2.73 \pm 1.23 ^a	2.19 \pm 0.14 ^a
3-Methyl-1-pentanol	Fruity	2.56 \pm 0.06 ^a	1.16 \pm 0.02 ^b	1.47 \pm 0.60 ^b
4-Methyl-1-pentanol	Tropical fruit	0.33 \pm 0.08 ^a	0.18 \pm 0.13 ^a	0.32 \pm 0.08 ^a
Acetoin	Buttery	0.45 \pm 0.04 ^a	0.28 \pm 0.04 ^b	0.33 \pm 0.05 ^b
Butanol	Medicinal, alcohol	4.33 \pm 0.40 ^a	1.95 \pm 0.72 ^b	2.84 \pm 0.66 ^b
Hexanol	Grassy	2.97 \pm 0.38 ^a	2.86 \pm 0.42 ^a	2.68 \pm 0.37 ^a
Isoamyl alcohol	Alcohol	284.55 \pm 35.59 ^a	251.03 \pm 23.81 ^{ab}	207.18 \pm 22.21 ^b
Isobutanol	Fusel, alcohol	34.12 \pm 3.74 ^{ab}	51.63 \pm 12.99 ^a	30.69 \pm 2.26 ^b
Pentanol	Ripe banana	0.30 \pm 0.01 ^a	0.19 \pm 0.13 ^a	0.29 \pm 0.02 ^a
n-Propanol	Alcohol, ripe fruit	90.18 \pm 10.81 ^a	33.52 \pm 26.88 ^b	77.41 \pm 5.52 ^a
Acids				
Acetic acid	Vinegar	295.95 \pm 11.72 ^a	364.09 \pm 87.52 ^a	270.46 \pm 22.03 ^a
Butyric acid	Rancid, cheesy, sweaty	0.23 \pm 0.09 ^a	0.12 \pm 0.06 ^a	0.17 \pm 0.06 ^a
Hexanoic acid	Cheesy, sweaty	7.47 \pm 7.19 ^a	5.47 \pm 5.75 ^a	11.62 \pm 4.75 ^a
Isobutyric acid	Rancid, cheesy, buttery	1.77 \pm 0.16 ^a	1.72 \pm 0.47 ^a	1.10 \pm 0.26 ^a

Isovaleric acid	Cheesy, mouldy	1.88 ± 0.17 ^a	1.55 ± 0.30 ^a	1.37 ± 0.16 ^a
Octanoic acid	Rancid, cheesy, sweaty	1.37 ± 0.11 ^a	1.12 ± 0.14 ^a	1.20 ± 0.13 ^a
Propionic acid	Rancid, pungent	2.37 ± 0.8 ^a	1.12 ± 0.42 ^b	1.84 ± 0.28 ^{ab}
Valeric acid	Roast barley	0.93 ± 0.05 ^a	0.68 ± 0.03 ^b	0.83 ± 0.04 ^a

*Mean totals with the same letter in the same row are not significantly different as calculated by Fisher's least significant difference (LSD) at $P \leq 0.05$

** Dzedze *et al.*, 2019; Liu *et al.*, 2015

Table 4. Major wine volatile aroma compounds analysed in small-scale Shiraz wines produced in 2017 at the Nietvoorbij research cellar, following fermentation by two commercial red wine yeasts *i.e.* WE372 (Anchor Yeast, South Africa) and MERIT (Chr. Hansen, Denmark); and NI6 (yeast previously isolated from Paarl Shiraz grapes), respectively.

Aroma compounds (mg/L)	Aroma descriptor**	WE372	MERIT	NI6
		Mean ± Std Dev*	Mean ± Std Dev*	Mean ± Std Dev*
Esters				
2-Phenylethyl acetate	Rose, honey, fruity	0.49 ± 0.02 ^a	0.56 ± 0.04 ^a	0.51 ± 0.20 ^a
Diethyl succinate	Fruity	7.46 ± 0.09 ^a	6.91 ± 0.33 ^a	5.71 ± 3.51 ^a
Ethyl acetate	Cherry, floral	47.64 ± 2.64 ^a	49.32 ± 2.33 ^a	48.60 ± 12.78 ^a
Ethyl butyrate	Fruity	0.22 ± 0.01 ^a	0.21 ± 0.03 ^a	0.23 ± 0.08 ^a
Ethyl caprate	Fruity	0.08 ± 0.00 ^a	0.08 ± 0.01 ^a	0.07 ± 0.03 ^a
Ethyl caprylate	Fruity, floral	0.13 ± 0.01 ^a	0.15 ± 0.01 ^a	0.15 ± 0.07 ^a
Ethyl hexanoate	Fruity	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.03 ± 0.04 ^a
Ethyl-3-hydroxybutanoate	Sweet-associated, berry	0.55 ± 0.02 ^{ab}	0.60 ± 0.03 ^a	0.45 ± 0.08 ^b
Ethyl lactate	Strawberry, buttery	33.55 ± 1.41 ^a	27.19 ± 1.84 ^a	24.82 ± 7.27 ^a
Ethyl phenylacetate	Sweet-associated	0.72 ± 0.01 ^b	0.78 ± 0.01 ^a	0.75 ± 0.02 ^{ab}
Hexyl acetate	Cherry, floral	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
Isoamyl acetate	Banana, tropical fruit	2.42 ± 0.31 ^a	2.40 ± 0.21 ^a	2.18 ± 0.87 ^a
Isobutyl acetate	Banana, tropical fruit	0.07 ± 0.02 ^a	0.11 ± 0.03 ^a	0.09 ± 0.02 ^a
Alcohols				
2-Phenyl ethanol	Roses, honey-like	46.86 ± 3.61 ^a	30.64 ± 2.31 ^b	18.93 ± 1.08 ^c
3-Ethoxy-1-propanol	Fruity	8.01 ± 0.15 ^a	1.60 ± 0.02 ^c	6.34 ± 0.95 ^b
3-Methyl-1-pentanol	Fruity	6.74 ± 0.28 ^a	5.46 ± 0.37 ^a	4.99 ± 1.46 ^a
4-Methyl-1-pentanol	Tropical fruit	0.31 ± 0.07 ^a	0.23 ± 0.01 ^a	0.26 ± 0.03 ^a
Acetoin	Buttery	0.45 ± 0.03 ^a	0.25 ± 0.01 ^a	0.38 ± 0.17 ^a
Butanol	Medicinal, alcohol	3.44 ± 0.07 ^a	1.22 ± 0.10 ^b	2.55 ± 0.98 ^{ab}
Hexanol	Grassy	2.52 ± 0.23 ^a	2.54 ± 0.16 ^a	3.26 ± 1.13 ^a
Isoamyl alcohol	Alcohol	255.29 ± 18.80 ^a	247.36 ± 15.93 ^a	249.04 ± 96.50 ^a
Isobutanol	Fusel, alcohol	37.78 ± 4.42 ^b	65.97 ± 1.58 ^a	41.63 ± 9.16 ^b
Pentanol	Ripe banana	0.31 ± 0.01 ^{ab}	0.29 ± 0.02 ^a	0.33 ± 0.02 ^a
n-Propanol	Alcohol, ripe fruit	229.17 ± 10.36 ^a	82.26 ± 1.31 ^b	196.07 ± 25.85 ^a
Acids				
Acetic acid	Vinegar	357.48 ± 6.88 ^a	412.66 ± 8.79 ^a	285.22 ± 40.60 ^b
Butyric acid	Rancid, cheesy, sweaty	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
Hexanoic acid	Cheesy, sweaty	0.00 ± 0.00 ^a	0.69 ± 0.98 ^a	3.53 ± 2.63 ^a
Isobutyric acid	Rancid, cheesy, buttery	1.33 ± 0.13 ^a	1.91 ± 0.05 ^a	1.26 ± 0.50 ^a

Isovaleric acid	Cheesy, mouldy	1.07 ± 0.06^a	1.18 ± 0.05^a	1.17 ± 0.37^a
Octanoic acid	Rancid, cheesy, sweaty	1.19 ± 0.03^a	1.36 ± 0.08^a	1.31 ± 0.48^a
Propionic acid	Rancid, pungent	6.16 ± 0.54^a	1.96 ± 0.09^b	3.62 ± 1.12^b
Valeric acid	Roast barley	0.73 ± 0.04^a	0.51 ± 0.03^b	0.58 ± 0.15^{ab}

*Mean totals with the same letter in the same row are not significantly different as calculated by Fisher's least significant difference (LSD) at $P \leq 0.05$

** [Dzedze et al., 2019](#); [Liu et al., 2015](#)

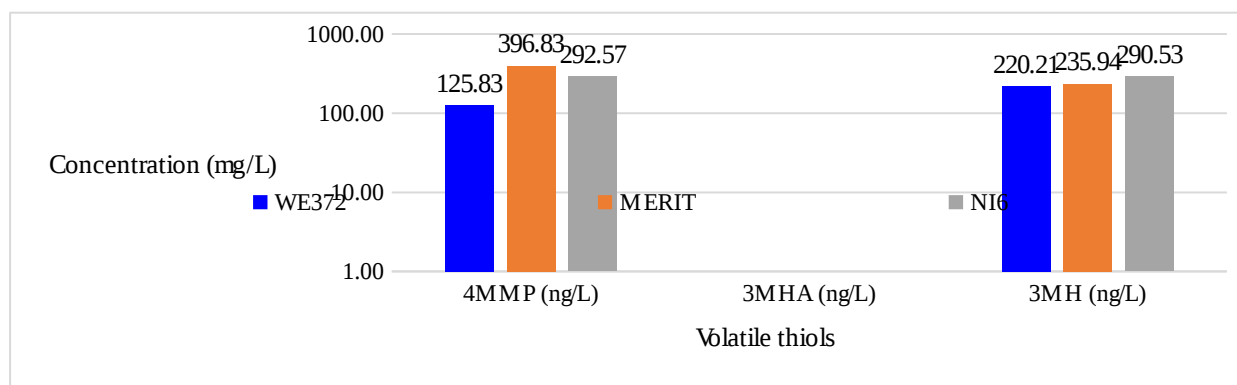


Figure 6. Concentrations of volatile thiols (4-mercapto-4-methylpentan-2-one, 4MMP; 3-mercaptohexan-1-ol, 3MH; and 3-mercaptohexyl acetate, 3MHA) measured using GC-FID for 2016 Shiraz wines produced with two commercial red wine yeasts *i.e.* WE372 (Anchor Yeast, South Africa) (blue bars) and MERIT (Chr. Hansen, Denmark) (orange bars); and NI6 (yeast previously isolated from Paarl Shiraz grapes) (grey bars), respectively.

*Mean totals with the same letter are not significantly different as calculated by Fisher's least significant difference (LSD) at $P \leq 0.05$

** [Dzedze et al., 2019](#); [Liu et al., 2015](#)

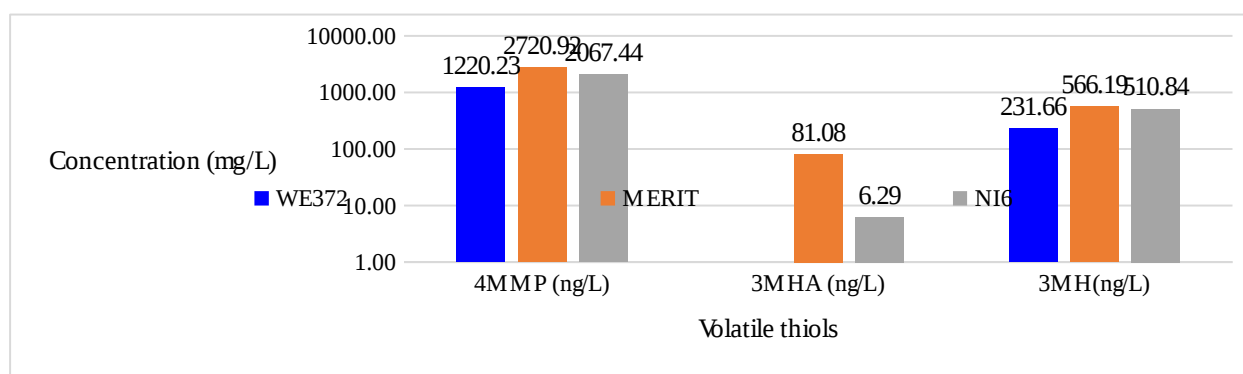


Figure 7. Concentrations of volatile thiols (4-mercapto-4-methylpentan-2-one, 4MMP; 3-mercaptohexan-1-ol, 3MH; and 3-mercaptohexyl acetate, 3MHA) measured using GC-FID for 2017 Shiraz wines produced with two commercial red wine yeasts *i.e.* WE372 (Anchor Yeast, South Africa)

(blue bars) and MERIT (Chr. Hansen, Denmark) (orange bars); and NI6 (yeast previously isolated from Paarl Shiraz grapes) (grey bars), respectively.

*Mean totals with the same letter are not significantly different as calculated by Fisher's least significant difference (LSD) at $P \leq 0.05$

** *Dzedze et al., 2019*; *Liu et al., 2015*

4. Discussion

As previously mentioned, the yeast starter culture is instrumental to enhance wine varietal aroma and flavour by modulating non-volatile bound compounds present in grape juice (*González-Barreiro et al., 2015*). For that reason, wines can be considered the metabolic footprint of the wine yeast used to conduct the alcoholic fermentation. It is, therefore, important for oenologists to inoculate with a starter culture with the desired characteristics that can complete the fermentation, hence CHEF DNA karyotyping was deployed to identify the yeast inoculum as the fermentation progressed. As CHEF DNA karyotypes of yeast isolates at the end fermentation matched the respective ADWY inoculums, it can be safely stated that all ADWY inoculums propagated sufficiently to be present at the end of fermentation. As a result, variation in the chemical, sensory and metabolite levels of the final wine can be attributed to the inoculated yeast strain, as it was the only variable in this study.

Differences observed in the duration of fermentation between 2016 and 2017 vintages highlight the effect of vintage on the winemaking process, hence, trials were conducted over more than one vintage. Nonetheless, no noticeable differences in fermentation rate were observed amongst different yeast strains across vintages. Higher VA elicits unpleasant vinegar-like aromas in wines, hence it is a major problem in the wine industry (*Vilela et al., 2013*). As MERIT and NI6 produced Shiraz wines with a negative association with VA, these strains can be considered an asset in this regard. All yeast strains produced wines with acceptable pH values for locally produced red wines (*Britz and Tracey, 1990*), as average pH values for the 2016 and 2017 vintages were 3.83 ± 0.03 and 3.76 ± 0.08 , respectively. Furthermore, wines within this pH

range were previously reported to have a higher perceived mouthfeel and velvety texture (Demiglio *et al.*, 2008). Overall, NI6-produced wines on average had marginally higher pH values than wines produced with references during both vintages.

Baker and Ross (2014) reported that higher levels of alcohol positively contributed to the perceived sensory profile of red wines, as these wines tend to have a 'longer finish' or 'aftertaste'. This is an important attribute of wine quality and is defined as the lingering taste and aroma after swallowing. Therefore, indications are that the yeast NI6 contributed towards varietal typicity of Shiraz wines, as all wines produced with the yeast, irrespective of the vintage had higher alcohol levels compared to wines produced with WE372 and MERIT, respectively. Wines produced with NI6 also had a more favourable association with 'finish' and 'body', which complements a previous study that showed a positive correlation between increasing alcohol levels in red wines and higher aroma intensity and mouthfeel (King and Heymann, 2014). The MERIT-produced 2016 wines did, however, had higher alcohol levels than the WE372-produced wines. This was complemented by the descriptive sensory evaluation, as the former had a better association with 'body'. On the other hand, MERIT-produced 2017 wines, had marginally less alcohol than that produced with NI6 and WE372, respectively. This observation was again reflected in the descriptive sensory evaluation, as the former had a weaker association with 'body'. This observation further supports the study of King and Heymann (2014) that highlighted the positive correlation between increasing alcohol and better mouthfeel.

In terms of malic acid degradation, indications are that NI6 had a stimulative effect on malolactic fermentation (MLF), as wines produced with NI6 during both vintages had lower malic acid levels. This observation links to marginally higher pH values measured in these wines, as $\text{pH} > 3.5$ was previously reported to be favourable for malic acid degradation (Bauer and Dicks, 2004). Red wines that successfully underwent MLF were reported to have superior aroma and flavour (Du Plessis *et al.*, 2017). Therefore, NI6 has characteristics to enhance varietal aroma

and flavour. Overall, NI6 consistently produced Shiraz wines with chemical parameters better than that produced by reference strains during both vintages.

Descriptive sensory evaluation showed that both WE372 and MERIT produced 2016 wines with a positive association with typical Shiraz varietal aromas viz. 'berry', 'jammy', and 'smokey', respectively. These wines also had a positive association with 'spicy and peppery' aroma, which can be attributed to the aroma compound rotundone that is abundant in Shiraz grapes and wines (Herderich *et al.*, 2012; Wood *et al.*, 2008). This observation complements the recommendations by the respective active dried yeast manufacturers. Strain NI6 also showed its potential for the production of aromatic Shiraz, as 2016 wines had a positive association with the aforementioned sensory aromas, as well as overall quality.

Conversely, MERIT-produced 2017 wines, had a negative association with the aforementioned varietal aromas, whilst having a positive association with vegetative (herbaceous) aromas, which include Shiraz varietal aromas like olive and herbs (Albanese *et al.*, 2013; Moran *et al.*, 2021). Even though vintage differences were observed for this strain, wines from both vintages had Shiraz typical varietal aromas. Moreover, the wines were not negatively perceived by judges. Shiraz wines produced with NI6 during 2017 had a favourable association with 'spicy and peppery' aromas. As mentioned above, rotundone, the compound responsible for the 'peppery' and 'spicy' aroma in Shiraz wines (Caputi *et al.*, 2011), may have been present at higher levels during the 2017 vintage. This observation highlights the effect of vintage on the grape matrix, and consequently varietal aroma compounds and/or precursor levels (Roullier-Gall *et al.*, 2014). Nonetheless, the NI6-produced 2017 still showed a positive association with 'berry' aromas, as was observed during the 2016 vintage. Wines produced with WE372 during the 2017 vintage again had a positive association with 'berry' and 'jammy' aromas, as well as 'finish' and overall quality. The strain, therefore, consistently shows why it comes highly recommended for the

production of varietal Shiraz wines. Overall, NI6 consistently produced Shiraz wines equal in sensory quality to that produced by commercially available strains recommended for varietal red winemaking during both vintages. It can be tentatively said that NI6 being isolated from Shiraz grapes is advantageous in accentuating the varietal characteristics.

Analyses of total fatty acids (contributes to wine 'fruity' aromas and complexity in moderate concentrations)(Liu *et al.*, 2019) of 2016 Shiraz wines showed that both WE372 and MERIT produced wines with profoundly more undesirable acetic acid (the main contributor to volatile acidity) than strain NI6. This observation was again made during the 2017 vintage, which addresses one key problem statement, as higher VA negatively affects wine organoleptic quality. Overall, all wines were perceived to have varietal aromas and flavours (Fig. 4 & 5). As the concentration of the remaining fatty acids *viz.* butyric acid, hexanoic acid, isobutyric acid, isovaleric acid, octanoic acid, propionic acid and valeric acid was not excessive, it can be envisaged they contributed positively to the sensory quality of the respective wines. Overall, the yeast NI6 produced noticeably less of the unwanted acetic acid than both references during both vintages, which is a positive attribute towards enhancing varietal aromas that are known to be masked by excessively high VA. This observation was complemented by FTIR analyses for both vintages, as NI6 on average produced wines with the lower VA (Tables 1 and 2).

Reference strain, namely WE372 produced 2016 Shiraz wines with the highest concentration of total esters, which imparts red- and blackberry aromas (Lytra *et al.*, 2013). Nonetheless, all strains produced these aroma compounds above their sensory detection threshold (Francis and Newton, 2005; Lapalus, 2016; Niu *et al.*, 2019), hence descriptive sensory evaluations showed that all strains produced wines that positively associated with Shiraz varietal aromas *i.e.* 'jammy' and 'berry' (Fig. 4). Interestingly, the concentration of diethyl succinate, a metabolite that imparts 'fruity' and 'caramel' aromas (Manolache *et al.*, 2018; Pineau *et al.*, 2009) in MERIT-produced 2016 wine were similar compared to wines produced with WE372 and NI6,

respectively. This observation was complemented by descriptive sensory evaluation as the wines also had a comparable association with 'berry' and 'jammy' both 'sweet associated' aromas (Fig. 4).

Overall, WE372 again produced 2017 wines with higher total ester concentration, especially those associated with varietal aromas *viz.* 'rose', 'honey', 'fruity', 'cherry' and strawberry' etc. aromas. This observation, therefore, complements recommendations by the yeast manufacturer for the yeast to be used to enhance and/or modulated wine 'berry' aromas, as the wine was associated with 'berry' aroma (Fig. 5). As mentioned above, MERIT-produced 2017 wines had a negative association with 'berry' aromas (Fig. 5), despite the detection of *i.a.* ethyl acetate (imparts a 'fruity' aroma), 2-phenylethyl acetate (imparts 'rose', 'honey' and 'fruity' aromas), and ethyl-3- hydroxybutanoate (imparts 'berry' aromas)(Pineau *et al.*, 2009) at concentrations above the respective sensory detection thresholds (Francis and Newton, 2005). It can be speculated that the perceived vegetative and herbaceous aromas, somehow masked the 'berry' aromas.

With reference to higher alcohols, strain WE372 produced wines during both vintages with more 2-phenyl ethanol which imparts 'honey-like' and 'rose' aromas (Musarurwa *et al.*, 2016), than wines produced with MERIT and NI6, respectively. Descriptive sensory evaluation mentioned above complemented this observation, as WE372-produced wines had a stronger association with 'jammy' aromas (Fig. 5), which has a sweet connotation. On the other hand, MERIT-produced wines during both vintages had more isobutanol than wines produced with NI6 and WE372, respectively. It is noteworthy that the differences were significantly more during the 2017 vintage. As isobutanol can elicit spirit or solvent notes (De-La-Fuente-Blanco *et al.*, 2016), it can be speculated that it masked the 'berry' aromas, thus the MERIT produced 2017 wine was perceived to be more vegetative. The remainder of the higher alcohols was produced at lower concentrations by all strains, and it can be envisioned that there their associated notes mentioned

above *i.a.* 'i.a. 'tropical fruit, 'buttery' 'grassy', 'alcohol' and 'banana' (Dzedze *et al.*, 2019; Liu *et al.*, 2015) had no discernible negative effect on sensory quality wines, as all wines were perceived to have varietal aroma and flavour.

With reference to volatile thiols, MERIT released the highest concentration of the volatile thiol *i.e.* 4MMP (imparts 'passion fruit' 'berry' [blackcurrant] aromas in both 2016 and 2017 wines (Fig. 6 & 7). However, the descriptive sensory evaluation showed that wines produced with WE372 and NI6 had a stronger association with the 'berry' aroma than wines produced with MERIT during both vintages (Fig. 4 & 5). Indications, therefore are that other volatile thiols, as well as previously discussed ester compounds, also enhances 'berry' aromas.

It was previously reported that some wine yeast strains, referred to as '3MH converters' enzymatically convert 3MH to 3MHA (Swiegers *et al.*, 2007). It can be postulated that none of the yeasts included in this study expressed proteins (enzyme) responsible for acetylation of 3MH to produce 3MHA (Santiago and Gardner, 2015) during the 2016 vintage, as the volatile thiol 3MHA, which imparts 'red fruit' aromas (Rigou *et al.*, 2014), was not detected in any of the 2016 wines (Fig. 6). Strains MERIT and NI6 did however produced 2017 wines with 3MHA levels above the compound's sensory detection threshold. This observation can be attributed to vintage differences, as the concentration of bound aroma-inactive precursor on this aroma compound might have been too low. This observation will be further investigated as part of another study. Swiegers *et al.*, (2005) also found that different yeast strains varied in their abilities to convert 3MH to 3MHA.

Overall, 3MH (imparts blackcurrant ['berry'] aromas in red wines)(Rigou *et al.*, 2014) was detected in all 2016 wines, but NI6 wine had a marginally higher concentration than wines produced with both the red wine reference strains. Both MERIT and NI6 produced 2017 wines with significantly higher concentrations of 3MH than WE372. However, the descriptive sensory evaluation data showed that wines produced with WE372 had the best association with 'berry'

aromas of all yeast strains included in this study, whilst wines produced with NI6 had a better association with 'berry' aromas compared to wines produced with MERIT, which surprisingly had a negative association with 'berry' aroma (Fig. 5). Overall, all yeast strains produced Shiraz wines with 4MMP, 3MH, and 3MHA at concentrations exceeding their respective olfactory perception thresholds (Musumeci *et al.*, 2015). Thus indications are that all volatile thiols analysed in this study, *i.e.* 4MMP, 3MH, and 3MHA, that was traditionally associated with white cultivars, especially Sauvignon blanc contributed to Shiraz wine varietal aromas.

3 Conclusion

In conclusion, the indigenous yeast strain NI6 produced small-scale Shiraz wines during the 2016 and 2017 vintages, with equal quality to that produced with both red wine reference strains *i.e.* WE372 and MERIT, respectively. All wines produced with NI6 had lower VA and the lowest acetic acid, irrespective of vintage. This is a quality characteristic of NI6 as it proves that the strain is a low VA producer. Descriptive sensory evaluation showed another quality characteristic of NI6, as it produced wines with typical varietal aromas, especially 'smoky' and 'spicy' aromas, during both vintages. In terms of aroma compounds, *i.e.* esters (associated with fruity aromas), both red wine reference strains produced Shiraz wines with higher ester concentrations than NI6. Merit also produced wines with higher volatile thiols than both WE372 and NI6, except during 2016 when NI6 produced wines with more 3MH than both red wine reference strains. Nonetheless, NI6 consistently produced less of the undesirable compounds that are associated with wine off-odours. Overall, this study showed that wines with a positive correlation with black and/or fruits aromas and flavours also had volatile thiol levels above its sensory detection thresholds, which indicates that ester compounds are not solely responsible for Shiraz wine fruity aromas and flavours as was traditionally reported.

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