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Wine chemical, sensory, aroma compound and protein analysis of wines produced from chemical and biological fungicide treated Chenin blanc grapes

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ABSTRACT

Fungal diseases in vineyards are one of the main causes leading to economic losses within the viticulture sector and are continuously increasing with time. The most common of these fungal diseases are powdery mildew, downy mildew and grey mould which are controlled by the use of fungicides. However, fungicide residues can alter the fermentation process and prevent some biochemical pathways of yeast metabolism involved in phenolic and/or aroma compound production that are critical for sensory quality. In this study, Chenin blanc grapes treated with chemical and natural fungicides were used to produce small-scale wines and laboratory-scale fermentations. Treatments consisted of a control (an approved chemical used in industry), a 1x and 2x treatment (new formulations). Laboratory-scale fermentations were conducted in duplicate using the commercial *Saccharomyces cerevisiae (S. cerevisiae)* Active Dry Wine Yeast (ADWY) strains VIN 13 and VIN 7. The fermentations were monitored by weighing frequently until they stabilised (CO_2 weight loss). Small-scale wines were made according to the standard Nietvoorbij experimental wine making procedure. At the end of fermentation, lees samples were subjected to CHEF gel electrophoreses to confirm that the inoculated yeast strain completed the fermentations. Moreover, fermenting wine samples, collected at the start and end of the fermentation, were subjected to protein extraction, quantification and characterisation the yeast proteins expressed at the various stages of fermentation. Resultant wines were subject to chemical and sensory evaluations five months after bottling. From the results obtained in the above study, it was concluded that the inoculated yeast strains used for wine making completed the fermentations at a similar rate to their respective controls (registered fungicide). In addition, small-scale cellar fermentations showed that the fungicide treatments (1x the recommended dosage [Treatment 1] and 2x the recommended dosage [Treatment 2]) compared to the controls had no notable negative effects on wine aroma and sensory profiles although differences were observed in the proteins expressed after the fermentation. The chemical and biological fungicides had no notable effects on the wine making procedure and quality of resulting wines compared to the registered fungicide.

1. Introduction

The wine making process involves the conversion of grape juice into wine during which various biochemical reactions occur (Hart, Ndimba, & Jolly, 2017a & b; Ugliano & Henschke, 2009, pp. 327–343). These biochemical reactions start during the ripening phase of the grapes, proceeds during harvesting, fermentation and lastly in the bottle (Gao, Zietsman, Vivier, & Moore, 2019; Goold et al., 2017; Mojsov, Andronikov, Janevski, Jordeva, & Zhezhova, 2015; Romano, Fiore, Paraggio, Caruso, & Capece, 2003). Wine quality strongly depends on the quality of grapes used, i.e., the greater the quality of the grapes, the higher the quality of the wine produced (Caboni & Cabras, 2010, pp. 43–59). During the wine making process, metabolites from both the grapes and yeast are released and contributes to overall wine flavour and aroma which are critical factors influencing the overall quality of the wine (Li, Tao, Wang, & Zhang, 2008; Perestrelo, Fernandes, Albuquerque, Marques, & Camara, 2006; Villamor & Ross, 2013). Moreover, these aroma and flavour compounds are released from the grapes by yeast-derived enzymes during the fermentation process (Hart et al., 2017a & b; Li et al., 2008; Perestrelo et al., 2006; Ugliano &

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Henschke, 2009, pp. 327–343). Various factors such as the presence of fungicides residue may negatively influence the biochemical pathways responsible for flavour and aroma compound production, including the presence of fungicide residues (Barba, Oliva, & Payá, 2010, pp. 421–440). However, their effects on the wine quality differ between yeast strains, due to the yeast starter culture's fermentation potential, which is based on the grape must composition and fermentation conditions (Escudero, Campo, Fariña, Cacho, & Ferreira, 2007; Moio et al., 2004; Ugliano & Henschke, 2009, pp. 327–343).

Nonetheless, fungal diseases in vineyards are one of the main factors leading to economic losses in the viticulture sector, therefore, outh it is important that these diseases are treated and controlled (Nigro et al., 2006; Romanazzi, Lichter, Gabler, & Smilanick, 2012). Various fungicides are commercially available and applied under Good Agricultural Practices (GAP). Moreover, in South Africa these fungicides have to be administered as stipulated in Department of Agriculture, Forestry and Fisheries (DAFF) Act 36 of 1947 to meet safety regulations. For this reason, thorough testing is required before registration (DAFF Act No.36 of, 1947; Nigro et al., 2006; Romanazzi et al., 2012). Although fungicides are applied under GAP, residues may be present in the grape must, which can lead to stuck and sluggish alcoholic fermentations (Barba et al., 2010, pp. 421–440; González-Rodríguez, Noguerol-Pato, González-Barreiro, Cancho-Grande, & Simal-Gándara, 2011).

Moreover, fungicide residues present on grapes berries and/or in grape must (juice) could affect wine sensory quality and stability, due to the fact that they can inhibit yeast metabolic pathways involved in phenolic and/or aroma compound production that are critical for sensory quality (Noguerol-Pato, Sieiro-Sampedro, González-Barreiro, Cancho-Grande, & Simal-Gándara, 2014; 2015). Additionally, yeast-derived (released) enzymes was previously reported to play a significant role in wine aroma and flavour. Therefore, the fungicide residues might also have an effect on the proteins expressed, as wine yeast might be exposed to it within the grape must. However, studies focusing on the effect of fungicides on fermenting wine yeast proteome and metabolome, and final wine organoleptic properties have not yet been conducted. The aim of this study was to compare the effect of new fungicide treatments on the fermentation rate, wine yeast protein expression and aroma compounds (metabolites) released, as well as final wine sensory profile with that of a registered fungicide.

2. Material and methods

2.1. Field trials

Chenin blanc grapes were treated (sprayed) with chemical and biological fungicides originating from different Agrochemical companies (hereafter coded experiments A, B and C) at 1x treatment the recommended dosage (Treatment 1 hereafter T1) and 2x the recommended dosage (Treatment 2 hereafter T2) according to Good Agricultural Practises (GAP). Grapes from experiments (sites) A, B and C treated with fungicides containing methyl-1H-pyrazole carboxylic acid phenylethyl, boscalid and *Gelania africana* and penconazole as active ingredients originated from the Durbanville, Agter-Paarl region and Stellenbosch wine regions, respectively.

Agrochemical companies applied the fungicides 14–28 days before harvesting in compliance with protocol. Control grapes, treated with a registered chemical fungicide was included in trial to serve as reference to compare the new fungicide treated grapes. Grapes were harvested and delivered to the ARC Nietvoorbij research cellar, where after laboratory-scale fermentations and small-scale wines were produced (Fig. 1).

2.2. Small-scale wine making

The white wine making process was conducted according to Hart et al. (2017a) following the ARC Infruitec-Nietvoorbij harvest programme 2014 (ARC Infruitec-Nietvoorbij experimental wine evaluation committee). Total sugar (g/L), total acidity (g/L), pH, ethanol (%), malic acid (g/L) and volatile acidity (g/L) of base grape must, fermenting grape must sampled under CO_2, as well as resultant wines were determined using the OenoFoss™ (Foss, Hillerød, Denmark). In addition, an alcolyzer (Anton Paar) was used to verify the alcohol values obtained with the OenoFoss™. All fermentations were conducted in triplicate in a completely randomised block design (Addelman, 1970).

2.3. Laboratory-scale fermentations

Laboratory-scale fermentations were conducted in triplicate in a completely randomised block design (Addelman, 1970) using 500 mL grape must (juice) aliquots for each yeast strain, transferred into 750 mL glass bottles, whereafter the respective yeast strains (i.e., VIN 13 and VIN 7) were inoculated at a concentration 2% (v/v). Thereafter bottles were closed using plastic fermentation caps filled with sterile distilled water, sealed tightly with parafilm to ensure anaerobic conditions, and placed in a temperature-controlled room at 15 °C. Fermentations were monitored by CO_2 weight loss thrice a week, and allowed to proceed until the fermentation stabilised (< 5 g/L). Fermentation curves were constructed to monitor yeast performance. All fermentations were conducted in triplicate in a completely randomised block design (Addelman, 1970).

2.4. Proteomic analysis

Samples used for proteomic analyses were 50 mL Chenin blanc grape must under fermentation. Sampling was performed aseptically at two day-intervals with the use of food-grade CO_2 gas, subsequently, samples were stored at −80 °C until required for proteomic analyses. Proteins were tripsinised and resultant peptides were separated by nanoscale liquid chromatography (nano-LC) using a Thermo Scientific EASY-nLC II at the accredited National Agricultural Proteomics Research & Services Unit method (obtainable from the National Agricultural Proteomics Research & Services Unit (NAPRSU), University of the Western Cape, South Africa). Thereafter, matrix-assisted laser-desorption/ionisation time-of-flight/time-of-flight mass spectrometer in conjunction with LIFT (MALDI LIFT-TOF/TOF MS) was performed using an UltrafleXtreme MALDI ToF/ToF system (Bruker Daltonics, Bremen, Germany). Resultant mass spectra was subjected to database (SwissProt) interrogation using the Mascot algorithm on a ProteinScape 3.0 workstation.

2.5. Metabolites analysis

Metabolite analyses was done in 27 bottled Chenin blanc wines. These wines were produced from grapes treated with the respective registered fungicides, as well as the two treatments (1x and 2x dosages) of the experimental fungicide originating from the three trial sites (3 × 3 x 3) were analysed for aroma compounds (i.e., esters, total acids and higher alcohols [fusel oils] by deploying gas chromatography (GC) in conjunction with calibration mixtures of the applicable aroma compounds at the accredited Gas chromatography–mass spectrometry (GC-MS) division, Mass spectroscopy unit, Central Analytical Facility (CAF), University of Stellenbosch (US). The GC-MS/MS system used in this study comprised of a GC Trace 1300/TSQ8000 mass selective detector equipped with an AI 1310 auto sampler (Thermo Scientific™ Inc, USA). Aroma compounds were separated using a 30 m × 0.25 mm x 0.25 μm Zebron WAX plus column (Phenomenex Inc., Torrance, CA, USA).

2.6. Sensory evaluation

Small-scale Chenin blanc wines were evaluated by a panel of 12 trained wine judges using a descriptive sensory evaluation method. The

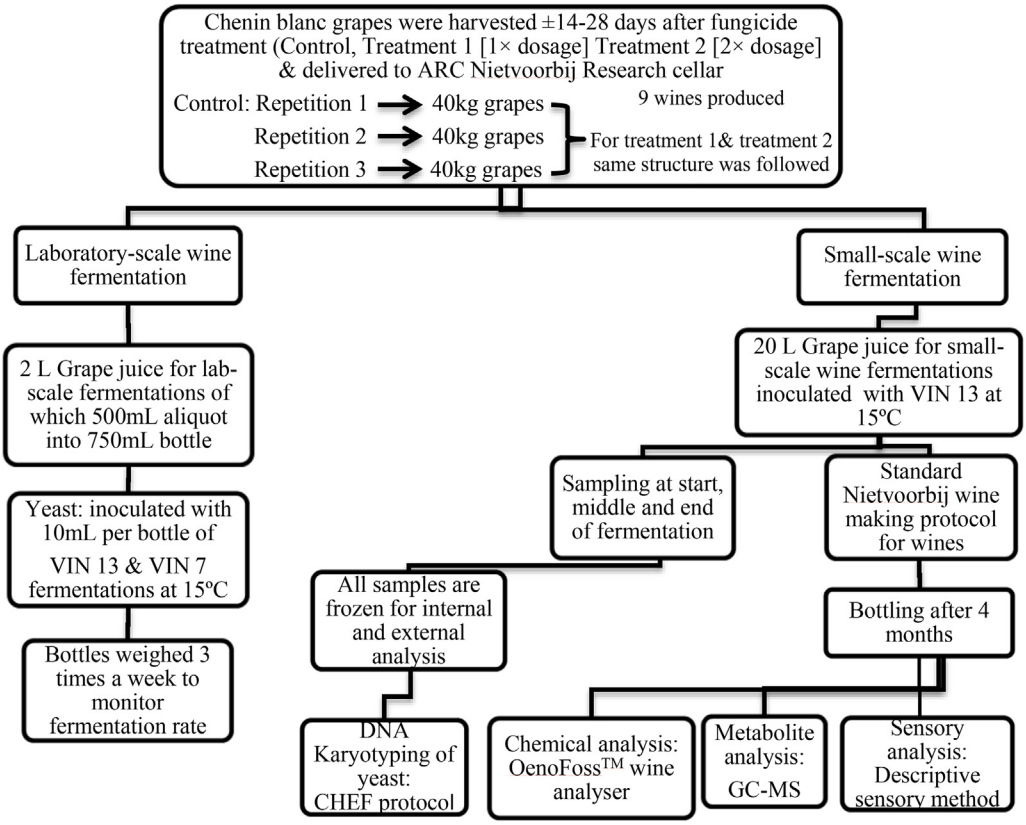

Fig. 1. Summary of experimental design of the study.

panel was provided with tasting sheets that allowed them to evaluate the wine according to an unstructured line scale. The judges were asked to rate 'colour' (unacceptable to excellent), aroma and flavour, namely 'tree fruit', 'tropical fruit ' and 'wine foreign' (usually not associated with, or desirable in Chenin blanc wines) (undetectable to prominent), 'body' (watery/thin to full-bodies) 'acidity' (low, balanced and excessively) and 'overall quality' (unacceptable to excellent). All wines were served in a randomised order, using international wine-tasting glasses.

2.7. Statistical analyses

Chemical, sensory and metabolomic data was subjected to analysis of variance (ANOVA) and multiple factor analysis (MFA) to study the linear relationship between the chemical, sensory and metabolomic variables, and to standardise the data prior to performing the MFA using XLSTAT software (Addinsoft, 2016).

3. Results and discussion

3.1. Chemical and sensory analyses of small-scale wine making

All 27 bottled Chenin blanc wines, produced from grapes treated with the respective registered fungicides, as well as the two treatments (1x and 2x dosages) of the experimental fungicide originating from the three trial sites fermented to dryness, as the residual glucose/fructose values were < 5 g/L, whilst ethanol (alcohol) concentration of all samples ranged between 11 and 14% v/v, which comply with white wine legal limits (≤15% v/v) reported by Anon, 1989 (Table 1). In addition, volatile acidity (VA) values for experiments A, B and C ranged from 0.04 to 0.07 g/L, 0.06–0.11 g/L and 0.017–0.19 g/L, respectively, fell within acceptable legal limits for SA white wines (≤1.2 g/L) (DAFF Act No.60 of, 1989; Du Toit, 2001). Moreover, pH values experiment A, B and C ranged from 3.10 to 3.20, 3.11 to 3.47 and 3.22 to 3.29, respectively. The overall pH values for all experiments ranged from 3.0 to 3.4, which is the typical range for SA white wines, although the T2 for experiment B, had a slightly higher pH. The total acidity (TA) for the various experiments was as follows: experiments A, B and C ranged from 3.96 to 4.38 g/L, 4.73–5.14 g/L and 5.82–6.15 g/L, respectively. A previous study showed that the presence of residues of certain fungicides could affect acidity of the wines (Barba et al., 2010, pp. 421–440). Nonetheless, all wine chemical parameters were within acceptable limits for SA wines. Therefore, indication are that the fungicides did not negatively affect the Chenin blanc wine chemical parameters (Table 1). Moreover, sensory ANOVA results showed no significant difference between the control and treatments (T1 and T2) in sensory attributes from all experiments (Table 2).

3.2. Laboratory-scale fermentations

All laboratory-scale fermentations stabilised after 15 days, as no CO_2 weight loss was observed. Fermentations of must originating from control and experimental fungicide treated (T1 and T2) grapes had a similar rate of fermentation (data not shown).

3.3. Proteomics analyses

3.3.1. Bradford assay and SDS-PAGE

Concentrations of all six pooled samples of the control as well as treatments 1 (1x dosage) and 2 (2x dosage) taken at the start and end of small-scale winemaking (fermentation) were > 30 ng (0.03 μg). Therefore, all the samples had adequate proteins in order to proceed with SDS-PAGE assays (Kang, Gho, Suh, & Kang, 2002). The SDS-PAGE showed no noticeable difference in protein band distribution and intensity between proteins extracted from ferments originating from control Chenin blanc grapes and treated Chenin blanc grapes sampled

Table 1
Chemical profile of Chenin blanc wines produced in small-scale fermentations at 15 °C[a].

Experiment	Treatments	Gluc/Fruc (g L^{-1})	Total acidity (g L^{-1})	pH	Ethanol (%$^{v}/_{v}$)	Malic acid (g L^{-1})	Volatile acidity (g L^{-1})
A	Control (C)	0 ± 0	3.9 ± 0.2	3.2 ± 0.2	14.0 ± 0.2	0 ± 0	0.0 ± 0.0
	1x Treatment (T1)	0 ± 0	4.2 ± 0.6	3.1 ± 0.1	14.0 ± 0.2	0 ± 0	0.1 ± 0.0
	2x Treatment (T2)	0 ± 0	4.4 ± 0.2	3.1 ± 0.0	13.0 ± 0.9	0 ± 0	0.1 ± 0.0
B	C	0 ± 0	5.1 ± 0.6	3.1 ± 0.0	11.0 ± 0.5	0.4 ± 0.2	0.1 ± 0.2
	T1	0 ± 0	5.0 ± 0.1	3.3 ± 0.1	12.0 ± 0.3	0.5 ± 0.1	0.1 ± 0.1
	T2	0 ± 0	4.7 ± 0.7	3.5 ± 0.2	13.0 ± 0.3	0.9 ± 0.9	0.1 ± 0.1
C	C	0 ± 0	6.2 ± 0.5	3.3 ± 0.1	13.0 ± 0.6	2.0 ± 0.1	0.2 ± 0.0
	T1	0 ± 0	6.2 ± 0.4	3.2 ± 0.1	13.0 ± 1.3	2.0 ± 0.2	0.2 ± 0.0
	T2	0 ± 0	5.8 ± 0.2	3.3 ± 0.0	12.0 ± 1.1	2.0 ± 0.4	0.2 ± 0.0

[a] Means ± standard deviation (n = 3).

Table 2
ANOVA sensory analysis of Chenin blanc wines produced in small-scale fermentations.

Experiment	Treatment	Colour	Tree Fruit	Tropical	Wine Foreign	Overall Quality
A	Control (C)	69.9a[a]	54.3a	57.4a	20.7a	52.1a
	1X Treatment (T1)	69.7a	53.8a	59.9a	22.5a	54.7a
	2X Treatment (T2)	71.4a	55.2a	56.7a	21.1a	56.0a
B	C	76.4a	48.7a	51.7a	15.5a	57.3a
	T1	79.3a	53.0a	49.9a	19.9a	58.8a
	T2	76.3a	45.5a	47.0a	16.0a	58.0a
C	C	76.8a	47.6a	52.4a	15.9a	58.59a
	T1	77.8a	51.3a	52.7a	13.9a	62.7a
	T2	74.1a	46.9a	53.3a	15.9a	57.2a

[a] Values within columns followed by the same letter do not differ significantly ($p > 0.05$).

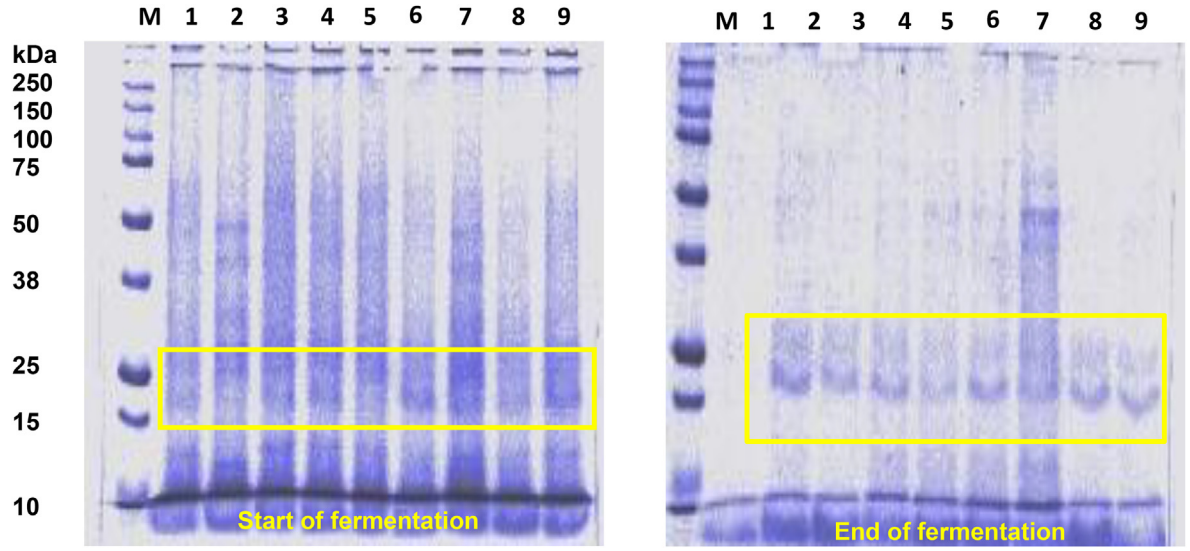

Fig. 2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of protein extracts originating from samples collected at the start and end of fermentation for experiment A: Lane M: Protein ladder (Precision Plus Protein™, Bio-Rad, Madrid, Spain); Lanes1 to 3: Control; Lanes 4 to 6: Treatment 1(1x dosage); Lanes 7 to 9: Treatment 2 (2x dosage).

at the start and end of fermentation, respectively (Fig. 2). However, at the end of fermentation, higher protein intensity was observed within the 50–75 kDa range for T2 in experiment A. Similar results were observed for experiments B and C (data not shown).

3.3.2. Matrix-assisted laser-desorption/ionisation time-of-flight/time-of-flight mass spectrometer in conjunction with LIFT (MALDI LIFT-TOF/TOF MS)

The MALDI LIFT-TOF/TOF MS analyses showed noticeable differences in peptide mass spectra between proteins extracted from ferments originating from control Chenin blanc grapes and treated Chenin blanc grapes sampled at the start of fermentation, respectively (Fig. 3a). This indicates that the matrix of the fermenting grape must differed as a result of the different treatment, hence the differential wine yeast protein expression observed between the control compared to the treatments. The same observation was made with regard to proteins extracted at the end of fermentation (Fig. 3b).

Mass spectra of the control at the start and end of fermentation differed, which indicated that the yeast starter culture reacted to changes within the grape must matrix as the fermentation progressed

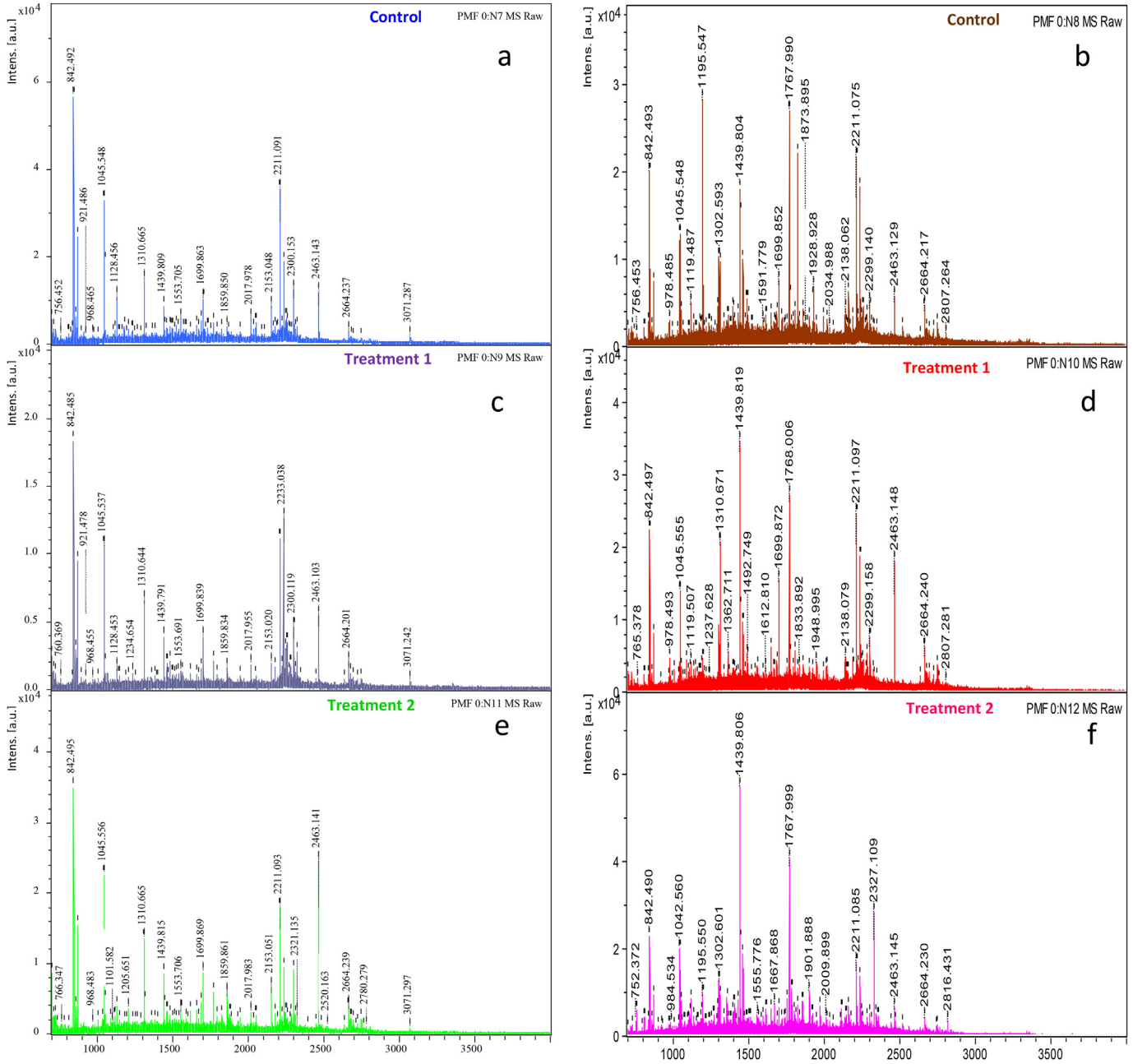

Fig. 3. Experiment A: Mass spectra showing tripsinised wine yeast protein (peptides) peak intensities at the start of fermentation and end of fermentation following inoculation of Chenin blanc grape juice (must) originating from grapes subjected to different treatments, i.e. control (grapes treated with a registered chemical spray) (a & b), 1x the recommended dosage (Treatment 1) (c & d) and 2x the recommended dosage (Treatment 2) (e & f), respectively. * peak intensity are shown on y-axis, and mass to charges (0–3500 Da) are shown on x-axis. * pooled samples of triplicate fermentations. * peak intensity are shown on y-axis, and mass to charges (0–3500 Da) are shown on x-axis.

(Fig. 3a and b). The same observations were made for both treatments (T1 and T2). Similar results for experiments B and C were observed (data not shown). Peptide mass fingerprinting (PMF) in conjunction with MALDI-TOF/MS characterised one protein for each treatment (control, T1 and T2) at the start and end of the fermentation. A general protein database search using UniProtKB (http://www.uniprot.org/uniprot/?query=HOSC&sort=score) identified three VIN 13 expressed proteins in all samples with the following accession numbers YD177, PGK1 and PSP2, respectively (Table 3). The protein PGK, identified as phosphoglycerate kinase, was expressed in the control at the start of fermentation. The enzyme was reported to be involved in glycolysis through which a hexose sugar, e.g. glucose or fructose is metabolised into pyruvate and other metabolites, such as alcohol and glycerol (Ghaemmaghami et al., 2003).

This observation, therefore, complements the other results of this study as the grape juice used for wine making had a total sugar content of 23 g/L which comprised mostly of glucose and fructose, which are metabolised into various metabolites as the fermentation progresses (Conde et al., 2007; Vianna & Ebeler, 2001). The Polymerase suppressor protein 2 (PSP2), on the other hand, was measured/found in the control at the end of fermentation (Table 3). The PSP2 was reported as a suppressor of wine yeast DNA polymerases, which are involved in DNA replication (Formosa & Nittis, 1998; Waldherr et al., 1993). It can, therefore, be speculated that, as the fermentation was in the stationary phase, yeast cell proliferation was inhibited by expressing this enzyme that suppresses DNA replication. The last protein identified was

Table 3
Proteins expressed by the commercial yeast strain, namely VIN 13 during the fermentation of 2017 Chenin blanc grape must that was identified by peptide mass fingerprinting (PMF) in conjunction with MALDI-TOF/MS.

Sample	Spot position	Accession	Protein	Molecular weight (kDa)	Isoelectric point (pI)	Score	Peptides	Sequence coverage (%)
Control–start of fermentation (AC)	N13	PGK_YEAST	Phosphoglycerate kinase OS = *Saccharomyces cerevisiae* (strain ATCC 204508/S288c) GN = PGK1 PE = 1 SV = 2	44.7	7.1	32	2	7
Control–end of fermentation (AC)	N14	PSP2_YEAST	Protein PSP2 OS = *Saccharomyces cerevisiae* (strain ATCC 204508/S288c) GN = PSP2 PE = 1 SV = 2	65.5	8.7	28.4	1	2.2
Treatment 1–start of fermentation (A1)	N9	YD177_YEAST	IMPACT family member YDL177C OS = *Saccharomyces cerevisiae* (strain ATCC 204508/S288c) GN = YDL177C PE = 1 SV = 1	19	9	53.5	1	14.1
Treatment 1–end of fermentation (A1)	-	-	-	-	-	-	-	-
Treatment 2–start of fermentation (A2)	N11	YD177_YEAST	IMPACT family member YDL177C OS = *Saccharomyces cerevisiae* (strain ATCC 204508/S288c) GN = YDL177C PE = 1 SV = 1	19	9.9	35.5	1	14.1
Treatment 2–end of fermentation (A2)	N10	YD177_YEAST	IMPACT family member YDL177C OS = *Saccharomyces cerevisiae* (strain ATCC 204508/S288c) GN = YDL177C PE = 1 SV = 1	19	9.9	27	1	14

IMPACT family member YDL177C in the initial stage and at the end of fermentation. It was reported to be a miscellaneous enzyme that is found in the exponential growth phase where it is known to assist with breaking down grape pulp and skin cells (Ghaemmaghami et al., 2003). Therefore, treatments (experimental fungicide) did not have a negative effect since the fermentation was completed within the standard duration of a typical white wine fermentation (± 10 days).

3.4. Metabolite analysis

Wines were bottled and allowed to mature for four months, where after they were analysed for aroma compounds (metabolites). Metabolites were categorised into esters, higher alcohols and fatty acids, respectively.

The data was statistically analysed using Analysis of Variance (ANOVA) and Multiple Factor Analysis (MFA). The ANOVA results for experiment A showed that the metabolite compounds were not significant difference between the control and treatments ($p > 0.05$) (Table 4). The ester compounds present in the highest concentration were ethyl acetate (24.86, 20.89 and 19.89 g/L in control, T1 and T2, respectively), isoamyl acetate (2.39, 1.51 and 1.84 g/L, respectively), ethyl hexanoate (6.94 g/L control and the rest were less than 1 g/L), ethyl lactates (24.81, 24.68 and 23.93 g/L, respectively) and diethyl succinate (2.21 g/L, T1, while control and T2 were around 1 g/L or less). The remainder were present at concentrations around 1 g/L or less. The odour threshold of ethyl acetate is 17.62 g/L, hence in this study it was found in concentrations above the threshold value. Ethyl acetate primarily contributes to aromas such as “solvent”, “varnish” and “fruity” which were perceived in the wines and by judges during sensory evaluation with comments such as “sulphur (rotten egg)”, “chemical taste and smell” (Escudero et al., 2007; Gomez-Miguez, Cacho, Ferreira, Vicario, & Heredia, 2007; King et al., 2008; Lee & Noble, 2003; Li et al., 2008). Plata, Millan, Mauricio, and Ortega (2003).

Plata et al. (2003) reported that ethyl acetate production is yeast-dependent and involves an enzymatic reaction. Furthermore, the isoamyl acetate threshold is 0.03 g/L, ethyl hexanoate 0.014 g/L, ethyl lactate 0.58 g/L and diethyl succinate 200 g/L in white wine (Gomez-Miguez et al., 2007; Li et al., 2008). Therefore, the wines had aromas such as banana, pear, fruity and apple since the concentrations of the aforementioned compounds were above the threshold values. However, the diethyl succinate was detected in concentrations less than the threshold value of 200 g/L (Escudero et al., 2007; Gomez-Miguez et al., 2007; King et al., 2008; Lee & Noble, 2003; Li et al., 2008). A study by Pretorius and Lambrechts (2000) also reported that acetate esters are responsible for the pleasantly fruity, flowery and ester-like character in wines made from neutral cultivars such as Chenin blanc and Colombar.

Higher alcohol compounds also showed no statistically significant difference ($p > 0.05$) between the control and treated wine in experiment A. However, the following compounds were present in the highest concentration, 1-propanol (58.20, 110.50 and 143.80 g/L for the control, T1 and T2, respectively) which can be associated with “ripe fruit” (Feng et al., 2015), isobutanol (4.55, 11.14 and 16.41 g/L, respectively), isoamyl alcohol (49.09, 133.19 and 93.09 g/L, respectively), 3-methyl-1-pentanol (4.97, 4.89 and 4.81 g/L, respectively), hexanol (1.76, 1.41 and 1.29 g/L, respectively), 3-ethoxyl-1-propanol (7.26, 9.24 and 10.93 g/L, respectively) and 2-phenyl-ethanol (3.16, 8.76 and 4.05 g/L, respectively). The rest were present at concentrations around 1 g/L or less. Additionally, the higher alcohols were still within the normal range, with concentrations less than 400 g/L, the threshold value beyond which higher alcohols cause the wine to have a harsh, solvent-like odour. However, in concentrations below 300 g/L, higher alcohols contribute to aromas such as rose, grassy, honey and herb-like (Campo, Ferreira, Escudero, Marqués, & Cacho, 2006; Ferreira, López, & Cacho, 2000; Gil, Cabellos, Arroyo, & Prodanov, 2006).

Table 4
Experiment A: Volatile metabolites of Chenin blanc wines detected using Gas chromatography-flame ionisation detector (GC – FID).

Aroma compounds (mg L^{-1})	Odour description[b]	Control	1x treatment	2x treatment
Esters				
Ethyl_Acetate	Varnish, fruity, solvent	24.86a[a]	20.89a	19.89a
Isobutyl-Acetate	Banana	0.02a	0.02a	0.02a
Ethyl_butyrate	Acidic, fruity, apple	0.28a	0.27a	0.27a
Isoamyl_Acetate	Banana, pear	2.39a	1.51a	1.84a
Ethyl_Hexanoate	Green apple	6.94a	0.27a	0.34a
Hexyl_Acetate	Apple, cherry, pear,floral	0.08a	0.05a	0.09a
Ethyl_Lactate	Lactic, buttery, fruity	24.81a	24.68a	23.93a
Ethyl_Caprylate	Fruity, flower	0.46a	0.54a	0.51a
Ethyl-3-hydroxybutanoate	Fruity, sweet	1.11a	0.85a	0.77a
Ethyl_Caprate	Fruity, melon	0.17a	0.17a	0.17a
Diethyl_Succinate	Wine, fruit	0.49a	2.21a	1.14a
Ethyl_phenylacetate	Fruit, sweet	0.26a	0.24a	0.22a
2-Phenylethyl_Acetate	Rose, honey, tobacco, fruity, cooked apple	0.98a	1.13a	1.19a
Higher alcohols				
n-propanol	Alcohol, ripe fruit	58.20a	110.50a	143.08a
Isobutanol	Fusel, alcohol	4.55a	11.14a	16.41a
Butanol	Fusel odour, medicinal	0.65a	0.88a	0.92a
Isoamyl_alcohol	Alcohol, harsh	49.09a	133.19a	93.09a
Pentanol	Ripe banana	0.00	0.00	0.00
4-methyl-1-pentanol	Tropical	0.20a	0.21a	0.21a
Acetoin	Buttery	0.20a	0.20a	0.18a
3-methyl-1-pentanol	Fruity, Wine	4.97a	4.89a	4.81a
Hexanol	Grassy	1.76a	1.41a	1.29a
3-ethoxy-1-propanol	Fruity	7.26a	9.24a	10.93a
2-Phenyl_Ethanol	Roses	3.16a	8.76a	4.05a
Fatty acids				
Acetic_Acid	Vinegar	133.72a	142.20a	107.94a
Propionic_Acid	Rancid, pungent	6.81a	1.26a	1.62a
Isobutyric_acid	Acidic	0.44a	0.68a	0.49a
Butyric_Acid	Rancid, cheese, sweat	0.68a	0.62a	0.64a
Iso-Valeric_Acid	Blue cheese	0.67a	0.99a	0.65a
Valeric_Acid	Roast barley	0.17a	0.24a	0.16a
Hexanoic_Acid	Sweat, cheesy	50.47a	2.95a	31.72a
Octanoic_Acid	Rancid, harsh, sweaty	3.49a	4.48a	4.10a

[a] Values between columns followed by the same letter do not differ significantly ($p > 0.05$).
[b] Lee and Noble (2003); Escudero et al. (2007); King et al. (2008).

Fatty acids present at highest concentrations were acetic acid (133.72, 142.20 and 107.94 g/L for the control, T1 and T2, respectively), propionic acid (6.18, 1.26 and 1.62 g/L, respectively), hexanoic acid (50.47, 21.95 and 31.72 g/L, respectively) and octanoic acids (3.49, 4.48 and 4.10 g/L, respectively), with the remainder present at concentrations around 1 g/L or less. It is noteworthy that acetic acid is the major contributor to total fatty acids and reported to impart undesired "vinegar-like" off-odours when present at a threshold of 200 g/L (Cheng, Liu, Yue, & Zhang, 2015; Hart et al., 2017a & b; Jiang and Zhang, 2010). Hexanoic acid and octanoic acid were within permissible concentration ranges for white wine (1–73 g/L and 2–717 g/L, respectively). Hexanoic acid imparts "sweaty" nuances (Francis & Newton, 2005; Lawrence, 2012; Pretorius & Lambrechts, 2000). In addition, a study by Lawrence (2012) found that octanoic acids at concentrations between 3.5 and 12.0 g/L produced fresh and fruity Chenin blanc wines. Therefore, these results showed that the fungicide treatments did not produce wines that were sensorially significantly different to the control.

In experiment B, generally, there were no statistical significant difference between the control and treatments ($p > 0.05$) (Table 5). The ester compounds present in the highest concentration were ethyl acetate (11.13, 17.07 and 5.69 g/L for the control, T1 and T2, respectively) and ethyl lactate (17.23, 13.74 and 17.43 g/L, respectively), with the rest once more present at concentrations around 1 g/L or less. Ethyl acetate contributes "varnish" and "solvent" aromas at a threshold of 17.62 g/L (Gomez-Miguez et al., 2007; Li et al., 2008; Pretorius & Lambrechts, 2000). In this study, ethyl acetate was found in concentrations lower than the threshold value (17.62 g/L) when compared to experiment A where it was above. Previous research showed that ethyl lactate at a threshold of 14 g/L contributes to fruity and flowery aromas (Li et al., 2008). Hence, there is no doubt that these wines will have the aromas mentioned above, since the ethyl lactate was detected at concentrations above the threshold value 14 g/L (Escudero et al., 2007; Gomez-Miguez et al., 2007; King et al., 2008; Lee & Noble, 2003; Li et al., 2008; Pretorius & Lambrechts, 2000). In addition, the comments from the judges such as "bit acetone", "foreign rotten smell" also support the results obtained.

No statistical significant difference was observed between the higher alcohols of the control and treated wines in experiment B ($p > 0.05$). The higher alcohols present at the highest concentration were 1-propanol (50.45, 51.19 and 81.82 g/L for the control, T1 and T2, respectively), isobutanol (7.71, 7.96 and 0.06 g/L, respectively), isoamyl alcohol (59.12, 67.18 and 1.53 g/L, respectively), 3-methyl-1-pentanol (3.47, 2.76 and 3.52 g/L, respectively), 3-ethoxy-1-propanol (2.82, 2.89 and 5.09 g/L, respectively), and 2-phenyl-ethanol (3.10, 3.44 and 0.79 g/L, respectively). The aforementioned compounds could contribute to alcohol and ripe fruit aromas. Additionally, it was notable that isobutanol, isoamyl alcohol and 2-phenyl-ethanol concentrations were higher in the control and T1, compared to T2. Moreover, 2-phenyl-ethanol, associated with floral and rose notes (Knoll et al., 2011; Rocha et al., 2010), was also detected in the wines at higher concentrations, but lower than the threshold value (400 g/L). The higher alcohols were detected at concentrations lower than 300 g/L at which they contribute rose, grassy, honey and herb-like aromas. However, in concentrations above 400 g/L they give harsh, solvent-like odours. Moreover, statistically, the data showed no significant differences

Table 5
Experiment B: Volatile metabolites of Chenin blanc wines detected using Gas chromatography-flame ionisation detector (GC – FID).

Aroma compounds (mg L^{-1})	Odour description[b]	Control	1x treatment	2x treatment
Esters				
Ethyl_Acetate	Varnish, fruity, solvent	11.13a[a]	17.07a	5.69a
Isobutyl-Acetate	Banana	0.03a	0.03a	0.00
Ethyl_butyrate	Acidic, fruity, apple	0.14a	0.10a	0.00
Isoamyl_Acetate	Banana, pear	0.32a	0.20a	0.08a
Ethyl_Hexanoate	Green apple	0.12a	0.00	0.00
Hexyl_Acetate	Apple, cherry, pear, floral	0.00	0.00	0.00
Ethyl_Lactate	Lactic, buttery, fruity	17.23a	13.74a	17.43a
Ethyl_Caprylate	Flower, fruity	0.19a	0.10a	0.69a
Ethyl-3-hydroxybutanoate Fruity, sweet		0.52a	0.59a	0.77a
Ethyl_Caprate	Fruity, melon	0.06a	0.04a	0.00
Diethyl_Succinate	Wine, fruity	0.64a	0.67a	0.14a
Ethyl_phenylacetate	Fruit, sweet	0.21a	0.23a	0.23a
2-Phenylethyl_Acetate	Rose, honey, tobacco	0.63a	0.45a	0.07a
Higher alcohols				
n-propanol	Alcohol, ripe fruit	50.45a	51.19a	81.82a
Isobutanol	Fusel, alcohol	7.71a	7.96a	0.06a
Butanol	Fusel odour, medicinal	0.13a	0.09a	0.00
Isoamyl_alcohol	Alcohol, harsh	59.12a	67.18a	1.53a
Pentanol	Ripe banana	0.00	0.00	0.00
4-methyl-1-pentanol	Tropical	0.00	0.00	0.07a
Acetoin	Buttery	0.07a	0.07a	0.00
3-methyl-1-pentanol	Fruity, Wine	3.47a	2.76a	3.52a
Hexanol	Grassy	1.82a	1.49a	1.22a
3-ethoxy-1-propanol	Fruity	2.82a	2.89a	5.09a
2-Phenyl_Ethanol	Roses	3.10a	3.44a	0.79a
Fatty acids				
Acetic_Acid	Vinegar	91.81b	108.25 ab	149.92a
Propionic_Acid	Rancid, pungent	0.07a	0.04a	0.18a
Isobutyric_acid	Acidic	0.32a	0.36a	0.08a
Butyric_Acid	Rancid, cheese, sweat	0.13a	0.07a	0.00
Iso-Valeric_Acid	Blue cheese	0.49a	0.48a	0.24a
Valeric_Acid	Roast barley	0.17a	0.17a	0.13a
Hexanoic_Acid	Sweat, cheesy	62.42a	53.70a	97,01a
Octanoic_Acid	Rancid, harsh, sweaty	2.20a	1.44a	0,29a

[a] Values between columns followed by the same letter do not differ significantly ($p > 0.05$).
[b] Lee and Noble (2003); Escudero et al. (2007); King et al. (2008).

($p > 0.05$).

The overall fatty acids showed no significant differences between the control and treatments in experiment B ($p < 0.05$), except for acetic acid. Acetic acid was significantly higher in T2 (149.92 g/L) than in the control (91.81 g/L) ($p > 0.05$). It is noteworthy that although the concentration was significantly higher than in the control, it was still below the threshold value of 200 g/L and the wines did not have "vinegar-like" off-odours (Cheng et al., 2015; Hart et al., 2017a & b; Jiang, 2010). Additionally, other fatty acids present at higher concentrations were hexanoic acid (62.42, 53.70 and 97.01 g/L for the control, T1 and T2, respectively) and octanoic acid (2.20, 1.44 and 0.29 g/L, respectively). The rest were present at concentrations around 1 g/L or less. The aforementioned fatty acids are within the acceptable concentration ranges.

There were no statistical significant differences in the esters produced in experiment C between the control and the treatments ($p > 0.05$) (Table 6). The ester compounds present in the highest concentrations were ethyl acetate (4.44, 4.13 and 5.04 g/L for the control, T1 and T2), respectively), ethyl lactate (14.12, 15.63 and 15.18 g/L, respectively) and ethyl caprylate (1.05, 2.01 g/L for the control and T1, respectively, not detected in T2), with the remainder present at concentrations around 1 g/L or less. It is noteworthy that ethyl acetate was detected at concentrations below its threshold value (17.62 g/L). Therefore, the undesirable aromas usually associated with it were not perceived in this experiment. Furthermore, ethyl caprylate was detected at concentrations higher than the threshold value (0.58 g/L) and can thus contribute to the fruity and floral aromas perceived.

The higher alcohol compounds also showed no significant differences between the control and treatments ($p > 0.05$), except for acetoin. Acetoin was significantly lower in the control (0.24 g/L) when compared to the treatments (0.31 g/L, 0.32 g/L for T1 and T2, respectively). However, it is important to highlight that acetoin contributes to the "buttery" character in wine at a threshold value of 150 g/L (Francis & Newton, 2005). This was substantiated by the sensory results, as the panel did not perceive "buttery". Wines produced from T1-treated grapes had the highest concentration of 1-propanol (13.72 g/L), whilst the remainder of wines had less than 1 g/L. Concentrations of other higher alcohols, i.e. isoamyl alcohol (12.99, 20.33 and 8.78 g/L), 3-methyl-1-pentanol (2.84, 3.19 and 3.23 g/L), 3-ethoxyl-1-propanol (3.09, 2.23 and 2.22 g/L) and 2-phenyl ethanol (1.22, 1.59 and 1.96 g/L) for the control, T1 and T2 wines were respectively. The rest of the higher alcohols were present at concentrations around 1 g/L or less in all wines. Therefore, their aroma contribution to the wines was also below a detectable threshold.

Fatty acids showed no statistical significant differences between the control and the treatments ($p > 0.05$), except for iso-valeric acid. Iso-valeric acid was significantly lower in the control (0.25 g/L) when compared to the treatments (0.33 g/L, 0.35 g/L for the T1 and T2, respectively). Additionally, the threshold value of iso-valeric acid in wines is 0.033 g/L and it imparts sweet, acid, rancid, and floral aromas to wine (Peťka, Ferreira, González-Viñas, & Cacho, 2006; Sánchez-Palomo, Gómez García-Carpintero, Alonso-Villegas, & González-Viñas, 2010). Therefore, in this experiment, it was detected in concentrations exceeding the threshold value. Fatty acids present at higher concentrations were acetic acid (195.64, 208.62 and 188.46 g/L for the control, T1 and T2, respectively), propionic acid (1.09, 1.14 and 1.15 g/

Table 6
Experiment C: Volatile metabolites of Chenin blanc wines detected using Gas chromatography-flame ionisation detector (GC – FID).

Aroma compounds (mg L^{-1})	Odour description[b]	Control	1x treatment	2x treatment
Esters				
Ethyl_Acetate	Varnish, fruity, solvent	4.44a[a]	4.13a	5.04a
Isobutyl-Acetate	Banana	0.00	0.00	0.00
Ethyl_butyrate	Acidic, fruity, apple	0.01a	0.01a	0.03a
Isoamyl_Acetate	Banana, pear	0.09a	0.09a	0.15a
Ethyl_Hexanoate	Green apple	0.00	0.00	0.00
Hexyl_Acetate	Apple, cherry, pear, floral	0.00	0.00	0.00
Ethyl_Lactate	Lactic, buttery, fruity	14.12a	15.63a	15.18a
Ethyl_Caprylate	Fruity, flower	1.05a	2.01a	0.00
Ethyl-3-hydroxybutanoate Fruity, sweet		0.56a	0.54a	0.50a
Ethyl_Caprate	Fruity, melon	0.01a	0.01a	0.01a
Diethyl_Succinate	Wine, fruit	0.19a	0.25a	0.35a
Ethyl_phenylacetate	Fruit, sweet	0.25a	0.26a	0.26a
2-Phenylethyl_Acetate	Rose, honey, tobacco	0.09a	0.09a	0.17a
Higher alcohols				
n-propanol	Alcohol, ripe fruit	0.05a	13.72a	0.05a
Isobutanol	Fusel, alcohol	0.19a	0.19a	0.06a
Butanol	Fusel odour, medicinal	0.00	0.00	0.00
Isoamyl_alcohol	Alcohol, harsh	12.99a	20.33a	8.78a
Pentanol	Ripe banana	0.00	0.00	0.00
4-methyl-1-pentanol	Tropical	0.22a	0.22a	0.27a
Acetoin	Buttery	0.24b	0.31a	0.32a
3-methyl-1-pentanol	Fruity, Wine	2.84a	3.19a	3.23a
Hexanol	Grassy	0.99a	1.07a	1.07a
3-ethoxy-1-propanol	Fruity	3.09a	2.23a	2.22a
2-Phenyl_Ethanol	Roses	1.22a	1.59a	1.96a
Fatty acids				
Acetic_Acid	Vinegar	195.64a	208.62a	188.46a
Propionic_Acid	Rancid, pungent	1.09a	1.14a	1.15a
Isobutyric_acid	Acidic	0.12a	0.12a	0.15a
Butyric_Acid	Rancid, cheese, sweat	0.00	0.00	0.01a
Iso-Valeric_Acid	Blue cheese	0.25b	0.33a	0.35a
Valeric_Acid	Roast barley	0.13a	0.15a	0.14a
Hexanoic_Acid	Sweat, cheesy	98.49a	99.09a	94.34a
Octanoic_Acid	Rancid, harsh, sweaty	0.52a	0.55a	0.92a

[a] Values between columns followed by the same letter do not differ significantly ($p > 0.05$).
[b] Lee and Noble (2003); Escudero et al. (2007); King et al. (2008).

Table 7
ANOVA of thiol compounds of 2016 small-scale Chenin blanc wines using Gas chromatography-mass spectrometry (GC-MS).

Experiment	Treatment	4MMP[a]	3MHA[b]	3 MH[c]
A	Control (C)	48.7a[d]	527.3a	193.0a
	1*X* Treatment (T1)	28.9a	568.9a	181.1a
	2*X* Treatment (T2)	98.7a	395.5a	185.4a
B	C	166.8a	15.13a	128.7a
	T1	156.7a	15.99a	104.7b
	T2	148.3a	50.51a	125.1a
C	C	627.6a	41.9a	250.2a
	T1	752.2a	0a	222.6a
	T2	480.4a	0a	189.2a

[a] 4MMP – 4-mercapto-4-methylpentan-2-one.
[b] 3MHA – 3-mercaptohexyl acetate.
[c] 3 MH – 3-mercaptohexan-1-ol.
[d] Values within columns followed by the same letter do not differ significantly ($p > 0.05$).

L, respectively) and hexanoic acids (98.49, 99.09 and 94.34 g/L, respectively), with the remainder present at concentrations around 1 g/L or less. Furthermore, it was notable that acetic acid in T1 was marginally higher than its threshold value (200 g/L), while in the control and T2, it was detected at lower concentrations. As previously mentioned, acetic acid contributes to undesired "vinegar-like" off-odours when above the threshold value of 200 g/L, hence T1 in this experiment would have been expected to have those off-odours. Hexanoic acid was found in concentrations higher than the typical concentration range of 1–73 g/L for white wine, and is known to contribute to "sweaty" nuances (Francis & Newton, 2005; Lawrence, 2012; Pretorius & Lambrechts, 2000).

3.5. Thiol analysis

The ANOVA results for experiment A, showed that although there was no statistical significant differences between the control and the treatments ($p > 0.05$), however 3MHA was present at higher concentrations (527.30, 568.90 and 395.50 g/L for the control, T1 and T2, respectively) when compared to 4MMP (48.66, 28.91 and 98.72 g/L, respectively) and 3 MH (193.04, 181.12 and 185.38 g/L, respectively) (Table 7). In experiment B, the ANOVA results showed no statistical significant difference in thiol compounds produced between the control and both treatments (T1 and T2) ($p > 0.05$), with the exception of 3 MH for T1 (104.71 g/L) which showed a significant difference when compared to the control (128.67 g/L) and T2 (125.06 g/L) (Table 7). The ANOVA results for experiment C, also showed no statistical significant difference between the control and both treatments (T1 and T2) ($p > 0.05$). However, 3MHA was not detected in the final wines originating from T1 and T2 grapes (Table 7).

3.6. Metabolite, thiol and sensory Multiple Factor Analyses (MFA)

Multiple Factor Analyses (MFA) was used to analyse the association between metabolites, thiols and sensory data. experiment A (Fig. 4)

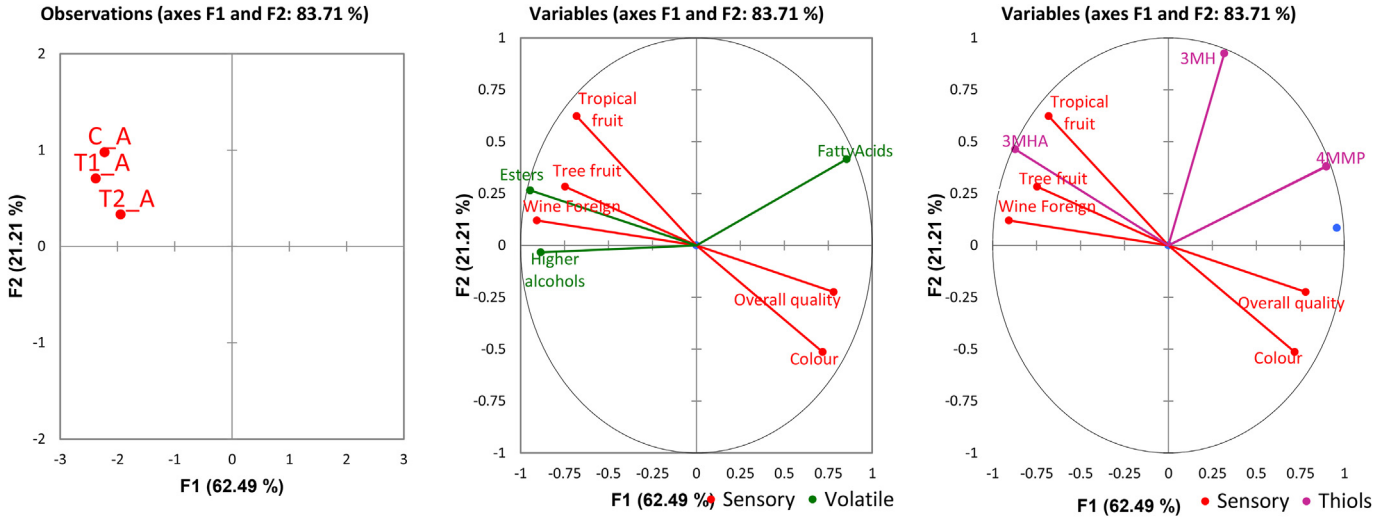

Fig. 4. Experiment A: Multiple Factor Analysis (MFA) of wine sensory profiles and the association with esters, higher alcohols, fatty acids and thiol compounds i.e. 3-mercaptohexanol (3 MH), 3-mercaptohexanol acetate (3MHA) and 4-mercapto-4-methylpentan-2-one (4MMP) when comparing chemical spray treatments (i.e. Control [C], 1x dosage [T1] and 2x dosage [T2]. *Mean totals of triplicate fermentations.

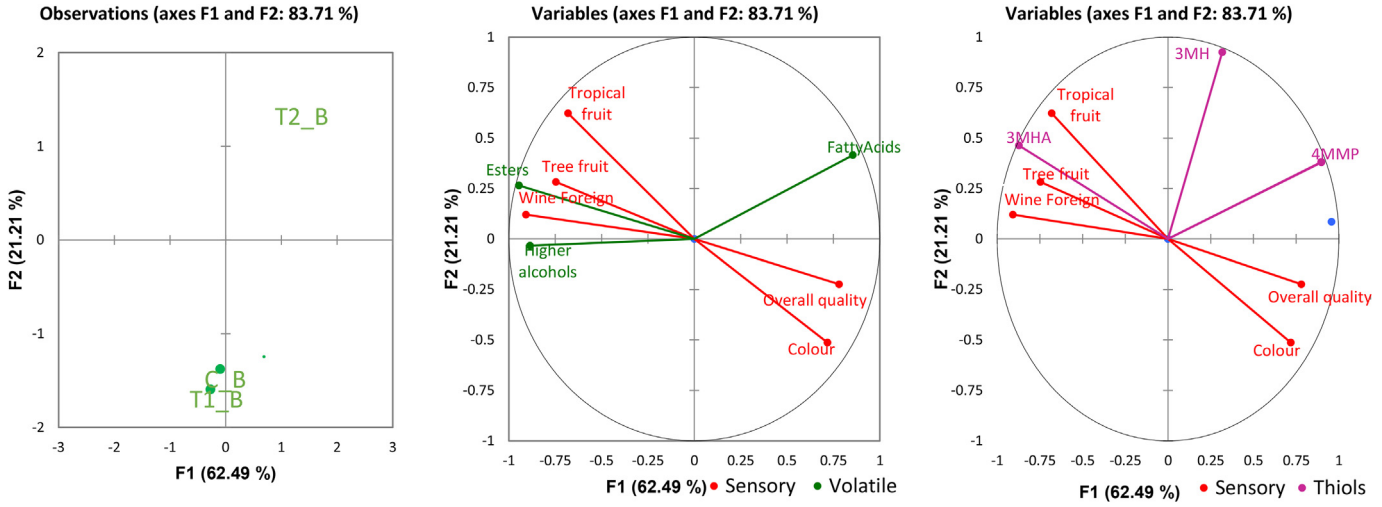

Fig. 5. Experiment B: Multiple Factor Analysis (MFA) of wine sensory profiles and the association with esters, higher alcohols, fatty acids and thiol compounds i.e. 3-mercaptohexanol (3 MH), 3-mercaptohexanol acetate (3MHA) and 4-mercapto-4-methylpentan-2-one (4MMP) when comparing chemical spray treatments (i.e. Control [C], 1x dosage [T1] and 2x dosage [T2]. *Mean totals of triplicate fermentations.

showed that the control and treatments (T1 and T2) clustered in the top left quadrant and produced wines with a positive association with esters and higher alcohols, which can contribute to positive aromas such as rose and honey. However, the wines showed negative association with the fatty acidsbut a, positive association with 3MHA, which is known to positively affect wine aroma (associated with “box tree”, “tropical fruit”, “passion fruit” and “citrus” aromas (Buica, Brand, & Wilson, 2018; King et al., 2008; Tominaga, Baltenweck-Guyot, Des Gachons, & Dubourdieu, 2000). Moreover, although the wines showed a negative association with 3 MH and 4MMP (Fig. 4), the wines had a positive association with “Tropical fruit” and “Tree fruit” aromas. Some judges detected an association with “Wine Foreign” aromas, which are usually not associated with Chenin blanc. This could be the reason why the wines had a negative association with “Overall Quality” (Fig. 4). However, the metabolites and thiols formation, when observing the sensory analyses, indications are that the tested chemical sprays did not have a negative effect on the overall wine sensory quality as the treatments showed similar results to the control (registered fungicide).

The MFA data for experiment B showed that the control and T1 clustered in the bottom left quadrant indicating a positive association with higher alcohols, which can contribute to rose and honey aromas (Fig. 5). Moreover, the wines were also negatively associated with 3MHA, 3 MH and 4MMP. It is important to note that the wines had a positive association with “Overall Quality” and “Colour”. Furthermore, even though T2 showed a positive association with total fatty acids as it clustered in the same top right quadrant, it also had a positive association with “Tropical Fruit” and “Tree fruit” aromas based on descriptive sensory evaluation (Fig. 5). The latter observation confirmed the positive association of T2 with 3 MH and 4MMP that are linked to “box tree”, “tropical fruit”, “passion fruit” and “citrus” aromas (Buica et al., 2018; King et al., 2008; Piano et al., 2015; Tominaga et al., 2000). Therefore, the results indicated that the chemical sprays in terms of sensory quality did not negatively affect the final wines. It should however, be noted that although the T2 had a lower association with quality, it also had a negative association with “Wine Foreign” aromas, therefore the fungicide did not negatively affect the wine compared to the control (registered fungicide).

Experiment C MFA data showed that the control and both treatments clustered in the top right quadrant indicating that they the wines were associated with fatty acids (Fig. 6). Furthermore, the wines also

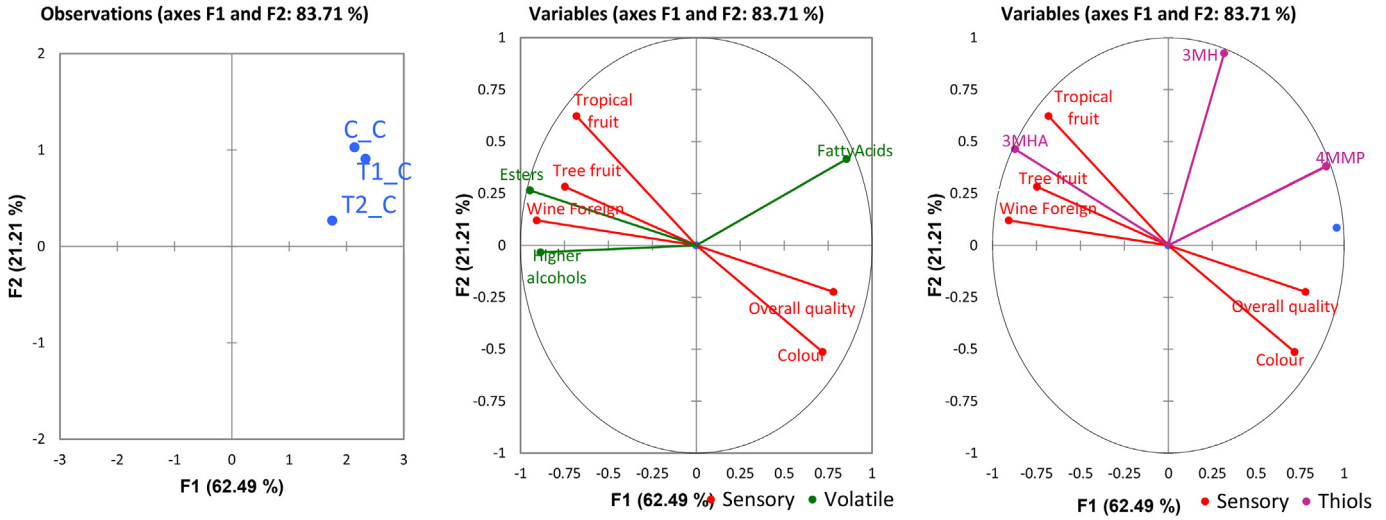

Fig. 6. Experiment C: Multiple Factor Analysis (MFA) of wine sensory profiles and the association with esters, higher alcohols, fatty acids and thiol compounds i.e. 3-mercaptohexanol (3 MH), 3-mercaptohexanol acetate (3MHA) and 4-mercapto-4-methylpentan-2-one (4MMP) when comparing chemical spray treatments (i.e. Control [C], 1x dosage [T1] and 2x dosage [T2]. *Mean totals of triplicate fermentations.

showed a positive association with the 3 MH and 4MMP compounds. These thiol are known to contribute to the aroma profile of wine by producing aromas such as “box tree”, “tropical fruit”, “passion fruit” and “citrus” (Buica et al., 2018; King et al., 2008; Piano et al., 2015; Tominaga et al., 2000). This is desirable, as studies conducted by Du Plessis and Augustyn (1981) suggested 4MMP releases guava-like aromas. Moreover, although the wines associated with fatty acids (Fig. 6), they also had a positive association with “Tropical Fruit” and “Tree fruit” aromas. This observation complemented the sensory analyses, which indicated that the chemical sprays did not have a negative effect on wine sensory quality as the treatments showed similar results to the control.

4. Conclusion

From the results, it was concluded that both yeast strains used for wine making completed their respective fermentations at a similar rate compared to the relevant control fermentation. In addition, small-scale winemaking showed that the fungicide name the fungicides had no notable negative effects on wine aroma and sensory profiles relatively to the registered fungicide control. However, differences in wine yeast protein expression extracted in fermenting samples originating from the control (registered fungicide) and experimental fungicide-treated grapes indicates that fungicides had an effect on the fermenting yeast strain, as the fungicide was the only variable. As some yeast-derived proteins are required to release some wine aroma enhancing compounds (metabolites) e.g. volatile thiols during fermentation, it can be concluded that differences in metabolite levels observed was indirectly influenced by fungicide treatments. Therefore, the use of fungicides that will have a negative effect on the protein expression of yeast starter cultures is not recommended, as wine producers will incur financial losses. Reason being, lower perceived wine aroma and flavour is synonymous with wine quality and will therefore, have a negative influence consumer preferences. Nonetheless, the fungicide treatments did not negatively affect the yeast performance and final wine sensory quality.

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