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Inactive dry yeast application on grapes modify Sauvignon Blanc wine aroma

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ABSTRACT

This study investigated the potential to improve wine aroma by applying two inactive dry yeast products (IDYs) at the onset of ripening on Sauvignon Blanc grapes. Both products led to increased reduced glutathione concentrations in the grape juice and corresponding wines, as well as differences in individual higher alcohol acetates (HAAs) and ethyl esters of straight chain fatty acids (EEFAs) at the end of fermentation. After two months of storage, a significantly slower decrease of EEFAs and to a lesser extent of HAAs was found for wines made from grapes with IDY applications. These wines also resulted in significantly slower synthesis of ethyl esters of branched acids, whereas varietal thiols were altered in a product-specific manner. The modifications in the wine chemical composition were also sensorially corroborated. This study showed that vineyard additions of IDY products directly on the grapes at the onset of ripening have a subsequent benefit to the production and preservation of aroma in wines.

1. Introduction

In recent years several commercial products based on inactive dry yeast (IDY) preparations have been used during the winemaking process to enhance or preserve wine aromatic composition and/or improve desired mouthfeel properties. The IDYs are typically *Saccharomyces cerevisiae* derivatives from different manufacturing processes and can be divided into four commercially available groups of products namely, inactive yeasts, yeast autolysates, yeast hulls and yeast extracts (Pozo-Bayón, Andujar-Ortiz, Alcaide-Hidalgo, Martín-Álvarez, & Moreno-Arribas, 2009; Rodríguez-Bencomo et al., 2014). The IDY additions are generally made to juice before, during or after fermentation (Comuzzo et al., 2012; Del Barrio-Galán, Pérez-Magariño, Ortega-Heras, Williams, & Doco, 2011). The IDY products are used as alcoholic fermentation enhancers, promoting yeast resistance to osmotic stress, improving nitrogen compound assimilation and enhancing wine sensory profiles, amongst other listed benefits (Pozo-Bayón et al., 2009; Pozo-Bayón, Andújar-Ortiz, & Moreno-Arribas, 2009a, 2009b). IDY products are also promoted as "wine quality enhancers", said to decrease perception of bitterness and increase sweetness of wines, efficiency in tartaric acid stabilisation, provide antioxidant properties and increases the structure and mouthfeel of wines (Andujar-Ortiz, Pozo-Bayón, Moreno-Arribas, Martín-Álvarez, & Rodríguez-Bencomo, 2012; Pozo-Bayón et al., 2009a, 2009b). IDYs are also used in wines that undergo malolactic fermentation, providing nutrients for bacteria, enhancing growth and malolactic fermentation rate and reducing the risk of malolactic fermentations being carried out by undesired bacteria (Pozo-Bayón et al., 2009, 2009a, 2009b). Despite their relative wide use in wine production, no study has aimed to verify their

Abbreviations: ANOVA, analysis of variance; CD, common dimension; CCSWA, common component specific weight analyses; EEBAs, ethyl esters of branched acids; EEFAs, ethyl esters of straight-chain fatty acids; GABA, γ-aminobutyric acid; GC–FID, gas chromatography–flame ionisation detection; GC–MS, gas chromatography–mass spectrometry; GSH, reduced glutathione; GSSG, oxidised glutathione; HAAs, higher alcohol acetates; HCA, hierarchical cluster analysis; HPLC, high pressure liquid chromatography; HS-SPME, headspace solid-phase microextraction; IBMP, 3-isobutyl-2-methoxypyrazine; IDYs, inactive dry yeasts; IDY1, treatment with application of product IDY1; IDY2, treatment with application of product IDY2; MDS, non-metric multidimensional scaling; SPME, solid phase microextraction; UPLC, ultra high pressure liquid chromatography; 3SH, 3-sulfanylhexan-1-ol; 3SHA, 3-sulfanylhexyl acetate; TA, titratable acidity; TSS, total soluble solids.

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application in the vineyard on grapes. Summarising the current literature and as suggested by Andújar-Ortiz, Chaya, Martín-Álvarez, Moreno-Arribas, and Pozo-Bayón (2013), based on the type and timing of IDYs supplementation to the must or wine, the resulting modifications in wine volatile and non-volatile fractions could be a consequence of: (i) modification of yeast metabolism and subsequently its by-products (Saerens et al., 2008); (ii) release of amino acids, mannoproteins, lipids, peptides, vitamins, minerals and volatiles from IDYs (Andújar-Ortiz et al., 2013; Guadalupe, Martínez, & Ayestarán, 2010; Pozo-Bayón et al., 2009, 2009a, 2009b); (iii) retention of wine volatiles by mannoproteins and peptides from IDYs (Chalier, Angot, Delteil, Doco, & Gunata, 2007; Comuzzo, Tat, Tonizzo, & Battistutta, 2006; Comuzzo et al., 2012; Pozo-Bayón et al., 2009, 2009a, 2009b) and; (iv) possible antioxidant effects of IDYs (Del Barrio-Galán et al., 2011; Kritzinger, Stander, & Du Toit, 2012; Rodríguez-Bencomo et al., 2014). Glutathione-enriched IDYs are claimed to increase wine reduced glutathione (GSH) concentrations either by direct liberation of GSH into juice/wine or by providing precursors for GSH synthesis during fermentation (Kritzinger, Bauer, & du Toit, 2012). GSH is an important antioxidant whose additions to juice and wine have been reported to prevent browning, increase production of some volatile thiols during fermentations and provide a proposed protective role against the loss of certain terpenes, esters and thiols in wine during ageing (Andújar-Ortiz et al., 2013; Kritzinger et al., 2012; Makhotkina et al., 2014).

Sauvignon Blanc wines have received recognition around the globe due to their specific, varietal aromatic profiles, often characterised by green and tropical fruit nuances attributed to methoxypyrazines, thiols, esters and higher alcohols (Benkwitz et al., 2012; Dubourdieu, Tominaga, Masneuf, Peyrot des Gachons, & Murat, 2006; Lund et al., 2009). With the exception of methoxypyrazines, which remain almost unchanged during wine ageing, thiols and esters are subject to more dynamic modifications, with concentrations declining or increasing during wine ageing depending on storage conditions (Antalick, Perello, & de Revel, 2014; Brajkovich et al., 2005; Coetzee, Lisjak, Nicolau, Kilmartin, & du Toit, 2013; Herbst-Johnstone, Nicolau, & Kilmartin, 2011). Therefore, the use of IDYs either to increase the production of volatiles or to moderate the decrease of desired aromatic constituents of Sauvignon Blanc wines is of interest.

This study aimed to follow and quantitate the impact of two IDY preparations, designed by their manufacturer, to improve wine aroma of Sauvignon Blanc. One of the novel aspects of this work was the fact that the IDY preparations were applied in the vineyard on grapes at the onset of ripening, instead of during the winemaking process. The chemical and sensorial analyses focused on the influence of these applications on Sauvignon Blanc grape berry ripening, juice parameters and wine aroma post-fermentation and after short-term ageing.

2. Materials and methods

2.1. Vineyard

This experiment was carried out in a commercial highly characterised *Vitis vinifera* L. cv. Sauvignon Blanc vineyard located in the Overberg region of the Southern coastal area, South Africa (34°9′53.10″S; 19°0′50.51″E) (Šuklje et al., 2012, 2014). The Sauvignon Blanc vines (clone 316 grafted on 101.14 Mgt) were planted in 2004 in a northwest to southeast row orientation with 2.5 m plant spacing between rows and 1.8 m in-row. The vines were trained on a double cordon with vertical shoot positioning and pruned to six two-bud spurs per running metre of cordon. Two inactive dry yeast derivative products (designated IDY1 and IDY2), produced by the same manufacturer and patented for foliar application technology WO/2014/024039, (Lallemand Inc., Montreal, Canada) were used in this study. These specific preparations were chosen for the study, since they could be both applied in a vineyard directly on grapes and are marketed for their wine quality promotion capacities. Product IDY1, which is not commercially cleared yet, was specified by the manufacturer as an inactive *S. cerevisiae* + sulphur derivative, which should increase concentrations of varietal thiols in wines. Product IDY2 (LalVigne™ AROMA, Lallemand Inc.) was described as consisting solely of inactive *S. cerevisiae* and was promoted to increase aroma precursors in grapes and complexity of final wines. A Latin square experimental design was set up across nine adjacent rows. A treatment repetition consisted of eight adjacent vines replicated across three rows. Application of IDY1 and IDY2 was carried out on the same day in accordance with producers' instructions. IDY1 and IDY2 was applied twice to the Sauvignon Blanc grapes at a rate per application of 4 kg/ha and 3 kg/ha, respectively. The first spraying was performed one week after 100% *véraison* (7 February 2013) at E-L stage 36, and the second spraying was performed ten days thereafter (E-L stage 37). The E-L stages were determined as outlined by Coombe (1995). Bunches were sprayed evenly, on a non-windy day using a small hand pump on both sides of the canopy. The control treatment received no applications. The IDY applications were prepared from single batches of the respective products to minimise batch-specific variation that could occur.

2.2. Grape sampling and basic parameters of maturity

Grape samples were collected before the first and second application of the IDYs, to assure that the chosen experimental plot was homogeneous in terms of ripening. Small parts of bunches were carefully sampled from both sides of the canopy avoiding damage to the berries and in total ~1 kg of grapes per replicate ($n = 3$ per treatment) was collected. Samples were transported in a cooled box to the analytical laboratory and subsamples of 100 berries were randomly selected ($n = 3$), placed in a re-sealable plastic bag and crushed by hand. The juice was collected and total soluble solids (TSS) were measured using a digital refractometer (Atago PAL-1, Tokyo, Japan) with temperature correction. The pH value and titratable acidity (TA) were determined through sodium hydroxide titration to the end point of 7.0 with a Metrohm titrator and sample changer (785 DMP Titrino with a LL-Unitrode Pt1000 FP, Metrohm AG, Herisau, Switzerland).

2.3. Wine making

Grapes were harvested on 11 March 2013 at around 22 °Brix. Approximately 20 kg of grapes per replicate ($n = 3$ per treatment), in total 60 kg per treatment (IDY 1 and 2 additions, respectively) and control, were harvested and replicates per treatment and control were pooled together and stored overnight at +4 °C prior to crushing. Grapes were destemmed and crushed with the addition of 40 mg/kg sulphur dioxide in the presence of solid carbon dioxide and a constant flow of nitrogen gas (N_2). Grape slurries were collected in plastic buckets in which a cup of ~300 g of solid carbon dioxide pellets was added and purged with N_2. Grape slurry was pressed off as soon as logistics allowed. Pressing also occurred in the presence of a constant flow of N_2 in combination with the addition of solid carbon dioxide to prevent oxidation of the must. To facilitate sedimentation an enzyme was added at 2 g/hL (Rapidase Vino Super; DSM Food Specialists B.V., Heerlen, The Netherlands) and the juice was clarified at +4 °C for 24 h. The clear musts (two treatments and untreated control) were divided into four equal volumes and fermentations carried out in quadruplicate per treatment. Per treatment, 4 L of the clear must were decanted into four 4.5-L fermenters, filled with N_2 beforehand. Before yeast

inoculation, 50 mL sample of must were taken for TSS, TA, pH value and amino acid analyses. A 15-mL sample of grape juice was also collected for GSH and oxidised glutathione (GSSG) analyses as described elsewhere (Kritzinger et al., 2012). Must was inoculated with 30 g/hL Lalvin YSEO QA 23 yeast (Lallemand Inc.). Fermentations were conducted in a temperature-controlled room at 15 °C. Three days after inoculation, 30 g/hL of an additional yeast nutrient (Fermaid K, Lallemand) was added to avoid stuck fermentation. Fermentation progress was monitored once per day by weighing the mass evolution of CO_2. The wines fermented dry, reaching a residual sugar level below 4 g/L. Afterwards, SO_2 was added at 40 mg/L and wines were cold stabilized at −4 °C for 10 days and thereafter the level of free SO_2 was adjusted to 35 mg/L and wines were bottled. The bottled wines were stored at +4 °C until the wine chemical analyses were conducted and sensory evaluation performed as outlined below.

2.4. Wine sampling

Wines were sampled immediately after completing the fermentation and 2 months after bottling. At these occasions 50-mL wine aliquots ($n = 4$ per treatment) were collected for wine aromatic compounds and amino acids analyses and, additionally, a 15-mL wine sample ($n = 4$ per treatment and control) was taken for GSH and GSSG analyses. Samples collected at the end of fermentation were stored at −20 °C until analysed, whereas samples collected two months after bottling were analysed immediately.

2.5. Reduced and oxidised glutathione analyses

The GSH and GSSG concentrations were monitored in grape juice before yeast inoculation, at the end of fermentation and after two months of wine ageing. Analyses were carried out by ultra-high-pressure liquid chromatography (UPLC) (Waters Acquity, Milford, MA) coupled to a Waters Xevo triple-quadrupole mass spectrometer using electrospray ionisation in the positive mode (Kritzinger et al., 2012). The separation was performed on a Waters Acquity BEH phenyl column (100 mm × 2.1 mm × 1.7 μm) with 0.4% trifluoroacetic acid as solvent A and acetonitrile as solvent B.

2.6. Amino acids analyses

Samples for amino acids analyses were collected from a clarified juice before fermentation and at the end of fermentation as described above. Collected sample was filtered through 0.45-μm Sartorius Minisart RC 25 filters (Fisher Scientific, Johannesburg, South Africa) in a vial. Analyses were performed by high-pressure liquid chromatography (HPLC) using Agilent 1100 (Agilent Technologies, Waldbronn, Germany). Separation of amino acids was achieved on a Zorbax Eclipse plus C18 Rapid Resolution column (4.6 × 150 mm, 3.5 μm particle size, Agilent Technologies) with eluent A consisting of 10 mM sodium tetraborate, 10 mM sodium phosphate and 5 mM sodium azide pH 8.2 and eluent B consisting of methanol:acetonitrile:water 45:45:10 (v:v) (Henderson & Brooks, 2010; Šuklje et al., 2015). A gradient at flow rate 1.5 mL/min was employed: 0–0.5 min (2% B); 0.5–10.7 min (27.5% B); 10.7–12.7 min (27.5% B); 12.7–17.1 min (38.5% B); 17.1–20.8 min (57% B); 20.8–20.9 min (100% B); 20.9–24.0 min (100% B); 24.0–24.1 min (2% B). Derivatisation was performed using three different reagents; iodoacetic acid (Sigma–Aldrich, Aston Manor, South Africa) for cysteine, *o*-phthaldialdehyde (Sigma–Aldrich) for primary amino acids and fluorenylmethyloxycarbonyl chloride (Sigma–Aldrich) for secondary amino acids (Šuklje et al., 2015). Quantification was performed with fluorescence detection and norvaline and sarcosine (both Sigma–Aldrich) were used as internal standards.

2.7. Analyses of higher alcohols, fatty acids and some minor compounds

The quantification of 20 compounds, including higher alcohols (8), esters (3, i.e. ethyl lactate, diethyl succinate and ethyl acetate), carboxylic acids (7), acetoin and acetic acid was performed using gas chromatography coupled to a flame ionisation detector (GC–FID) (Louw et al., 2009). Briefly, 5 mL of wine spiked with 4-methyl-2-pentanol as internal standard at final concentration of 10 mg/L were extracted with 1 mL of diethyl ether (Louw et al., 2009). The ether phase was dried over Na_2SO_4, filtered through glass wool and injected twice (Louw et al., 2009). Quantification was performed by an 6890 Plus GC (Agilent Technologies, Santa Clara, CA) equipped with a split/splitless injector and J&W DB-FFAP capillary GC column (Agilent Technologies) with dimensions 60 m × 0.32 mm × 0.5 μm. Identification of compounds was performed by comparing retention times with those of pure standards, whereas quantification was carried out by comparing the ratio of peak area and internal standard peak area with calibration graphs constructed using pure standards.

2.8. Esters analyses

Esters in wines were quantified according to a method previously published (Antalick, Perello, & de Revel, 2010) with some modifications as described (Šuklje et al., 2014). In brief, 10 mL of wine were spiked with a 20-μL mixture of deuterated esters composed of $[^2H_3]$-ethyl butyrate at 40 mg/L, $[^2H_{11}]$-ethyl hexanoate at 20 mg/L, $[^2H_{15}]$-ethyl octanoate at 20 mg/L, $[^2H_{23}]$-ethyl dodecanoate at 4 mg/L, and $[^2H_5]$-ethyl cinnamate at 12 mg/L, all obtained from CDN Isotopes (Pointe-Claire, QC, Canada). An aliquot of 5 mL of spiked wine was placed into a 20-mL solid-phase microextraction (SPME) vial previously filled with 1.5 g of NaCl. Samples were analysed as described using a DB-FFAP capillary column (60 m, 0.25 mm, 0.5 μm film thickness, Agilent Technologies) and a 6890 GC coupled to a 5975C mass spectrometer (Agilent Technologies) equipped with Enhanced Chemstation version D.01.02.16 software (Agilent Technologies).

2.9. Wine thiols analyzes

3-Sulfanylhexan-1-ol (3SH) and 3-sulfanylhexyl acetate (3SHA) were analysed in wines two months after bottling (Piano et al., 2015). Briefly, isotopically labelled 3SH ($[^2H_2]$-3SH) and 3SHA ($[^2H_2]$-3SHA), synthesised at Auckland University, New Zealand, were added to 180 mL of wine. Extraction was performed by adding 110 mL of dichloromethane to the samples (Merck, Modderfontein, South Africa). The derivatisation was carried out by the addition of 5 g/L *o*-phthaldialdehyde in methanol and 10 g/L of ethanolamine in 80 mM borate buffer to 50 μL of concentrated wine extract. Separation was performed with an Acquity UPLC (Waters) connected to a Waters Xevo triple quadrupole mass spectrometer using a Waters Acquity UPLC BEH C18 column (2.1 × 100 mm, 1.7 μm particle size), fitted with a guard cartridge (Waters VanGuard C18, 2.1 × 5 mm, 1.7 μm particle size) and the detection was performed in multiple reaction mode.

2.10. 3-Isobutyl-2-methoxypyrazine analyses

3-Isobutyl-2-methoxypyrazine (IBMP) analyses were conducted on wines two months after bottling, according to previously published methods (Parr, Green, White, & Sherlock, 2007; Šuklje et al., 2015). Briefly, 10 mL of wine were spiked with deuterated IBMP (CDN Isotopes) at a final concentration of 25 ng/L. NaCl (3 g) and 6.4 mL of MilliQ water were placed in an SPME vial before adding 1.6 mL of the spiked sample. Thereafter 2 mL of 4 M NaOH

Table 1
Attribute identification for data blocks.

No	Block index 1 Wine sensory attributes	Block index 2 Juice amino acids	Block index 3 Wine volatile composition
1	Tropical	Aspartic acid	Ethyl propionate
2	Pineapple	Glutamic acid	Ethyl isobutyrate
3	Guava	Cysteine	Propyl acetate
4	Passion fruit	Asparagine	Isobutyl acetate
5	Banana lolly	Serine	Methyl butyrate
6	Lychee	Glutamine	Ethyl butyrate
7	Gooseberry	Histidine	Ethyl 2-methylbutyrate
8	Papaya	Glycine	Ethyl isovalerate
9	Citrus	Threonine	Butyl acetate
10	Lemon	Arginine	Isoamyl acetate
11	Lime	Alanine	Isobutyl butyrate
12	Grapefruit	Tyrosine	Ethyl trans-2-butenoate
13	Stone fruit	γ-Aminobutyric acid	Ethyl valerate
14	Apple	Tryptophan	Methyl hexanoate
15	Pear	Phenylalanine	Ethyl hexanoate
16	Peach	Isoleucine	Isoamyl butyrate
17	Apricot	Ornithine	Hexyl acetate
18	Floral	Leucine	Ethyl heptanoate
19	Orange blossom	Lysine	Ethyl trans-2-hexenoate
20	Honey blossom	Hydroxyproline	Isobutyl hexanoate
21	Grass	Proline	Methyl octanoate
22	Green pepper		Hexyl butyrate
23	Canned beans		Ethyl octanoate
24	Tomato leaf		Isoamyl hexanoate
25	Sweaty		Octyl acetate
26	Cat urine		Ethyl nonanoate
27			Isobutyl octanoate
28			Methyl decanoate
29			Hexyl hexanoate
30			Ethyl decanoate
31			Isoamyl octanoate
32			Ethyl undecanoate
33			Isobutyl decanoate
34			Hexyl octanoate
35			Ethyl dodecanoate
36			Ethyl phenylacetate
37			Phenylethyl acetate
38			Ethyl dihydrocinnamate
39			Butyl phenylacetate
40			Hexyl phenylacetate
41			Octyl phenylacetate
42			Ethyl acetate
43			Methanol
44			Propanol
45			Isobutanol
46			Butanol
47			Isoamyl alcohol
48			Acetoin
49			Ethyl lactate
50			1-Hexanol
51			3-Ethoxy-1-propanol
52			Acetic acid
53			Propionic acid
54			Isobutyric acid
55			Isovaleric acid
56			Diethyl succinate
57			Valeric acid
58			Hexanoic acid
59			2-Phenylethanol
60			Octanoic acid
61			Decanoic acid
62			3-Sulfanylhexan-1-ol
63			3-Sulfanylhexyl acetate
64			3-Isopropyl-2-methoxypyrazine

were added and the vial was crimped and the sample mixed by vortex (IKA, Staufen, Germany). Samples were extracted by headspace solid-phase microextraction (HS-SPME) using a DVB-CAR-PDMS fibre (Supelco, Bellefonte, PA). Quantification was performed by gas chromatography-mass spectrometry (GC–MS) using an HP-5MS fused silica capillary column (30 m, 0.25 mm, 0.25 μm film thickness; Agilent Technologies) and a 6890 gas chromatograph coupled to a 5975C mass spectrometer (Agilent Technologies) equipped with Enhanced Chemstation version D.01.02.16 software (Agilent Technologies).

2.11. Wine sensory evaluation

Wine sensory analyses were performed three months after bottling. An expert panel of thirty judges (between the ages of 22 and 62), which was not trained beforehand for chosen sensory attributes, performed a sorting task. The panel consisted of wine industry professionals and post-graduate students from the Stellenbosch area (South Africa). All post-graduate students had experience in winemaking and participated in previous studies as sensory judges. A sorting task followed by a description of each group was used to characterise the differences between the wines (Valentin, Chollet, Lelièvre, & Abdi, 2012). Samples were presented simultaneously and in a different randomised order for each participant. Samples were served in covered black ISO glasses (to avoid dispersion of odorants) and coded with random three-digit numbers. Participants were asked to smell and taste the wines and sort them into groups according to similarity. They were allowed to form any number of groups as long as they formed more than one group and less groups than the total number of samples. They could include as many or as few wines as they wished in each group. Thereafter, the participants were asked to use 3–5 descriptors to describe each of the groups that they formed. Descriptors cited by more than 3 panellists were considered for further data processing and are listed in Table 1. Descriptors used by less than 3 panellists were either combined with a similar attribute or deleted where no synonyms were used by other panellists. The number of times that an attribute was used to describe a sample was calculated. This was done for all attributes as well as all samples and recorded in a contingency table.

2.12. Statistical analysis

One-way and two-way analysis of variance (ANOVA) were performed on the chemical data using Statistica, Version 12 (StatSoft, Tulsa, OK) and the means were separated using Stats-Fisher's LSD test (different letters account for significant differences at $p \leqslant 0.05$). All quoted uncertainty is standard deviation of four replicates of one treatment, except when stated otherwise. For wine sensory evaluation, dissimilarities between samples were analysed using non-metric multidimensional scaling (MDS). The Pearson's correlation coefficients between the attributes and the MDS configuration were calculated and projected onto the MDS. This was done using the coordinates of the first two dimensions of the MDS analysis and the contingency table, containing the sum of the attribute citations for all samples. Hierarchical cluster analysis (HCA) was performed on the coordinates of the first two dimensions of the MDS analysis to identify groupings of wine samples. Chemical data sets were related to wine sensory data by common component and specific weight analyses (CCSWA) using the SAISIR toolbox (SAISIR, 2010) (Cordella & Bertrand, 2014) on the centred and mean standardised matrices. For the purposes of clarity, multi-block analysis of datasets herein was organised and each data set was assigned a number as seen in Table 1.

3. Results and discussion

3.1. Grape maturity parameters

No significant difference were evident between the basic parameters of maturity, i.e. TSS, TA and pH, measured in the grapes

before the first and second application of the IDYs (Table S1), confirming that the experimental plot was homogeneous and that the application of IDYs did not influence basic parameters of grape ripening. Grapes were harvested around 22.0 °Brix and 8.3 g/L TA, whereas pH values were around 3.25 (Table S1).

3.2. Glutathione before, after fermentation and after two months of wine ageing

Significantly lower GSH concentrations were measured in the juice (before yeast inoculation) of the untreated control compared to those of the IDY1 and IDY2 treatments (Table 2). The concentrations measured were in agreement with previously reported values in musts which can range from almost non-detectable to 100 mg/L (Coetzee et al., 2013; du Toit, Lisjak, Stander, & Prevoo, 2007). The trend was maintained until the end of fermentation with the samples obtained from grapes treated with the IDY products resulting in the highest GSH concentrations (Table 2). The GSSG concentrations in juice before the fermentation were between 0.33 and 0.45 mg/L and the differences were not significant between treatments (Table 2). The values reported for reductive Sauvignon Blanc wine making were in the range 0.46–1.43 mg/L (du Toit et al., 2007; Kritzinger et al., 2012) indicating that the juices in this study were processed with minimum oxygen exposure. A significant ($p < 0.001$) but similar decrease in GSH concentrations for all three treatments was observed after 2 months of wine storage (Table 2). This is in accordance with the fast initial decrease in GSH concentrations observed by Herbst-Johnstone et al. (2011) in Sauvignon Blanc wines. Similar to what was seen at the end of fermentation, the IDY1 treatment resulted in significantly higher GSH concentrations compared to IDY2 and control wines that were the lowest after two months of storage (Table 2).

3.3. Amino acids before and after fermentation

Amino acids were divided into groups according to their importance in yeast metabolism during the fermentation (Ljungdahl & Daignan-Fornier, 2012). The IDY2 treatment resulted in significantly higher concentrations of aspartic and glutamic acids, hydroxyproline and proline in juice before fermentation compared to the IDY1 treatment and the untreated control (Table 3). However when considering the total concentration of yeast-preferred, branched and other amino acids, no significant differences between treatments before fermentation were noticed (Table 3). On the contrary, significant differences between treatments in the concentrations of all amino acids quantified were measured in wines at the end of fermentation (Table 3). The IDY1 treatment exhibited significantly higher concentrations of residual "yeast-preferred" and "other" amino acids in wines compared to IDY2 and the untreated control (Table 3). This is in agreement with another study where higher residual amino acid concentrations at the end of fermentation were also observed in Grenache rose wines, when supplemented, in that case, during the fermentation with glutathione-enriched IDYs (Andujar-Ortiz et al., 2012). γ-Aminobutyric acid (GABA) concentrations were ten-fold higher in the IDY1 treatment at the end of fermentation, compared to the untreated control and the IDY2 treatment. A ten-fold increase in GABA was also observed in the Grenache rose wines from the aforementioned study by Andujar-Ortiz et al. (2012). Previous work provided the hypothesis that GABA and arginine consumption during the fermentation is oxygen-dependent and that strict anaerobic conditions might therefore increase their final concentrations in wines produced reductively (Valero, Millán, Ortega, & Mauricio, 2003). If we consider the measured GSSG levels as an indicator of wine oxygen exposure (Kritzinger et al., 2012), the higher GABA and arginine concentrations in the IDY1 wines in this study cannot be attributed to more strict anaerobic conditions during the fermentation. Free amino acids and ammonium are the main source of nitrogen for yeast during fermentation (Bell & Henschke, 2005). Therefore differences in amino acid concentrations between treatments at the end of fermentation (Table 3) could be related to modified yeast metabolism or increase in yeast biomass during fermentation, either due to a different amino acids: ammonium ratio and/or possible modified nutrient yeast transport (Beltran, Novo, Rozès, Mas, & Guillamón, 2004), an interesting possibility that should be investigated further. IDYs are known to possess high levels of lipids, such as unsaturated fatty acids and sterols, which can be assimilated by yeast and incorporated into their cell membrane, improving their general stress tolerance and nutrition uptake (Pozo-Bayón et al., 2009, 2009a, 2009b). It is possible that IDY application on grapes could modify the lipid composition of grape juice and consequently of yeast cell membranes. It was reported that yeast nitrogen assimilation is dependent on yeast cell membrane composition (Beltran et al., 2004); the results obtained here also provide interesting scope for further analysis in this regard.

3.4. Wine volatile composition at the end of fermentation

Small variations in total higher alcohol acetates (HAAs) between treatments and some individual esters in wines at the end of fermentation were observed, whereas when considered as groups, no significant differences in concentration of either ethyl esters of straight-chain fatty acids (EEFAs) or ethyl esters of branched acids (EBBAs) were noticed (Table 4). At the end of fermentation, higher concentrations of some higher alcohols (1-propanol, isobutanol) and fatty acids (propionic acid, isobutyric acid, valeric acid) were found in the wines from the IDY2 treatment, compared to the untreated control or the IDY1 treatment (Table 4). Also, higher concentrations of corresponding HAAs, i.e. propyl acetate and isobutyl acetate, were measured in wines from the IDY2 treatment at the end of fermentation (Table 4). Similarly, higher concentrations of isobutryic acid and isovaleric acid resulted in higher concentration of ethyl isobutyrate and ethyl isovalerate, whereas the same was not observed for all EEFAs and EEBAs when compared to corresponding medium chain or branched fatty acids (Table 4). Even though the substrate availability is not the limiting factor for HAAs synthesis, higher alcohol

Table 2
Reduced glutathione (GSH) and oxidised glutathione (GSSG) concentrations (mg/L) before and at the end of fermentation and after 2 months of storage.

	Glutathione (GSH)				Oxidised glutathione (GSSG)			
	IDY1	IDY2	Control	*p*	IDY1	IDY2	Control	*p*
Before fermentation	51.7 ± 1.7a	52.2 ± 2.7a	40.6 ± 2.3b	**<0.001**	0.33 ± 0.07a	0.35 ± 0.09a	0.45 ± 0.06a	0.153
After fermentation	54.2 ± 1.8a	47.6 ± 1.5b	38.1 ± 2.0c	**<0.001**	0.78 ± 0.16a	0.53 ± 0.11b	0.77 ± 0.09a	**0.031**
2 months of storage	8.4 ± 0.22a	7.2 ± 0.79b	5.9 ± 0.84c	**0.002**	0.18 ± 0.02a	0.19 ± 0.09a	0.16 ± 0.04a	0.718

One-way ANOVA was used to compare data. Means followed by different letters in a row are significant at $p \leqslant 0.05$ (Fisher's LSD). All quoted uncertainty is the standard deviation of four replicates per treatment. Bold numbers indicate significant differences.
IDY1, treatment with application of IDY1 product on the grapes; IDY2 treatment with application of IDY2 product on the grapes; control, control treatment.

Table 3
Sauvignon Blanc grape juice amino acids average concentrations (mg/L) before fermentation and wine amino acids concentrations at the end of fermentation (mg/L).

Amino acids	Grape juice				End of fermentation			
	IDY1	IDY2	Control	*p*	IDY1	IDY2	Control	*p*
Yeast preferred amino acids								
Aspartic acid	13.1 ± 1.0b	15.5 ± 0.7a	12.1 ± 1.1b	**0.002**	3.21 ± 0.1a	3.11 ± 0.1a	2.37 ± 0.2b	**<0.001**
Glutamic acid	85.7 ± 2.3b	96.8 ± 6.6a	82.2 ± 3.2b	**0.003**	10.7 ± 0.3a	6.0 ± 0.3b	4.47 ± 0.2c	**<0.001**
Asparagine	5.8 ± 0.5a	6.1 ± 0.71a	6.2 ± .2.9a	0.642	33.5 ± 2.3a	20.4 ± 1.3b	11.9 ± 0.7c	**<0.001**
Serine	53.3 ± 2.9a	54.8 ± 5.8a	51.4 ± 1.5a	0.498	3.05 ± 0.14a	2.3 ± 0.1b	1.76 ± 0.1c	**<0.001**
Arginine	458 ± 75.3a	453 ± 51.7a	488 ± 45.7a	0.674	30.9 ± 3.2a	13.9 ± 1.3b	6.61 ± 0.4c	**<0.001**
Alanine	172 ± 9.0a	174 ± 17.1a	162 ± 4.4a	0.312	16.0 ± 0.64a	10.9 ± 0.4b	8.44 ± 0.4c	**<0.001**
Glutamine	135 ± 14.2a	136 ± 15.3a	128 ± 6.3a	0.651	28.7 ± 4.33a	7.4 ± 0.7b	3.99 ± 0.1c	**<0.001**
Total	923 ± 100a	935 ± 97.8a	929 ± 51.6a	0.979	126 ± 10.7a	64.2 ± 4.1b	39.5 ± 1.7c	**<0.001**
Branched amino acids								
Valine	15.5 ± 0.8a	15.7 ± 1.5a	15.7 ± 0.8a	0.925	n.d.	n.d	n.d.	
Leucine	13.0 ± 1.1a	13.4 ± 1.4a	12.9 ± 0.4a	0.855	4.02 ± 0.21b	5.0 ± 0.2a	3.4 ± 0.2c	**<0.001**
Phenylalanine	18.9 ± 1.0a	19.2 ± 1.8a	18.2 ± 0.5a	0.552	2.84 ± 0.17b	3.6 ± 0.1a	2.5 ± 0.2b	**<0.001**
Isoleucine	7.5 ± 0.3a	7.8 ± 0.6a	7.5 ± 0.2a	0.423	0.87 ± 0.05b	1.1 ± 0.0a	0.8 ± 0.1c	**<0.001**
Total	54.9 ± 3.1a	56.1 ± 5.1a	54.3 ± 1.8a	0.772	7.7 ± 0.33b	9.7 ± 0.4a	6.7 ± 0.4c	**<0.001**
Other amino acids								
Histidine	17.8 ± 4.8a	16.5 ± 2.1a	19.8 ± 3.3a	0.454	3.4 ± 0.2a	2.7 ± 0.1b	2.2 ± 0.1c	**<0.001**
Glycine	4.0 ± 0.4a	3.8 ± 0.5a	3.9 ± 0.2a	0.803	7.8 ± 0.5a	4.3 ± 0.2b	3.0 ± 0.1c	**<0.001**
Threonine	49.5 ± 2.4a	52.1 ± 4.6a	49.3 ± 1.21a	0.387	0.6 ± 0.1b	0.9 ± 0.2a	0.6 ± 0.02b	**<0.001**
γ-Aminobutyric acid	184 ± 27.2a	173 ± 24.2a	208 ± 16.9a	0.133	120 ± 11.5a	10.4 ± 0.3b	8.4 ± 1.0b	**<0.001**
Tyrosine	7.6 ± 0.6a	7.5 ± 0.7	7.2 ± 0.2a	0.504	2.5 ± 0.2a	1.9 ± 0.1b	1.4 ± 0.1c	**<0.001**
Cysteine	6.5 ± 0.6a	7.2 ± 1.5a	8.3 ± 1.2a	0.139	9.2 ± 0.8a	6.0 ± 0.3b	4.3 ± 0.4c	**<0.001**
Methionine	7.7 ± 4.5a	5.6 ± 0.6a	10.2 ± 3.5a	0.198	1.6 ± 0.1b	1.9 ± 0.0a	1.4 ± 0.05c	**<0.001**
Tryptophan	3.9 ± 0.3a	3.8 ± 0.4a	3.9 ± 0.2a	0.964	0.4 ± 0.01b	0.5 ± 0.0a	0.4 ± 0.02b	**0.002**
Lysine	5.6 ± 0.6a	6.0 ± 1.0a	5.6 ± 0.4a	0.646	3.1 ± 0.1a	3.3 ± 0.2a	2.4 ± 0.2b	**<0.001**
Hydroxyproline	2.5 ± 0.6ab	3.3 ± 0.3a	2.2 ± 0.4b	**0.038**	5.0 ± 0.1a	4.8 ± 0.2ab	4.6 ± 0.2b	**0.031**
Proline	161 ± 31.5b	218 ± 13.1a	160 ± 20.0b	**0.008**	542 ± 31.3b	593 ± 23.8a	525 ± 14.6b	**0.008**
Ornithine	3.6 ± 0.4a	3.5 ± 0.4a	3.7 ± 0.3a	0.753	24.9 ± 4.0a	7.8 ± 0.8b	3.1 ± 0.3c	**<0.001**
Total	453 ± 36a	500 ± 44a	482 ± 24a	0.221	720 ± 30a	638 ± 23b	556 ± 16c	**<0.001**

One-way ANOVA was used to compare data. Means followed by different letters in a row are significant at $p \leqslant 0.05$ (Fisher's LSD). All quoted uncertainty is the standard deviation of four replicates per treatment. Bold numbers indicate significant differences.
n.d. not detected.
IDY1, treatment with application of IDY1 product on the grapes; IDY2 treatment with application of IDY2 product on the grapes; control, control treatment.

production has been reported to influence HAAs concentrations in wine (Sumby, Grbin, & Jiranek, 2010). On the contrary, EEFAs and EEBAs production is largely substrate dependent; however higher concentrations of corresponding acids do not always result in higher concentrations of esters (Saerens et al., 2008; Sumby et al., 2010). Other esters, such as isoamyl esters and ethyl esters of odd carbon number fatty acids were not significantly altered by either of the treatments, whereas methyl fatty acid esters, hexyl and isobutyl esters were higher in the wines from the untreated control (Table 4).

3.5. Wine volatile composition after two months of storage

3.5.1. Esters after two months of storage

Methyl fatty acids, isoamyl, isobutyl, hexyl and phenylethyl esters of fatty acids were significantly altered during ageing (Table 4). A significant decrease in EEFAs was noticed during ageing irrespective of the treatments, with concentrations of longer carbon chain number EEFAs (ethyl decanoate, ethyl dodecanoate) decreasing more rapidly compared to lower carbon chain EEFAs (Table 4). It is known that the hydrolysis of EEFAs is faster with the increase in chain length (C10 and C12) due to the lower activation energies (Makhotkina & Kilmartin, 2012; Ramey & Ough, 1980). However, when comparing HAA and EEFA with the same C8 chain length, a faster decrease of octyl acetate compared to ethyl octanoate was observed (Table 4), which is in accordance with previously published work (Antalick et al., 2014; Ramey & Ough, 1980). Interestingly, the decrease in EEFAs was twofold slower in the IDY2 treatment (13.5%) compared to the untreated control (27.3%), whereas the IDY1 treatment resulted in a smaller decrease of the aforementioned esters compared to the control, but higher than observed in the IDY2 treatment (Table 4). Similarly, with the application of IDY2 only a 1.5% decrease in the HAAs was noticed, and it was not significant compared to initial concentrations (Table 4). The hydrolysis rate for HAAs was similar in the untreated control and the IDY1 treatment, i.e. 7.4% and 6.7%, respectively (Table 4).

A similar trend as for the decrease in EEFAs and HAAs concentrations was observed for EEBAs synthesis over ageing. It is known, that EEBAs are synthesised in a small proportion during the fermentation, whereas the majority of EEBAs is synthesised by esterification from the corresponding branched amino acids and ethanol during wine ageing (Antalick et al., 2014). The increase in EEBAs concentration for the untreated control was 80%, whereas the increase for IDY1 and IDY2 treatments was lower, 65% and 26% respectively (Table 4). Therefore, there is a clear positive relationship between the rates of EEFA and HAA hydrolysis and the rates of EEBA synthesis with the control treatment showing the highest rates and IDY2 the lowest. These results indicate that the IDY applications slowed down the kinetics of ester hydrolysis and synthesis.

It has already been shown that GSH could reduce ester hydrolysis in wine by inhibiting oxidation reactions whereas concentrations of fatty acids and some alcohols were not altered significantly (Andújar-Ortiz et al., 2013; Roussis, Lambropoulos, & Papadopoulou, 2005). This is however the first time that similar effects are suggested for ester synthesis in wine. According to our interpretation, it is unlikely that oxygen is directly involved in such reactions, specifically since ester synthesis and hydrolysis mainly depend on acid:ester ratios, pH and temperature (Ramey & Ough, 1980). It is likely that oxygen might be indirectly involved, impacting potential catalysts of these chemical reactions (Nikolantonaki, Chichuc, Teissedre, & Darriet, 2010). For instance

Table 4
Wine volatiles analysed after fermentation and two months after fermentation.

	Perception threshold (μg/L)	End of fermentation			2 months of storage			Time	Treatment	*T*T*
		IDY1	IDY2	Control	IDY1	IDY2	Control			
Ethyl esters of straight-chain fatty acids (μg/L)										
Ethyl butyrate	20[1]	530 ± 18d	574 ± 17a	575 ± 21a	542 ± 24cd	566 ± 13ab	589 ± 11a	0.376	**<0.001**	0.425
Ethyl hexanoate	5[1]	1733 ± 60bc	1817 ± 61ab	1857 ± 108a	1593 ± 39d	1677 ± 28cd	1639 ± 28d	**<0.001**	**0.016**	0.351
Ethyl octanoate	2[1]	1797 ± 119ab	1697 ± 91abc	1879 ± 288a	1609 ± 63cd	1569 ± 15d	1551 ± 62d	**0.001**	0.457	0.353
Ethyl decanoate	200[2]	1002 ± 84ab	861 ± 60b	1156 ± 145a	428 ± 77c	541 ± 19c	239 ± 51d	**<0.001**	0.442	**0.002**
Ethyl dodecanoate	640[3]	175 ± 17a	128 ± 15b	192 ± 63a	39 ± 6.59c	38 ± 8.52c	17 ± 5.26d	**<0.001**	0.186	**0.026**
Total		5239 ± 288a	5079 ± 216a	5553 ± 705a	4212 ± 196b	4390 ± 30b	4035 ± 112b	**<0.001**	0.907	0.070
Higher alcohol acetates (μg/L)										
Propyl acetate	–	197 ± 5de	237 ± 9a	209 ± 12c	189 ± 3b	223 ± 3d	200 ± 2cd	**0.001**	**<0.001**	0.518
Isobutyl acetate	2100[4]	315 ± 14b	339 ± 15a	307 ± 16bc	292 ± 12cd	324 ± 6ab	286 ± 6d	**0.001**	**<0.001**	0.788
Butyl acetate	1830[4]	3.44 ± 0.12a	3.54 ± 0.22a	3.07 ± 0.07bc	3.21 ± 0.08b	3.46 ± 0.81a	2.93 ± 0.07c	**0.007**	**<0.001**	0.451
Isoamyl acetate	860[4]	9746 ± 241ab	10276 ± 428a	9663 ± 1092abc	8930 ± 246c	10328 ± 119a	9135 ± 169bc	0.051	**0.002**	0.244
Hexyl acetate	670[4]	289 ± 5c	378 ± 13a	348 ± 22b	241 ± 6d	342 ± 18b	280 ± 3c	**<0.001**	**<0.001**	0.075
Octyl acetate	800[4]	4.82 ± 0.35b	4.76 ± 0.38b	6.4 ± 1.43a	3.52 ± 0.25c	4.28 ± 0.08bc	3.52 ± 033c	**<0.001**	0.060	**0.008**
Phenylethyl acetate	250[1]	752 ± 29de	864 ± 18c	946 ± 87b	814 ± 42cd	702 ± 5e	1027 ± 47a	0.736	**<0.001**	**<0.001**
Total		11307 ± 276bc	12103 ± 439a	11481 ± 1216abc	10474 ± 252e	11926 ± 93ab	10709 ± 305de	**0.011**	**<0.001**	0.431
Ethyl esters of branched acid esters (μg/L)										
Ethyl isobutyrate	15[1]	8.41 ± 0.12e	9.87 ± 0.25d	8.57 ± 1.03e	14.11 ± 0.70b	12.21 ± 0.15c	15.44 ± 0.69a	**<0.001**	**0.011**	**<0.001**
Ethyl 2-methylbutyrate	1[1]	0.58 ± 0.02d	0.69 ± 0.02d	0.62 ± 0.07d	1.02 ± 0.03b	0.81 ± 0.14c	1.17 ± 0.02a	**<0.001**	**0.002**	**<0.001**
Ethyl isovalerate	3[2]	1.36 ± 0.05e	1.54 ± 0.05d	1.41 ± 0.15de	2.29 ± 0.12b	2.05 ± 0.98c	2.63 ± 0.07a	**<0.001**	**<0.001**	**<0.001**
Ethyl phenylacetate	73[5]	0.40 ± 0.01b	0.33 ± 0.08b	0.38 ± 0.07b	0.41 ± 0.04b	0.63 ± 0.06a	0.63 ± 0.17a	**<0.001**	0.075	**0.008**
Total		10.76 ± 0.17e	12.42 ± 0.37d	11.00 ± 1.22e	17.83 ± 0.83b	15.70 ± 0.26c	19.87 ± 0.91a	**<0.001**	**0.003**	**<0.001**
Cinnamates (μg/L)										
Ethyl dihydrocinnamate	1.6[2]	0.24 ± 0.006a	0.30 ± 0.03a	0.41 ± 0.06a	0.28 ± 0.02a	0.29 ± 0.02a	0.47 ± 0.02a	0.262	0.566	0.362
Methyl fatty acid esters (μg/L)										
Methyl butyrate	–	1.04 ± 0.16abc	0.87 ± 0.05de	1.09 ± 0.08ab	0.81 ± 0.18d	0.94 ± 0.09cde	1.19 ± 0.15a	0.685	**0.002**	**0.035**
Methyl hexanoate	–	1.73 ± 0.03b	1.71±.09b	1.94 ± 0.08a	1.57 ± 0.05c	1.77 ± 0.03b	1.78 ± 0.02b	**0.003**	**<0.001**	**0.002**
Methyl octanoate	–	1.38 ± 0.09b	1.37 ± 0.06b	1.69 ± 0.28a	0.96 ± 0.05c	1.25 ± 0.02b	1.04 ± 0.04c	**<0.001**	**0.017**	**0.002**
Methyl decanoate	–	0.74 ± 0.09a	0.62 ± 0.04b	0.97 ± 0.32b	0.26 ± 0.04c	0.37 ± 0.01c	0.17 ± 0.02c	**<0.001**	0.468	**0.003**
Total		4.89 ± 0.12b	4.57 ± 0.19bc	5.70 ± 0.74a	3.61 ± 0.23d	4.34 ± 0.11c	4.18 ± 0.18c	**<0.001**	**0.002**	**0.003**
Isoamyl esters of fatty acid (μg/L)										
Isoamyl butyrate	–	0.73 ± 0.07b	0.76 ± 0.05b	0.85 ± 0.07a	0.63 ± 0.06d	0.71 ± 0.04bc	0.64 ± 0.03cd	**<0.001**	0.062	**0.032**
Isoamyl hexanoate	–	2.52 ± 0.26b	2.22 ± 0.14bc	3.21 ± 0.82a	1.55 ± 0.20d	1.86 ± 0.04bc	1.37 ± 0.10d	**<0.001**	0.312	**0.003**
Isoamyl octanoate	125[2]	9.95 ± 1.54b	8.35 ± 1.02b	13.45 ± 4.60a	2.46 ± 0.54c	3.36 ± 0.30c	1.28 ± 0.30c	**<0.001**	**0.318**	**0.008**
Total		13.20 ± 1.86a	11.33 ± 1.19a	17.51 ± 5.41a	4.64 ± 0.79b	5.93 ± 0.30b	3.29 ± 0.43b	**<0.001**	0.855	0.652
Isobutyl esters of fatty acid (μg/L)										
Isobutyl butyrate	–	0.09 ± 0.007a	0.08 ± 0.007a	0.09 ± 0.02a	0.08 ± 0.003b	0.09 ± 0.002a	0.09 ± 0.008a	0.644	0.492	0.186
Isobutyl hexanoate	–	0.25 ± 0.03b	0.25 ± 0.01b	0.30 ± 0.05a	0.17 ± 0.02c	0.22 ± 0.01b	0.15 ± 0.02c	**<0.001**	0.253	**0.002**
Isobutyl octanoate	–	0.42 ± 0.06b	0.35 ± 0.04b	0.55 ± 0.19a	0.15 ± 0.03c	0.22 ± 0.01c	0.09 ± 0.02c	**<0.001**	0.554	**0.004**
Isobutyl decanoate	–	0.08 ± 0.01b	0.06 ± 0.01b	0.90 ± 0.03b	0.15 ± 0.03a	0.18 ± 0.03a	0.05 ± 0.02b	**<0.001**	**0.001**	**<0.001**
Total		0.84 ± 0.09b	0.75 ± 0.06bc	1.04 ± 0.28a	0.56 ± 0.08cd	0.70 ± 0.04bc	0.39 ± 0.05d	**<0.001**	0.902	**0.002**
Ethyl esters of odd carbon number fatty acids (μg/L)										
Ethyl valerate	–	0.91 ± 0.13a	0.83 ± 0.07ab	0.82 ± 0.09ab	0.70 ± 0.02b	0.75 ± 0.07b	0.82 ± 0.24b	0.068	0.863	0.229
Ethyl heptanoate	–	0.20 ± 0.01b	0.24 ± 0.02a	0.26 ± 0.04a	0.59 ± 0.86a	0.22 ± 0.01a	0.20 ± 0.01a	0.460	0.555	0.375
Ethyl nonanoate	–	0.36 ± 0.04b	0.35 ± 0.02b	0.50 ± 0.13a	0.32 ± 0.04b	0.28 ± 0.003b	0.32 ± 0.16b	**0.012**	0.109	0.256
Ethyl undecanoate	–	0.08 ± 0.008b	0.07 ± 0.007b	0.11 ± 0.04a	0.01 ± 0.003c	0.03 ± 0.004c	0.02 ± 0.004c	**<0.001**	0.379	**0.009**
Total		1.55 ± 0.13a	1.48 ± 0.10a	1.69 ± 0.19a	1.63 ± 0.86a	1.28 ± 0.07a	1.35 ± 0.22a	0.329	0.551	0.545
Hexyl esters of fatty acids (μg/L)										
Hexyl butyrate	–	0.01 ± 0.002a	0.02 ± 0.001a	0.01 ± 0.007a	0.01 ± 0.001a	0.01 ± 0.001a	0.01 ± 0.001a	0.349	0.165	0.956
Hexyl hexanoate	–	0.03 ± 0.003b	0.03 ± 0.004b	0.07 ± 0.02a	0.005 ± 0.001c	0.006 ± 0.003c	0.004 ± 0.001c	**<0.001**	**0.002**	**<0.001**

(*continued on next page*)

Table 4 (*continued*)

	Perception threshold (μg/L)	End of fermentation			2 months of storage			Time	Treatment	*T* * *T*
		IDY1	IDY2	Control	IDY1	IDY2	Control			
Hexyl octanoate	–	0.05 ± 0.008b	0.05 ± 0.008bc	0.08 ± 0.03a	0.01 ± 0.002d	0.03 ± 0.02cd	0.011 ± 0.03d	**<0.001**	0.262	**0.021**
Total		0.09 ± 0.01b	0.09 ± 0.01b	0.16 ± 0.05a	0.03 ± 0.003c	0.049 ± 0.02c	0.03 ± 0.00c	**<0.001**	0.057	**0.006**
Phenylethyl esters of fatty acids (μg/L)										
Phenylethyl butyrate	–	1.67 ± 0.11d	1.90 ± 0.07c	2.38 ± 0.24a	1.83 ± 0.12cd	1.77 ± 0.05cd	2.69 ± 0.12b	0.062	**<0.001**	**0.017**
Phenylethyl hexanoate	–	4.54 ± 0.24b	3.51 ± 0.13c	5.57 ± 1.07a	1.70 ± 0.13d	1.54 ± 0.03d	1.54 ± 0.15d	**<0.001**	**0.002**	**0.001**
Phenylethyl octanoate	–	4.70 ± 0.43b	3.61 ± 0.68b	8.04 ± 2.95a	0.16 ± 0.02c	0.20 ± 0.07c	0.13 ± 0.04c	**<0.001**	**0.007**	**0.006**
Total		10.91 ± 0.69a	9.02 ± 0.81a	16.00 ± 3.96a	3.69 ± 0.24b	3.52 ± 0.05b	4.36 ± 0.28b	**<0.001**	0.724	0.937
Miscellaneous esters										
Ethyl propionate (μg/L)	2100[4]	63.83 ± 2.86d	79.09 ± 3.52bc	79.71 ± 3.52bc	77.40 ± 2.50c	82 ± 3.019b	93 ± 1.87a	**<0.001**	**<0.001**	**0.002**
Ethyl trans-2-butenoate (μg/L)	–	8.54 ± 0.31b	8.46 ± 0.38b	7.37 ± 0.33c	8.98 ± 0.37a	8.43 ± 0.03b	8.21 ± 0.16b	**0.002**	**<0.001**	**0.024**
Ethyl trans-2-hexenoate (μg/L)	–	1.45 ± 0.05c	2.69 ± 0.15a	2.17 ± 0.10b	1.41 ± 0.05c	2.78 ± 0.56a	2.08 ± 0.04b	0.891	**<0.001**	0.726
Ethyl acetate (mg/L)	12270[6]	81.5 ± 1.6de	90.7 ± 3.7a	85.9 ± 1.9b	80.2 ± 1.2d	85.3 ± 2.6bc	83.1 ± 3.2bcd	**0.005**	**<0.001**	0.319
Ethyl lactate (mg/L)	157810[6]	14.5 ± 0.1c	17.05 ± 0.5a	16.2 ± 0.1a	14.9 ± 0.2bc	16.1 ± 2.1ab	16.9 ± 0.3a	0.925	**<0.001**	0.174
Diethyl succinate (mg/L)	200000[6]	0.32 ± 0.01a	0.32 ± 0.002a	0.33 ± 0.01a	0.32 ± 0.004a	0.33 ± 0.003a	0.33 ± 0.02a	0.514	0.199	0.484
Higher alcohols (mg/L)										
Methanol	–	70.6 ± 1.7a	71.9 ± 0.86a	71.8 ± 0.9a	69.4 ± 2.6a	70.4 ± 1.1a	73.4 ± 7.6a	0.776	0.339	0.611
Propanol	–	56.0 ± 1.2b	62.8 ± 2.49a	52.6 ± 1.6b	55.0 ± 1.0bc	60.8 ± 2.3a	54.0 ± 1.5bc	0.497	**<0.001**	0.178
Isobutanol	40000[2]	19.7 ± 0.6bc	21.1 ± 0.95a	19.9 ± 0.2bc	19.5 ± 0.2c	20.8 ± 0.5a	20.5 ± 05ab	0.792	**<0.001**	0.231
Butanol	150000[6]	1.76 ± 0.02a	1.80 ± 0.05a	1.63 ± 0.02b	1.50 ± 0.45a	1.76 ± 0.08a	1.64 ± 0.03a	0.316	0.283	0.449
Isoamyl alcohol	30000[1]	151 ± 4b	166 ± 9a	161 ± 3a	153 ± 1b	165 ± 3a	168 ± 6a	0.285	**<0.001**	0.379
Hexanol	8000[1]	0.60 ± 0.003a	0.60 ± 0.001a	0.60 ± 0.0003a	0.60 ± 0.001a	0.66 ± 0.13a	0.60 ± 0.0002a	0.348	0.379	0.394
3-Ethoxy-1-propanol	–	8.3 ± 0.32a	6.8 ± 0.31b	5.6 ± 0.17c	8.1 ± 0.2a	6.6 ± 0.18b	5.58 ± 0.18c	0.170	**<0.001**	0.654
2-Phenylethanol	10000[1]	13.0 ± 0.18c	16.4 ± 0.69b	18.6 ± 0.79a	13.0 ± 0.31c	15.8 ± 0.5b	18.9 ± 1.0a	0.690	**<0.001**	0.353
Carboxylic acids (mg/L)										
Propionic acid	–	1.92 ± 0.05b	2.39 ± 0.25a	2.17 ± 0.26ab	1.90 ± 0.11b	1.97 ± 0.06b	2.29 ± 0.28a	0.190	**0.011**	**0.034**
Isobutyric acid	2300[2]	1.28 ± 0.02b	1.43 ± 0.06a	1.33 ± 0.04b	1.29 ± 0.03b	1.33 ± 0.03b	1.36 ± 0.10ab	0.316	**0.009**	0.066
Isovaleric acid	33.4[2]	0.96 ± 0.02c	1.04 ± 0.04a	1.03 ± 0.03ab	0.98 ± 0.04c	0.99 ± 0.01bc	1.05 ± 0.0a	0.766	**0.002**	0.077
Valeric acid	–	0.55 ± 0.01ab	0.57 ± 0.05a	0.53 ± 0.02bc	0.52 ± 0.02c	0.55 ± 0.01ab	0.52 ± 0.01c	**<0.001**	**<0.001**	0.416
Hexanoic acid	3000[1]	5.57 ± 0.22c	5.97 ± 0.31b	6.39 ± 0.46a	5.52 ± 0.05c	5.7 ± 0.06bc	6.5 ± 0.20a	0.487	**<0.001**	0.469
Octanoic acid	500[2]	8.4 ± 0.15b	9.1 ± 0.59b	9.14 ± 0.25b	8.8 ± 0.19bc	8.8 ± 0.05bc	9.8 ± 0.45a	0.076	**<0.001**	**0.028**
Decanoic acid	1000[2]	2.91 ± 0.07a	2.77 ± 0.24a	2.93 ± 0.46a	3.01 ± 0.19a	2.78 ± 0.04a	2.79 ± 0.08b	0.930	0.298	0.565
Other compounds (mg/L)										
Acetoin	152600[6]	5.31 ± 0.13a	5.07 ± 0.07b	5.06 ± 0.13b	5.3 ± 0.1a	4.99 ± 0.14bc	4.87 ± 0.07c	0.115	**<0.001**	0.216
Acetic acid	200000[1]	317 ± 13a	251 ± 11b	220 ± 8c	322 ± 11a	244 ± 7b	226 ± 12c	0.775	**<0.001**	0.413
Pyrazine (ng/L)										
3-Isobutyl-2-methoxypyrazine	0.002[7]	n.a.	n.a.	n.a.	3.46 ± 0.48a	3.40 ± 0.62 a	4.01 ± 0.62a	0.305	n.a	n.a
Thiols (ng/L)										
3-Sulfanylhexan-1-ol	0.060[8]	n.a.	n.a.	n.a.	673.8 ± 31.6c	969.7 ± 43.0a	747.1 ± 17.0b	**<0.001**	n.a	n.a
3-Sulfanylhexyl acetate	0.004[9]	n.a.	n.a.	n.a.	99.0 ± 10.2b	133.3 ± 21.3a	97.9 ± 7.6b	**0.004**	n.a	n.a

Two-way ANOVA was used to compare data. Means followed by different letters in a row are significant at $p \leqslant 0.05$ (Fisher's LSD). All quoted uncertainty is the standard deviation of four replicates per treatment. Bold numbers indicate significant differences.
n.a. data not available.
T * *T* interaction treatment * time.
IDY1, treatment with application of IDY1 product on the grapes; IDY2 treatment with application of IDY2 product on the grapes; Control, control treatment.

[1] (Guth, 1997), odour thresholds reported were determined in 10% w/w aqueous ethanol.
[2] (Ferreira, López, & Cacho, 2000), odour thresholds reported were determined in 11% v/v ethanol, 7 g/L glycerine, 5 g/L tartaric acid and pH 3.4.
[3] (Salo, 1970), odour thresholds reported were determined in 9.5% w/w ethanol.
[4] (Pineau, Barbe, Van Leeuwen, & Dubourdieu, 2009), odour thresholds reported were determined in dearomatised red wine.
[5] (Tat, Comuzzo, Battistutta, & Zironi, 2007), odour thresholds reported were determined in red wine.
[6] (Etievant, 1991), odour thresholds reported were determined in 12% w/w aqueous ethanol.
[7] (Allen, Lacey, Harris, & Brown, 1991), odour thresholds reported were determined in white wine.
[8] (Tominaga, Furrer, Henry, & Dubourdieu, 1998), odour thresholds reported were determined in 12% ethanol, 5 g/L tartaric acid and pH 3.2.
[9] (Tominaga, Darriet, & Dubourdieu, 1996), odour thresholds reported were determined in 12% ethanol, 5 g/L tartaric acid and pH 3.2.

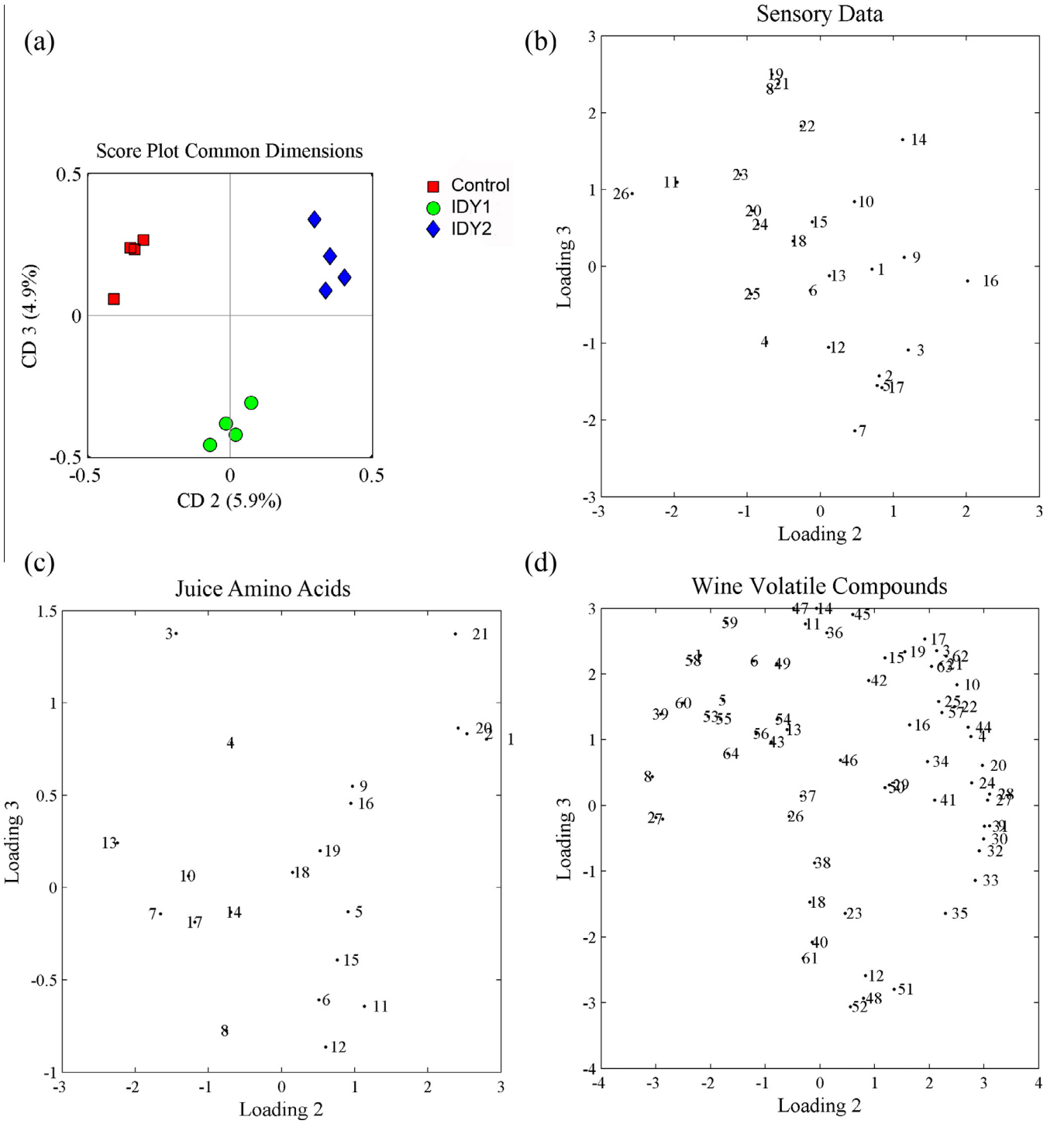

Fig. 1. Common components and specific weights analysis scores and loadings plots of the three data blocks: (a) scores of common dimensions 2 and 3 for samples with application of IDY1 and IDY2 in the vineyard and control treatment (b) loadings plot for data block 1 Sensory scores; (c) loadings plot for data block 2 Juice amino acids composition; (d) loadings plot for data block 3 Wine volatile composition. The numbered sensory attributes, juice amino acids and wine volatile chemical attributes are listed in Table 1.

some metal ions, such as iron or copper, are known to be involved in reactions leading to the oxidation of certain compounds in wine (Clark, Prenzler, & Scollary, 2007; Danilewicz, 2013; Nikolantonaki et al., 2010). Such reactions have not yet been reported to be involved in ester hydrolysis and synthesis in wine conditions. Metal oxides are however known to catalyse esterification and ester hydrolysis reactions in soybean oil and organic pollutants (Mello, Pousa, Pereira, Dias, & Suarez, 2011; Stone, 1991). As mentioned before, GSH is an important antioxidant present in grapes, must and wines, scavenging through its SH group for free *o*-quinones produced by the oxidation of caftaric acid and other polyphenols (Kritzinger et al., 2012). Many sulphur-containing compounds such as *N*-cysteineglycine and amino acids, i.e. cysteine and methionine, and other amino acids, such as tryptophan, tyrosine and phenylalanine, have been reported to have radical scavenging properties (Hernández-Ledesma, Dávalos, Bartolomé,

& Amigo, 2005; Rodríguez-Bencomo et al., 2014). Other compounds, such as small peptides containing methionine, tryptophan and tyrosine, which probably originate from IDYs, could also be involved in the preservation of some aromatic compounds as suggested (Rodríguez-Bencomo et al., 2014). Therefore, the presence of compounds with antioxidant activities in higher concentrations in IDY1 and IDY2 wines at the end of alcoholic fermentation, and after two months of storage could reduce the impact of oxygen on potential catalysts of EEFA and HAA hydrolysis and EEBA synthesis (Tables 2–4). This could positively contribute towards the slower loss of wine fruity aroma imparted by EEFAs and HAAs during ageing. Indeed, these groups of esters are known to impact much more on the aroma of young white wines than EEBAs (Antalick et al., 2014).

3.5.2. Thiols and 3-isobutyl-2-methoxypyrazine after two months of ageing

Thiols were only measured in wines after 2 months of ageing and substantial differences between treatments were noticed (Table 4). The IDY2 treatment resulted in significantly higher concentrations of 3SH and 3SHA in the corresponding wines (Table 4). Previous studies have reported contradicting data on the effect of GSH on the release of volatile thiols (Makhotkina et al., 2014; Patel et al., 2010; Roland, Vialaret, Razungles, Rigou, & Schneider, 2010). In the current study, thiol concentrations did not increase in the presence of higher GSH concentrations (in the case of the IDY1 treatment); also the rate of juice oxidation could not explain the variations observed, as the GSSG levels were similar for all three treatments. Higher 3SH release rate from its non-odorous precursors in the IDY2 treatment could be attributed to a higher concentration of glutamic acid as suggested by Pinu et al. (2014). However complex interactions between different amino acids and carboxylic acids have been shown to influence the release of 3SH during fermentation (Pinu et al., 2014). Higher 3SHA concentrations were found in wines from the IDY2 treatment, but the exact mechanism cannot be explained with our data. It could be either the result of a higher rate of 3SHA esterification from 3SH during fermentation, or a slower hydrolysis as observed for other HAAs in the IDY2 treatment. As expected, application of IDY1 and IDY2 had no effect on the grape-derived aromatic compounds such as IBMP (Table 4).

3.6. Wine sensory results and correlation with wine chemical composition

From the HCA graph it can be concluded that wines from the untreated control were clearly distinguished from the IDY1 treatment, whereas the separation between wines from IDY1 and IDY2 treatment and IDY2 and control was not so clear (Fig. S1). However, wines with application of IDYs resulted in higher perception of fruitier attributes, whereas control wines were associated with greener sensory descriptors (Fig. S2).

To correlate wine chemical composition with wine sensory results CCSWA was performed on GC–MS and GC–FID data obtained from the wines two months after bottling together with the grape amino acids analyses performed before fermentation and wine sensory results. The CCSWA define the common space and block weighting for the relative importance of multiple blocks of data in the same sample set for each common dimension (CD). The importance of each data block for each extracted CD is shown in Fig. S3. For the CD1 grape amino acids contributed the majority of variation (84.6%) to the distribution of the treatments. For the CD 2 and 3 wine sensory results and wine volatiles contributed to a higher proportion of variation (Fig. S3), accounting for ~11% of data variance that was able to clearly separate the treatments based on the abovementioned parameters (Fig. 1A). Separation of treatments based on other CDs is shown in Fig. S4. For clarity purposes a number was assigned to each measured chemical and sensory attribute (Table 1). Interestingly, control wines were associated with canned beans, cat urine and lime aromatic attributes together with IBMP (Fig. 1B and D). The wines from the IDY1 and IDY2 treatments were found in the positive dimension of CD1 and were associated with fruitier descriptors (Fig. 1A). The IDY1 wines were found in the dimension of sensory attributes such as gooseberry, apricot, banana lolly and pineapple, whereas wines from IDY2 treatment were described as having peach, citrus, apple, lemon and tropical characters (Fig. 1B). HAAs and thiols, which are known contributors to tropical fruit and fruity (pear, banana lolly) characters of white wines, were found in the positive dimension of the IDY2 treatment (Fig. 1D). Andújar-Ortiz et al. (2013) reported that Grenache rose wines supplemented with glutathione enriched IDY during the fermentation, not as in our study by application to the grapes in the vineyard, were described with higher banana and strawberry flavours after nine months of bottle ageing. Similarly, Verdejo wines with commercial IDY supplementation during the fermentation were judged as having better olfactory and mouthfeel properties after six months of ageing in the bottle (Del Barrio-Galán et al., 2011).

The present study evaluated the effect of two IDY products on fruit ripening, juice composition and wine chemical and sensory properties, including an analysis of the wines after short-term bottle ageing. The IDY products utilised here were applied already in the vineyard on grapes during early stages of ripening. The results confirmed that antioxidants, most notably GSH, as well as certain amino acids and compounds contributing to Sauvignon Blanc typical sensory expressions, such as thiols and esters were elevated in the juices and wines from the treatments. Interestingly, the data suggest that certain classes of volatiles are "protected", either by a reduction of their hydrolysis, and/or a change in their synthesis. The observed modifications in wine chemical and sensory composition between treatments and control wines could be attributed to the (i) alteration of vine/grape metabolic pathways (ii) modification of grape microflora and (iii) direct contribution of IDYs applied on grapes (by solubilisation during the grape crushing and pressing) to the grape juice. This study provides evidence that the specific IDY products used in this study, applied directly on grapes early in the ripening process, benefitted the production of aromatic wines and the study also provides a starting point to evaluate the mechanisms involved.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2015.11.105.

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