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The effect of grape juice dilution and complex nutrient addition on oenological fermentation and wine chemical composition

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The effect of grape juice dilution on fermentation

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Abstract

The impact of water addition and complex nutrient addition to grape juice in laboratory scale winemaking, on both alcoholic and malolactic fermentation duration and outcome has been examined using commercial wine yeasts, Lalvin EC1118™ and Lalvin R2™ and malolactic bacteria Lalvin VP41™. As expected, dilution with water did not impede fermentation, instead resulted in shortened duration, or in the case of malolactic fermentation enabled completion in these conditions. Addition of complex organic nutrient further shortened alcoholic fermentation by Lalvin R2™ and in some conditions also reduced the duration of malolactic fermentation. In general, compounds contributing to wine aroma and flavour were present at lower concentrations at the end of fermentation where juices were diluted and the addition of organic complex nutrient also influenced the concentration of some compound[illegible] wine. These findings are significant to commercial winemaking, highlighting that winemakers should consider potential impacts of juice dilution on processing efficiencies along with wine flavour and aroma.

Keywords: *fermentation, wine, grape juice dilution, yeast, nutrient, volatile compounds*

1. Introduction

The addition of water to grape juice before fermentation is legal and commonplace in some countries, including more recently, Australia. The wording of legislature around water additions varies between countries, for instance additions (noted as ameliorations) are allowed “to facilitate fermentation” (USA, Federal Regulation 27 CFR 24.178 and California State Law, Provisions

Applicable to Wine Produced in California 17 CCR 17010) or in Australia " water may only be added to wine pre-fermentation and does not dilute the must below 13.5 °Bé" (Standard 4.5.1 Section 5, 7C) or "oenological practices shall exclude the addition of water, except where required on account of a specific technical necessity" (European Union, Council Regulation (EC) 491/2009 Annex XVb A1). Fermentation difficulties can be encountered in high sugar musts where yeasts can fail to ferment all available sugars, leading to wines that are out of winemaker specifications. High-sugar musts are becoming increasingly common since elevated daily average temperatures during ripening (Schultz, 2016) can lead to accelerated and uneven phenological development of grapes, resulting in vintage compaction and delay of harvest by winemakers waiting for "flavour ripeness". Market limitations also exist for higher alcohol wines, where higher taxes are incurred and some consumers are increasingly avoiding these in favour of wines with lower alcohol, suiting some modern flavour and healthier lifestyle choices. The addition of water to must pre-fermentation to combat the problems associated with high sugar musts is a simple and inexpensive procedure for which the practical logistics have been discussed (Cowey, 2017). Previous studies have examined the effect of juice dilution with water on final concentrations and sensory impacts of various compounds in wine (Harbertson, Mireles, Harwood, Weller, & Ross, 2009; Petrie, Teng, Smith, & Bindon, 2019; Schelezki, Antalick, Šuklje, & Jeffery, 2020; Schelezki, Smith, Hranilovic, Bindon, & Jeffery, 2018; Schelezki, Suklje, Boss, & Jeffery, 2018; Teng, Petrie, Smith, & Bindon, 2020). Authors report variable effects on wine, from an overall decrease in wine volatiles (Schelezki et al., 2020) to an increase to specific compounds such as total higher alcohols and ethyl esters of fatty acids (Schelezki, Suklje, et al., 2018), and minor to measurable decreases in colour, tannin and phenolics (Schelezki, Smith, et al., 2018; Teng et al., 2020). Some wines were also reported as generally not being reduced in sensorial complexity, in fact some dilutions maintain many of the fuller bodied and richer flavours of wine from undiluted juices (Petrie et al., 2019). In terms of grape derived compounds, this presumably reflects that changing the juice to solid ratio, or indeed simply providing more water, enables increased extraction. The variability in the reported effect of dilution inevitably arises due the complexities of variety, vintage and experimental differences (Schelezki et al., 2020). Overall, authors suggest that impacts on wine of juice dilution pre-fermentation are surprisingly minor. Similarly it is hypothesised that these additions will not impede alcoholic and malolactic fermentation, however research has yet to specifically address this. We hypothesise that the addition of water to juice pre-fermentation will shorten fermentation time and effect the final concentration of some compounds in the final wine in proportion to the dilution. To test this, we analysed fermentation dynamics of two commercial wine yeast; Lalvin EC1118^{TM} (aromatically neutral) and Lalvin R2^{TM} (aromatic) over a range of juice dilutions (commercially relevant; 16 Bé juice diluted to 14.5, 13.5 and an extreme example 12.5 Bé, corresponding to water additions of 10.5, 17.2 and 24%). Pressed juice from white grapes was chosen so as to avoid the complexities introduced when winemaking with skin contact is undertaken. Furthermore, studies addressing the impact of juice dilution on extraction of colour and phenolics in red wines fermented in the presence of skins have been recently reported (Schelezki et al., 2020; Teng et al., 2020). Nitrogen was ameliorated to be equal across all juices, including those that were diluted, to ensure this was not impacting fermentation dynamics. Yeast nutrient (FERMAID$^{®}$ O, an organic complex nutrient; Lallemand) was also added to examine if any negative effects (potentially due to the dilution of micronutrients), could be reduced. It is well known that many compounds (volatile and non-volatile) in wine change with the addition of nitrogenous compounds (Bell & Henschke, 2005; Torrea, Fraile, Garde, & Ancin, 2003), thus inclusion of this analysis allowed us to examine the combined effects of dilution and nutrient supplementation.

2. Materials and methods

2.1 Strains and media

Saccharomyces cerevisiae strains Lalvin EC1118^{TM} and Lalvin R2^{TM} (Lallemand, Canada) were chosen due to their common commercial use, variation in nutrient requirements and contribution to wine aroma (Lavin EC1118^{TM} regarded as neutral and Lalvin R2^{TM} as aromatic). Yeast and bacteria were grown using methods that applicable to high throughput studies. Yeast were grown in 250 mL conical shake flasks from single colonies in 100 mL Yeast Peptone Dextrose (YPD) medium (1% w/v yeast extract, 2% w/v bacteriological peptone, and 2% w/v glucose) overnight at 28 °C with shaking (120 rpm). These cells were then inoculated at 2.5 x 10^6 cells mL^{-1} in 250 mL of diluted juice (45%, sterile (0.22 µm), 2018 Viognier-Marsanne blend, 10% YPD, 45% water) in 1 L conical shake flasks and grown overnight. This culture was used to inoculate experimental fermentations.

Oenococcus oeni malolactic bacteria strain Lalvin VP41™ (Lallemand) was chosen as it is widely used in the Australian wine industry. Lalvin VP41™ (2.5g) was rehydrated from a commercial packet, according to the manufacturer's instructions and grown in 50 mL of MRSAJ (de Man-Rogosa-Sharp medium; Amyl Media) in a 50 mL screw capped tube supplemented with 20% ($^v/_v$) apple juice (Golden Circle®) and 0.1% cyclohexamide ($^v/_v$, Sigma Aldrich) for 4 days at 30 °C and 20% CO_2. The *O. oeni* culture was centrifuged (10 min, 4600 x *g*), and the pellet was washed in phosphate buffered saline (PBS: 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, pH 7.4) and re-centrifuged. The cell pellet was washed with 50 mL Viognier-Marsanne juice (13.5 Bé, 0.22 µm), centrifuged (2 min, 4600 x *g*) and resuspended in 50 mL Viognier-Marsanne juice. This *O. oeni* culture was grown overnight at 30 °C and 20% CO_2 prior to inoculation at 1:100 (0.25 mL) in the experimental fermentations. Culture viability was analysed using spot plating (10 µL of serially diluted cultures were grown on MRSAJ with 2% agar, and colonies enumerated post growth).

A filter sterile (0.22 µm) 2018 Adelaide Hills Viognier-Marsanne (~75% Viognier) blended juice was used for experimental fermentations (16 Bé, pH 3.2, 3.13 g L^{-1} malic acid, 18 : 63 mg L^{-1} free:total SO_2, 60 mg L^{-1} ammonia, 196 mg L^{-1} alpha amino nitrogen, 245 mg L^{-1} yeast assimilable nitrogen (YAN)). Juices were diluted with ultrapure water (pH 7.0) to 14.5, 13.5 and 12.5 Bé (10.5, 17.2 and 24% water). Density after addition of water was calculated instead of measured as, based on experience, measurement at that stage is inaccurate. These dilutions were chosen as they encompass both typical and extreme (12.5 Bé) in industry. pH was measured post water addition and found to have limited change (<0.05). Nitrogen was also adjusted in diluted juices to 245 mg YAN L^{-1} with addition of diammonium phosphate and complex organic nutrient (FERMAID® O, Lallemand) where indicated at either 0 (control), 200 or 400 mg L^{-1}.

2.2 Experimental fermentation

Fermentations were conducted with a custom-made high throughput robotic platform 'Tee-bot v.2.0' built on an EVO freedom workdeck (Tecan, Männedorf, Switzerland) that can accommodate 384 discreet fermentations and sample automatically at programmable intervals. Fermentation vessels had custom-made airlocks that allowed sampling through a silicone septum. Experimental fermentations were mixed at inoculation and briefly before sampling with magnetic stir bars. Viognier-Marsanne juice was pressed from solids to alleviate the complexity of changing the juice:solid ratio by dilution in experimental fermentations. Also, typically white wine fermentation is undertaken without the presence of grape skins. Temperature was regulated to 17 °C by a water bath that housed the fermentation flasks. Juice (25 mL, ameliorated with dilution or addition of FERMAID® O) was inoculated with 5 x 10^{6} yeast cells mL^{-1} (either EC1118™ or Lalvin R2™). After 24 hours, experimental fermentations to be treated with malolactic acid bacteria (MLB+) were inoculated with 0.25 mL of cultured Lalvin VP41™. This method of co-inoculation was chosen instead of sequential inoculation for malolactic fermentation as it is fast becoming a common industrial method, particularly in high sugar musts, and also reduces the risk of excess oxidation of wines due to extended fermentation times, particularly important here due to small experimental volumes. Control (MLB-) fermentations had 0.25 mL sterile Viognier-Marsanne juice added. Fermentations were performed in triplicate. This combination of treatments of yeast (Lalvin EC1118™, Lalvin R2™ or none), dilution (16, 14.5, 13.5 or 12.5 Bé), nutrient addition (0, 200 or 400 mg L^{-1}) and MLB (+/-) resulted in 216 unique fermentations. During fermentation, sugars (glucose, fructose) and malic acid consumption were monitored using commercial enzymatic kits (Megazyme, Bray, Ireland) with some modifications as described in (Walker et al., 2014; Jiang, Sumby, Sundstrom, Grbin, & Jiranek, 2018).

2.3 Determination of wine composition by HPLC and GC-MS.

Major yeast metabolites, organic acids (malic, succinic, acetic), glucose, fructose, glycerol and ethanol were determined from 1 mL filter sterilised terminal fermentation samples by HPLC according to Lin, Boss, Walker, Sumby, Grbin, & Jiranek (2020). In brief, undiluted samples were injected onto an Aminex H7C-8H column (300 × 7.8 mm, Bio-Rad) on an Agilent 1100 series HPLC system (Agilent Technologies). The column temperature was 60 °C and the mobile phase was a 2.5 mM solution of H_2SO_4, at a flow rate of 0.5 mL min^{-1}. Signals were detected at 210 nm with an Agilent G1315B diode array and an Agilent G1362A refractive index detector. Compounds were identified by their retention time and quantified by comparison to known standard solutions using Agilent ChemStation software. Quantification was performed using calibration curves ($R^2 > 0.99$) relating to the concentration of analytes from standard solutions.

Terminal fermentation samples after alcoholic fermentation of MLB- wines were also analysed by solid phase micro-extraction (SPME)-GC/MS exactly according to Hranilovic et al. (2018) with extraction and chromatographic condition as outlined by Boss, Pearce, Zhao, Nicholson, Dennis, & Jeffery (2015). MLB+ wines were not analysed since MLF was not completed in all samples. In brief, each sample was diluted with water (1 : 2 and 1 : 100) to a final volume of 10 mL, with the addition of 3 g of sodium chloride and then spiked with five standards: d13- hexanol (920 mg L^{-1} for 1 : 2 dilution, 92 mg L^{-1} for 1 : 100 dilution; C/D/N Isotopes, Pointe- Claire, QC, Canada); d11- hexanoic acid (930 mg L^{-1} for 1 : 2 dilution, 93 μg L^{-1} for 1 : 100 dilution; C/D/N Isotopes); d16- octanal (82.1 mg L^{-1} for 1 : 2 dilution, 8.21 mg L^{-1} for 1 : 100 dilution; C/D/N Isotopes); d3- hexyl acetate- (17.5 mg for 1:2 dilution, 1.75 mg L^{-1} for 1 : 100 dilution; C/D/N Isotopes); d3- linalool (1.73 mg L^{-1} for 1 : 2 dilution and 0.17 mg L^{-1} for 1 : 100 dilution, C/D/N Isotopes). Volatile compounds were then identified by comparing mass spectra with those of authentic standards and spectral libraries. A laboratory generated library (328 compounds) as well as the US National Institute of Standards and Technology- 11 and the Wiley Registry 9th edition mass spectral libraries were used for identification purposes. Compounds were considered positively identified after matching of both mass spectra and linear retention indices (LRI) with that of authentic samples. LRI was calculated from a compounds retention time relative to the retention of a series of n-alkanes (C8-C26). Quantification of target compounds was achieved by relating ion peak areas with that of the relevant internal standard using MassHunter Version B.08.00 (Agilent Technologies). Calibration curves of respective analytes were used to determine concentration of all volatiles. Target compounds were chosen that are well known to impact wine aroma. Samples of un-inoculated juices, subjected to the same experimental conditions were also analysed to decipher between juice and fermentation related volatiles. The concentrations of volatiles measured in unfermented juices were very low or below detection (data not shown).

2.4 Data analysis

Statistical analysis was undertaken with GraphPad Prism v 7.02 (GraphPad Software, La Jolla, CA, USA) and XLSTAT (Addinsoft, New York, NY, USA). Data is reported as the mean values with standard deviation and one way analysis of variance (ANOVA) with Fisher's least significant difference (LSD) multiple comparison test ($p < 0.05$) to determine statistical significance. Principal component analysis (PCA) was conducted using The Unscrambler X v10.1 (CAMO Software, Oslo, Norway). Input variables for the PCA were the volatile compound concentrations measured in each wine sample which were scaled to unit variance. The NIPALS algorithm was used for the PCA with cross validation to test the model.

3. Results and Discussion

3.1 Dilution and addition of nutrients reduced alcoholic and malolactic fermentation duration

Both alcoholic (AF) and malolactic fermentation (MLF) duration were shortened in diluted juices, as is expected where initial sugar concentrations were reduced (AF, Fig. 1 and 2; MF, data not shown). For instance, AF of juice diluted from 16 Bé to 12.5 Bé was reduced in duration with the use of Lalvin EC1118^{TM} by 126 hours, or 42% (298 vs 172 h). AF of undiluted juices by Lalvin

R2™ was slightly longer (in comparison to Lalvin EC1118™), however, dilution resulted in a similar reduction of fermentation duration of 121 hours or 34% (355 v 234 h). Dilution to 13.5 Bé or below also allowed the completion of MLF (<0.1 g L^{-1} of malic acid) within 45 days in wines fermented by either yeast, whereas MLF of higher Baumé juices failed to complete in this time frame, leaving residual malic acid (>1.0 g L^{-1}) (Supp. Tab. 1). The very slow MLF in these conditions is thought to be particular to this juice, perhaps due to limitation of a micronutrient or presence of an inhibitor such as ethanol.

The effect on fermentation dynamics of diluted juices with nutrient addition was also evaluated, this was in an effort to alleviate effects, if any, of micronutrient dilution. Additions were made at an industry standard rate, as recommended by the manufacturer (200 mg L^{-1}) and also at a higher rate (400 mg L^{-1}). The addition of nutrient had little to no impact on AF duration by Lalvin EC1118™ in any juice (Supplementary Table 1A), however, fermentations by Lalvin R2 ™, were up to 76 hours (26%) shorter, for instance in 13.5 Bé juice (Fig. 3). Surprisingly, increasing the dose of nutrient addition (from 200 to 400 mg L^{-1}) did not result in consistent differences in alcoholic fermentation, except in a single condition with13.5 Bé juice (Fig. 3, Supplementary Table 1A). For instance, addition of 400 mg L^{-1} of nutrient to 13.5 Bé juices with Lalvin R2 ™ reduced fermentation by 76 hours in comparison to 52 hours when only 200mg L^{-1} of nutrient was added and malolactic acid bacteria (MLB) were present. The presence of MLB also had little to no effect on AF duration (Fig. 3, Supplementary Table 1A).

The addition of nutrient shortened MLF in some instances. For example, juice diluted to 13.5 Bé with 200 or 400 mg L^{-1} of nutrient and with AF undertaken by Lalvin EC1118™ completed MLF in 47 hours less (4.7% reduction) than when no nutrient was added (Supplementary Table 1B).

3.2 Dilution and nutrient addition changed the chemical composition of wines

The effect of juice dilution on volatile compounds and major yeast metabolites of the resulting wines that were not inoculated for malolactic fermentation was examined. This wine set was chosen since malolactic fermentation was not complete in the complete set of wines inoculated with lactic acid bacteria. Similar to other studies (Schelezki et al., 2020; Schelezki, Suklje, et al., 2018), the dilution of juices modified the final concentrations of many compounds. Of the 38 volatile compounds analysed, 22 were significantly different to the control in at least three treatments (Table 1) with 19 influenced by dilution and 17 by nutrient addition. One treatment (juice diluted to 13.5 Bé and fermented with Lalvin R2) altered the abundance of 15 volatile compounds. In almost all cases, dilution of juices reduced the concentration of volatiles, presumably by dilution of juice-derived precursors that arise from major metabolic pathways such as glycolysis. This outcome is similar to that seen by (Schelezki et al., 2020) where many compounds were reported to decrease upon addition of water to Shiraz juice prior to fermentation.

2-Phenylethanol, described as contributing aromas of rose, honey and spice, was detected in all fermentations above its aroma threshold of 14 mg L^{-1} (Ferreira, Lopez, & Cacho, 2000), and with almost all dilutions was significantly reduced (70 - 49% or -1.44 to -2.02 Fold change (FC)). Furthermore, its ester, 2-phenylethyl acetate was also reduced with dilution (62 – 19% or -1.62 to -5.31 FC). In undiluted fermentations, isoamyl alcohol and, in fermentations conducted with Lalvin R2^{TM}, its ester, isoamyl acetate were also detected above their aroma thresholds. Isoamyl acetate could be reduced to below its aroma threshold with any of the trialled dilutions (Fig. 4). Typically, isoamyl acetate was reduced to around 20% of the control (6 ±3 to 13 ± 9 vs 56 ± 3 μg L^{-1}, Supp. Tab. 2). This ester can contribute an overpowering banana aroma, especially in white wines, and as such, a dilution strategy (where grapes have an elevated Bé) may represent an option for desirable flavour modification during winemaking.

Acetic acid was also reduced in diluted fermentations conducted with Lalvin R2^{TM}, for instance, 3.57 mg L^{-1} was measured in wines using undiluted juice, whilst dilution to 13.5 Bé dramatically reduced acetic acid concentrations to 0.8 mg L^{-1}. Even though the aroma threshold of acetic acid is 200 mg L^{-1} (Ferreira et al., 2000), given that this compound is one of the most common faults in wine, even at low concentrations this small difference could be of industrial relevance. The measured values of acetic acid here are also quite low in comparison to what is routinely found in finished wines (200 – 600 mg L^{-1}), which is likely due to the sterility of juice used and terminal sample collection directly after completion of primary fermentation. The primary alcohols 3 and 4-methyl-1-pentanol were significantly reduced in almost all diluted juices. For instance, with the use of Lalvin EC1118™, 3-methyl-1-pentanol was reduced to 31% (-3.23 FC) of the control at the most extreme dilution (12.5 Bé). Aromatically, 3-methyl-1-pentanol is described as contributing earthy and green notes, whereas for 4-methyl-1-pentanol its main descriptor is 'nutty', however their aroma detection thresholds are near 500 and 50,000 µg L^{-1}, respectively (Moreno, Zea, Moyano, & Medina, 2005), many magnitudes above that detected here. If considering these compounds individually we would expect the contribution here to wine aroma to be minimal. It is however widely accepted that many subtle changes in volatile components could result in detectable differences in overall wine aroma, and the individual effects of compounds on the sensory attributes of wine can be complicated by many factors such as the interaction with other wine compounds (Escudero, Campo, Fariña, Cacho, & Ferreira, 2007; Escudero, Gogorza, Melús, Ortín, Cacho, & Ferreira, 2004; Voilley, Lamer, Dubois, & Feuillat, 1990). Ethyl acetate was also commonly reduced in wines made from diluted juices with either yeast, whereas many more compounds were detected as decreased in diluted juices fermented with Lalvin R2™ with no additional complex nutrient, i.e., butanoic acid, ethyl butanoate, 2-methyl-4-vinylphenol, ethyl decanoate, isobutyl acetate, benzyl alcohol and hexyl acetate. The single volatile that increased with dilution of juice in this analysis was methionol (sweet potato aroma), but was only significant for the dilution to 14.5 Bé and with the use of Lalvin R2™.

Some major yeast metabolites were also affected by juice dilution (Supp. Tab. 2 - 4). As expected these decreased in concentration in proportion to juice dilution, for example ratios of malic and succinic acid, glycerol and ethanol decreased (ranging from 0.92 to 0.67, Supp. Tab. 4) to very similar ratios to that of juice dilution (14.5–12.5 Bé being 0.89–0.76; juice:water). This confirms the expectation that the major determinate of the final concentrations of these compounds is the initial concentration of sugars in juice. Only the concentration of acetaldehyde increased when juices were diluted to 13.5 or 14.5 Bé and fermented by Lalvin R2$^{\text{TM}}$ (167 and 197% respectively). Pyruvate decarboxylase forms acetaldehyde from pyruvate in the latter stages of glycolysis and then alcohol dehydrogenase reduces it to ethanol (Pronk, Yde Steenema, & Van Dijken, 1996). This reaction importantly regenerates NAD$^+$ from NADH. The accumulation of acetaldehyde is influenced by the expression and subsequent activity of alcohol dehydrogenases and particularly by the availability of its cofactor, NADH (Xu, Bao, et al., 2019; Xu, Niu, Liu, & Li, 2019). Transient accumulation of acetaldehyde has also been linked to decreased activity of NADP-dependent acetaldehyde dehydrogenase, which converts acetaldehyde to acetate (Remize, Andrieu, & Dequin, 2000). This may also explain the reduction in acetic acid accumulation in the present study with fermentation by Lalvin R2$^{\text{TM}}$, especially as it is reduced beyond that expected from dilution alone (at 13.5 Bé reduced to 28% and 12.5 Bé to 22% (-3.54 and -4.45 FC) of undiluted juice fermentations, Table 1). Perhaps this indicates that the cumulative effect of juice dilution is a reduction of the activity of alcohol dehydrogenase and/or acetaldehyde dehydrogenase, through modification of the NAD$^+$/H or NADP/H pools. Dilution of juices is expected to change the external osmolarity that yeast experience at the beginning of fermentation, and it is well documented that many metabolic processes are affected (Blomberg & Adler, 1992; Varela & Mager, 1996). These minor adjustments of redox cofactors may reflect how the cell achieves balance under these different initial osmotic conditions and results in changes to compound concentrations, such as acetaldehyde and acetic acid reported here. Interestingly, glycerol, the main compound involved in balancing redox factors in response to osmotic stress, is relatively unaffected, only reducing in proportion to juice dilution (Supp. Tab. 4).

We were also particularly interested to see if complex nutrient addition could recover volatile concentrations to those similar to undiluted juices, supposedly by re-supplying diluted precursors. Of the volatile compounds that were significantly different with addition of nutrient, the vast majority were increased (44 from 56 data points, Table 1), however these rarely recovered the concentration found in undiluted juices. In some conditions, ethyl lactate could be recovered (Supp. Fig 1). Compounds found to increase with the addition of nutrient in more than one condition included 3 and 4-methyl-1-pentanol (up to 129%), butanoic acid (up to 166%), ethyl butanoate (up to 120%), hexanoic acid (up to 141%) and octanoic acid (up to 346%), ethyl lactate (up to 131%) and isobutyric acid (up to 168%; Table 1). Interestingly the occurrence of fatty acids, hexanoic and octanoic acid was unaffected by juice dilution, but consistently increased with nutrient addition (octanoic: 215–346%, hexanoic: 125–141%). This is akin to that seen by other studies (Rollero et al., 2015; Torrea, Varela, Ugliano, Ancin-Azpilicueta, Leigh Francis, & Henschke, 2011). Similarly, the fatty acid, butanoic acid, also increased with addition of nutrients, however in contrast to hexanoic and octanoic acids, in wines fermented by Lalvin R2^{TM}, dilution reduced butanoic acid. Torrea and colleagues (2003) suggest an increase in medium chain fatty acids could simply be due to a relative increase in fatty acid synthesis due to nitrogen supplementation. Fatty acids are produced during fermentation by the fatty acid synthase (FAS) complex from acetyl-CoA and malonyl-CoA during lipid synthesis (Marchesini & Poirier, 2003; Taylor & Kirsop, 1977) with the main source of acetyl CoA during anaerobiosis being acetic acid (Chen, Siewers, & Nielsen, 2012). A number of factors could influence the activity of the enzymes involved and availability of acetyl-CoA in the cytosol such as the supply of cofactors like NADPH (Bloem, Sanchez, Dequin, & Camarasa, 2016; Sheng & Feng, 2015) and availability of nutrients (Chen et al., 2012). The mechanism leading to accumulation of medium chain fatty acids under these conditions is still debated but likely involves premature release from the FAS complex due to feedback inhibition by saturated fatty acids and/or an increase in the fatty acid synthetic pathway (Duffour, Malcorps, & Silcock, 2003; Furukawa, Yamada, Mizoguchi, & Hara, 2003; Saerens, Delvaux, Verstrepen, Van Dijck, Thevelein, & Delvaux, 2008). Perhaps saturated fatty acids from the complex nutrient contributed to this release of medium chain fatty acids. These commercial preparations commonly contain inactivated yeast and as such have a component of fatty acids along with amino acids, peptides, proteins, polysaccharides, nucleotides, vitamins (thiamine, biotin, pantothenic acid) and minerals (magnesium and zinc) (Lallemand, 2019; Pozo-Bayón, Andújar-Ortiz, & Moreno-Arribas, 2009). However, yeasts for this purpose are grown with plentiful oxygen, and thus saturated fatty acids should far exceed the concentration of non-saturated fatty acids, thus reducing the possibility of feedback inhibition of the FAS complex. Changes to the redox status of yeast have also been shown to affect volatiles produced by yeast (Bloem et al., 2016; Fariña, Medina, Urruty, Boido, Dellacassa, & Carrau, 2012). Of particular relevance to this study, Bloem and colleagues (2016) report decreased accumulation of medium chain fatty acids (hexanoic and octanoic) during increased demand for NADPH or NADH. As hexanoic, octanoic and butanoic acids were detected many magnitudes above their aroma threshold (hexanoic: 420 µg L^{-1} (Guth, 1997), octanoic: 500 µg L^{-1} and butanoic: 173 µg L^{-1} (Ferreira et al., 2000)), we expect these difference would translate to a sensorial difference. This group of compounds is commonly described as sweaty and cheesy. The presence of fatty acids have also been shown to translate to increases in their fatty acid ethyl ester, for instance ethyl hexanoate and ethyl butanoate (Saerens et al., 2008; Saerens et al., 2006). In this study, the sensorially desirable esters, ethyl lactate and ethyl butanoate, increased with the addition of nutrient (up to 131 and 120%, respectively in wines fermented by Lalvin R2™). Whilst ethyl lactate is described as fruity and ethyl butanoate as floral, fruity, and strawberry like, the increases found in this study were below their aroma thresholds. Exogenously added amino acids have been suggested to be direct precursors of esters, however studies report variable effects of changes in nutrition upon the ester composition of wine, which may reflect changes in juice composition, yeast strain and amount and timing of additions (Hernández-Orte, Ibarz, Cacho, & Ferreira, 2005; Miller, Wolff, Bisson, & Ebeler, 2007; Sumby, Grbin, & Jiranek, 2010; Torrea et al., 2011).

Both 3 and 4-methyl-1-pentanol increased with the addition of nutrient in the order of 9-29%, in comparison to wine made from the juice of the same Bé. The most consistent increases of these compounds occurred with addition of 400 mg L^{-1} nutrient, however the magnitude of increases was always well below that of the effect of the initial dilution, thus not resulting in full recovery of reduced volatiles caused by dilution. An increase in these compounds may simply reflect an increase in flux through glycolysis and/or fatty acid synthesis as the 2-keto acid precursors for their formation might be originally derived from pyruvate. This has been shown possible with synthetic pathways built from a combination of yeast and bacterial genes with the goal of efficient production of these compounds for biofuel (Zhang, Sawaya, Eisenberg, & Liao, 2008). This is supported since the *Saccharomyces cerevisiae* enzyme Adh6p, an alcohol dehydrogenase with a strict specificity for NADPH, is capable of completing the final step in formation of methyl pentanols (Zhang et al., 2008). Other aliphatic alcohols can be formed by yeast via the Ehrlich pathway, however these are limited to 5-carbon chains as determined by amino acid precursors (Hazelwood, Daran, van Maris, Pronk, & Dickinson, 2008). 2-Methyl-4-vinylphenol, methyl octanoate, methyl decanoate, acetic acid and ethyl acetate and methionol were found to decrease with the addition of nutrient in selected conditions (Table 1), with methionol being previously reported to decrease with exogenous additions of nitrogen (ca. 70%; Hernández-Orte et al., 2005).

Few differences in major metabolites were detected with added nutrient (Supp. Tab. 4). The most prominent, as with dilution, was an increase of acetaldehyde. Whether this is simply a reflection of increased flux through the glycolytic pathway or accumulation due to reduced availability of cofactors to drive the activity of alcohol dehydrogenases remains to be proven. Acetaldehyde is generally regarded as undesirable when occurring above 100-125 mg L^{-1} (Zoecklein, 1995), as occurred in this study (120-330 mg L^{-1}).

Principal Component Analysis of measured volatiles highlighted the interaction between dilution and yeast strain, with the effect of both of these variables on wine volatile compound composition being explained by both of the first 2 principal components (Fig. 5). Wines made with either yeast and from diluted juices were clearly separated, with wines made with Lalvin EC1118^{TM} located more towards the right and the top of the score plot (i.e. they had more positive PC1 and PC2 values) compared to the comparable juices fermented with Lalvin R2^{TM} (Fig. 5A). The clear separation of these yeasts supports what is widely known in the winemaking industry as Lalvin R2^{TM} can make a significant contribution to wine aroma, thereby it is marketed as “aromatic”, whereas Lalvin EC1118^{TM} is considered “neutral”. The Lalvin EC1118^{TM} wines were associated with higher concentrations of methyl esters and the straight chain fatty acids hexanoic and octanoic acid (Fig. 5B). In contrast, wines fermented with Lalvin R2^{TM} had higher concentrations of branched-chain acids and alcohols, and their associated esters (Fig. 5B). Wines also grouped according to their juice dilution with higher Bé cultures being located toward the left and top of the plot compared to those with lower Bé (Fig. 5A). The undiluted wines were found to have higher concentrations of a number of ethyl and acetate esters, as well as benzyl and phenylethyl alcohol, compared to the diluted wines which were located at the opposite quarter of the plot, suggesting they have lower concentrations of these compounds due to dilution of the must (Fig. 5). No clear trend appears to be associated with nutrient additions, reflecting the differential effects depending upon the compound.

4. Conclusions

In this study juice dilution does not impede microbial fermentation, but instead results in reduction of both alcoholic and malolactic fermentation duration and changes to the chemical composition of wines. Nutrient addition was also effective in shortening fermentation duration by Lalvin R2^{TM} by up to 26%. These impacts are of great interest to the wine industry, in particular shortened alcoholic fermentation duration reported here as a reduction of up to 42% (or 28% when considering dilutions to 13.5 Bé or above in accordance with Australian standards). Thus use of juice dilution or addition of nutrient could allow more efficient use of often-limited tank space since turnover would be more rapid. The concentrations of 22 volatile compounds were significantly different in three or more treatments, with a single treatment resulting in modifications to as many as 15 different volatile compounds. Even if these individually are not above their aroma threshold values, they may act in concert to result in global changes to wine aroma, an outcome of great interest to winemakers. Compounds that were significantly affected in more than 3 treatments by either dilution or nutrient addition and detected above their aroma thresholds were isoamyl acetate, isoamyl alcohol, 2-phenylethanol, methionol and hexanoic, butanoic and octanoic acids. Isoamyl acetate and isoamyl alcohol (banana aroma) and 2-phenylethanol (rose) were reduced by dilution, but the effect upon methionol (sweet potato aroma) varied depending on the treatment. Furthermore, the addition of complex organic nutrient, irrespective of dilution rate, increased medium chain fatty acids (hexanoic, butanoic and octanoic characterised by sweaty, cheesy and rancid aromas). Sensory studies would be of great benefit to determine which of these impacts result in wines that are detectably different to consumers, given the large quantity of changes detected in this study, we suspect this likely. Further studies should examine the impact of juice dilution on an industrial scale and when other wine processing methods are utilised, for instance with the purposeful addition of oxygen. Depending on the target wine style, these changes may be regarded as a desirable outcome. Winemakers should take into consideration the potential impacts of juice dilution, as well as yeast choice and nutrient addition on both processing efficiencies as well as effect on the aroma and flavour of wine.

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Conflicts of Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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Figure Legends

Figure 1. Sugar catabolism by Lalvin EC1118^{TM} (A) or Lalvin R2^{TM} (B) of 16 Bé juice (●) and juices diluted with water to 14.5 (■), 13.5 (▲) and 12.5 (♦) Bé without the addition of malolactic bacteria or complex organic nutrient. Data presented are the average of triplicate fermentations, with the error bars representing the standard deviation.

Figure 2. Alcoholic fermentation duration of Lalvin EC1118^{TM} (EC) and Lalvin R2^{TM} (R2), with (MLB+) and without (MLB-) malolactic bacteria. 16 Bé juice was either fermented neat (■), or diluted with water to 14.5 (■), 13.5 (■) or 12.5 (□) Bé. Data presented is the average of triplicate fermentations and includes standard deviations. *Significantly different to fermentation duration of 16 Bé juice within the set (same yeast and MLB treatment), one way ANOVA, $p < 0.05$.

Figure 3. Alcoholic fermentation duration of Lalvin R2^{TM}, in juice diluted to 13.5 Bé with (MLB+) and without (MLB-) malolactic bacteria and with the addition of complex organic nutrient at 0 (□), 200 (■) or 400 (■) mg L^{-1}. Data presented is the average of triplicate fermentations and includes standard deviations. Values with different letters are significantly different, one way ANOVA, $p < 0.05$.

Figure 4. Isoamyl acetate measured in wines (GC-MS) fermented by Lalvin EC1118^{TM} or Lalvin R2^{TM} with no malolactic bacteria added. Wines were made from juice with initial Bé of 16 or diluted with water to 14.5, 13.5 or 12.5. Nutrient was also added to juices; 0 (☐), 200 (▨) or 400 (■) mg L^{-1}. Dashed line represents aroma threshold of isoamyl acetate (30 µg L^{-1} (Guth, 1997)). *significantly different to 16.0 Bé with no nutrient added (for the same yeast), one way ANOVA, $p < 0.05$).

Figure 5. Principal Component Analysis scores (A) and loadings (B) plots of the wines fermented by Lalvin EC1118^{TM} (EC) or Lalvin R2^{TM} (R2) based on the concentration of 39 volatile compounds measured by GC-MS. Wines made from juice with initial Bé of 16, 14.5 (14), 13.5 (13) and 12.5 (12) with the addition of 0, 200 (20) or 400 (40) mg L^{-1} nutrient. The first two principal components are shown (PC-1 and PC-2).

Table 1. Volatiles detected in final wines that were significantly different (one way ANOVA, $p < 0.05$) to the matched control (no nutrient added 16 Bé juice fermented by either Lalvin EC1118^{TM} or Lalvin R2^{TM} with no exogenous nutrient added, or where nutrients were added comparison was to the same Be juice with no nutrients added). Where a significant difference was detected, the fold change difference of treatment to control is shown. Volatiles that increased upon dilution are shaded in green and decreased in red. The symbol (–) denotes no significant difference. Actual values (µg L^{-1}) are shown for undiluted juices. Aroma threshold as reported in [a](Moreno et al., 2005), [b](Guth, 1997), [c](Ferreira et al., 2000), [d](Peinado, Moreno, Bueno, Moreno, & Mauricio, 2004; Salo, 1970), [e](Etievant, 1991), [f](Takeoka et al., 1989), [g](Gomez-Miguez, Cacho, Ferreira, Vicario, & Heredia, 2007). *Volatiles detected above the aroma threshold in at least one treatment.

Supplementary legends

Supplementary Table 1. (A) Alcoholic fermentation duration of Lalvin EC1118^{TM} and Lalvin R2^{TM}, without (MLB-) and with (MLB+) malolactic bacteria. 16 Bé juice was either fermented neat or diluted with water to 14.5, 13.5 or 12.5 Bé. Data presented is the average of triplicate fermentations and includes standard deviations. Significantly different data within the set (same yeast and MLB treatment) are highlighted by different letters, one way ANOVA, $p < 0.05$. (B) Residual malic acid after 1122 hours or hours taken to metabolise all malic acid (< 0.2 g L^{-1}) by Lalvin VP 41™ when co-inoculated with either Lalvin EC1118™ or Lalvin R2™ in juice at 16 Bé or a range of dilutions with either 0, 200 or 400 mg L^{-1} of complex organic nutrient. Values are the average of triplicates ± Standard deviations. *Significantly different to no nutrient added (same Bé), Student's t-test, $p<0.05$.

Supplementary Table 2. Volatile and major yeast metabolic compounds (µg L^{-1} ± standard deviations) detected in final wines for all treatments except for those inoculated with lactic acid bacteria for malolactic fermentation. 'No yeast' controls were also analysed, and in most cases volatiles were below detection limits. Where they were measured the volatiles were many magnitudes lower than in fermented treatments and thus the data is not shown.

Supplementary Table 3. Volatile and major yeast metabolic compounds (volatiles: µg L^{-1} and major metabolites g L^{-1}) detected in final wines for all treatments except for those inoculated with lactic acid bacteria for malolactic fermentation. 'No yeast' controls were also analysed, and in most cases volatiles were below detection limits. Where volatiles were measured, they were many magnitudes lower than fermented treatments and thus the data is not shown. Blue highlighted cells indicate those measurements significantly different due to juice dilution (compared to the same yeast at 16 Bé) and orange highlighted cells indicate those significantly different due to nutrient addition (compared to the same yeast and same initial Bé juice). Significant differences were determined by ANOVA and significantly different data are indicated by different letters.

Supplementary Table 4. Ratios of significantly different compounds measured by HPLC. Ratios of compounds detected in wines made from diluted juices are in comparison to 16 Bé juices fermented by the same yeast, or in the case of where nutrient is added, to wines made from juices of the same Bé. Increased ratios are highlighted in green and decreased in red. Results for wines made from 16 Bé juices with no nutrient addition are displayed as actual values (g L^{-1}) ± SD. Significant differences were determined by ANOVA.

Supplementary Figure 1. Ethyl lactate measured in wines (GC-MS) fermented by Lalvin R2$^{\text{TM}}$ with no malolactic bacteria added. Wines were made from juice with initial Bé of 16 or diluted with water to 14.5, 13.5 or 12.5. Nutrient was also added to juices; 0 (☐), 200 (▨) or 400 (■) mg L^{-1}. Dashed line represents aroma threshold of isoamyl acetate (30 μg L^{-1} (Guth, 1997)). Significantly different values are labelled with different letters or letter groups (a-d), one way ANOVA, $p < 0.05$).

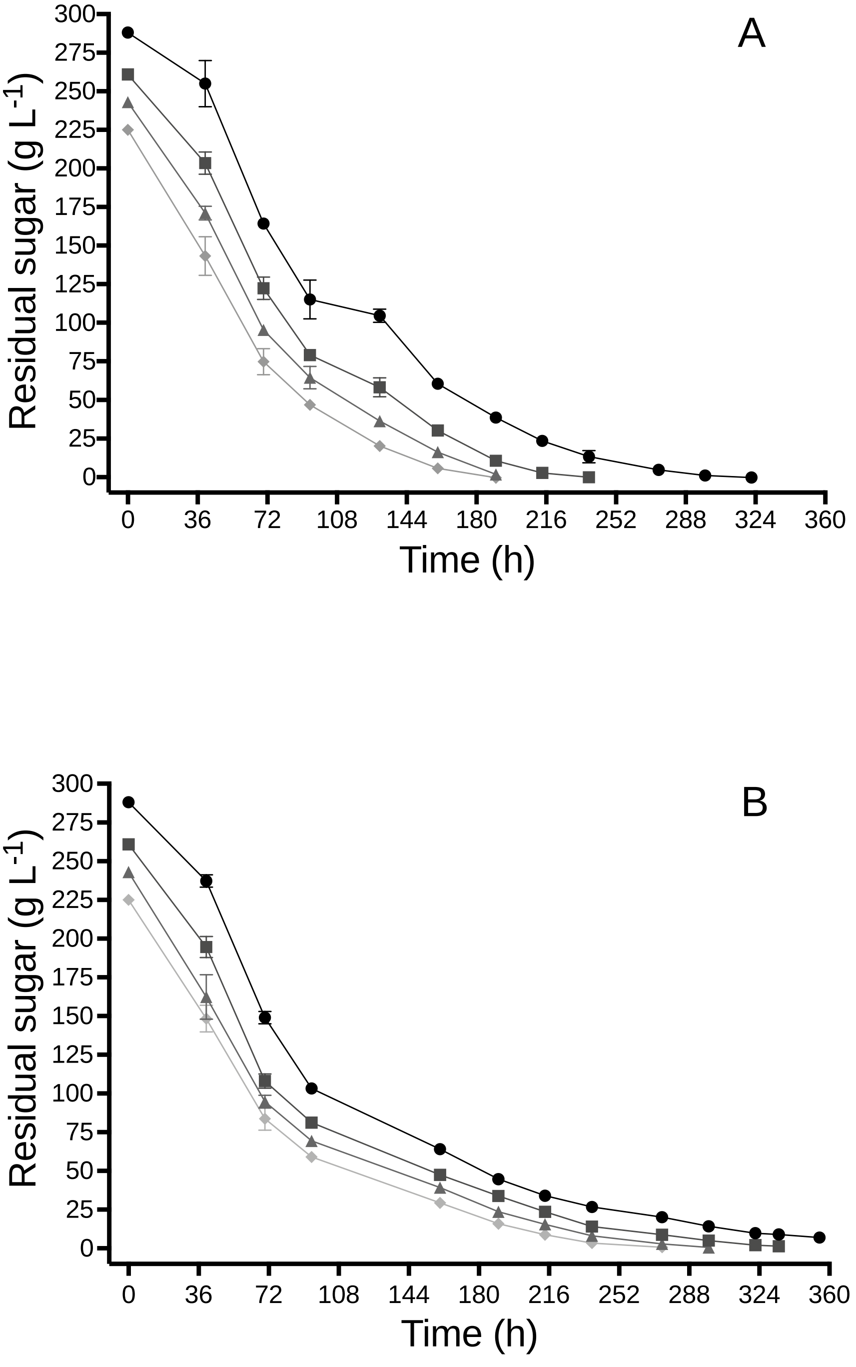

Figure 1. Gardner et al

Figure 2

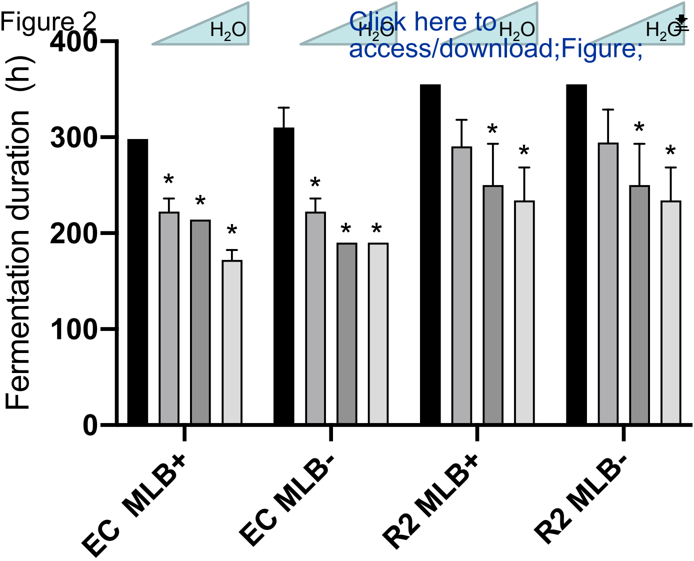

Figure 2. Gardner et al

Figure 3

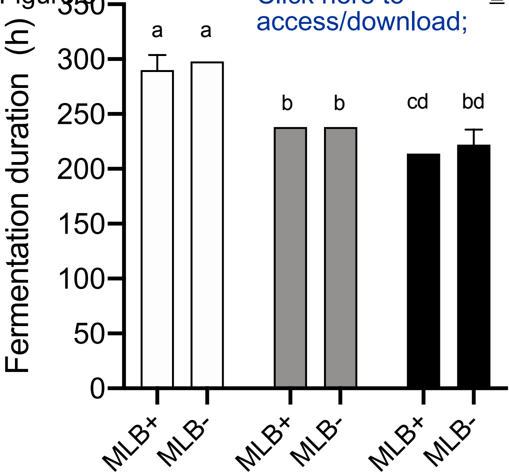

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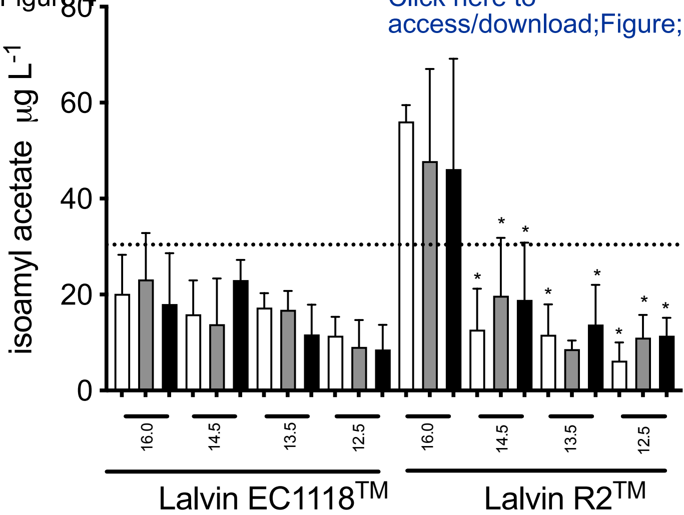
Figure 4



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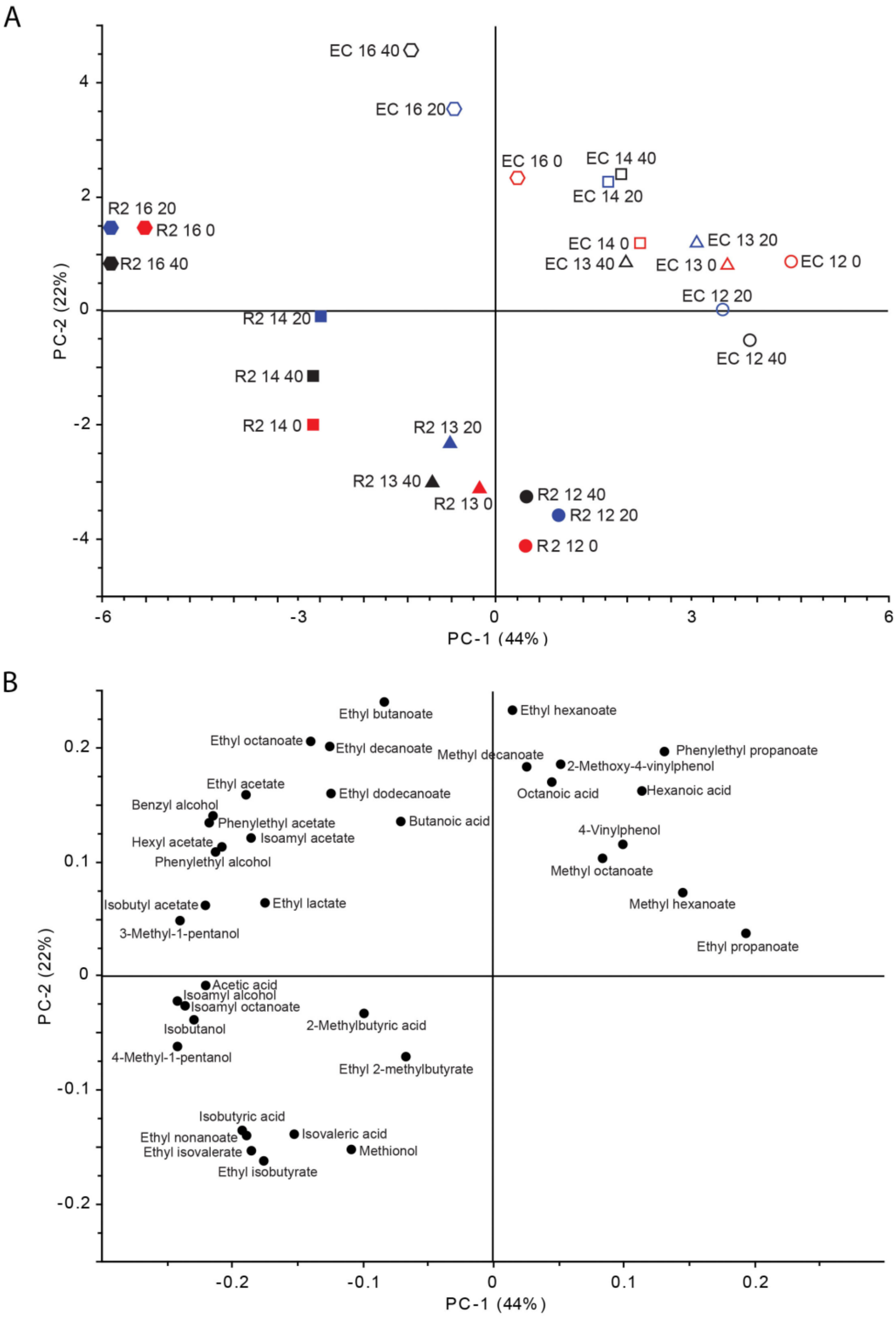

Figure 5. Gardner et al.

Table 1. Gardner et al.

	Baumé	Nutrient addition (mg L^{-1})	3-methyl-1-pentanol	4-methyl-1-pentanol	*hexanoic acid	ethyl acetate	*butanoic acid	2-phenylethyl acetate	ethyl butanoate	*octanoic acid	*2-phenylethanol	2-methyl-4-vinylphenol	ethyl decanoate	ethyl lactate	isobutyl acetate	*methionol	*isoamyl alcohol	methyl decanoate	isobutyric acid	methyl octanoate	acetic acid	benzyl alcohol	hexyl acetate	* isoamyl acetate
Aroma threshold (µg L^{-1})			500[a]	50000[a]	3000[b]	7500[b]	173[c]	250[b]	20[b]	500[c]	14000[c]	N/A	200[c]	14000[d]	1600[e]	1000[c]	30000[b]	N/A	2300[c]	200[f]	$2x10^{5}$[b]	$2x10^{5}$[g]	670[e]	30[b]
EC1118	**16 Bé**	**0**	217 ± 16	650 ± 21	6,107 ± 384	1,769 ± 228	895 ± 149	4.8 ± 1.6	6.9 ± 0.8	222 ± 49	171614 ± 17567	2544 ± 787	20 ± 3	3.91 ± 0.24	0.35 ± 0.1	1297 ± 43	145139 ± 13654	0.65 ± 0.46	601 ± 90	5.9 ± 3.54	260 ± 178	37 ± 5	0.34 ± 0.07	20 ± 8
		200	1.09	1.11	1.39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		400	1.24	1.22	1.37	1.16	-	-	-	3.13	-	-1.66	1.84	-	-	-	-	2.88	1.68	-	-	-	-	-
	14.5 Bé	**0**	-1.79	-1.45	-	-	-	-1.62	-	-	-1.44	-	-	-	-	-	-	-	-	-	-	-	-	-
		200	-	-	1.26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		400	-	1.11	-	-	-	-	-	2.30	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	13.5 Bé	**0**	-2.42	-1.79	-	-1.28	-	-2.16	-	-	-1.72	-	-	-	-	-	-1.23	-	-	-	-	-	-	-
		200	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		400	-	-	1.36	-	1.66	-	-	-	-	-1.61	-	-	-	-	-	-	1.54	-	-	-	-	-
	12.5 Bé	**0**	-3.23	-2.19	-	-1.47	-	-2.86	-	-	-2.02	-	-	-1.25	-	-1.79	-1.29	-	-	-	-	-	-	-
		200	-	-	1.41	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-5.01	-	-	-	-
		400	-	-	1.37	-	-	-	-	2.15	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lalvin R2	**16 Bé**	**0**	282 ± 17	1647 ± 49	5974 ± 1888	1942 ± 53	1019 ± 244	7.9 ± 0.8	7.7 ± 0.3	258 ± 118	181170 ± 36204	1851 ± 346	24 ± 9	3.89 ± 0.19	0.95 ± 0.1	1992 ± 113	177126 ± 17640	0.7 ± 0.56	1401 ± 297	7.93 ± 6.39	3577 ± 467	47 ± 3	0.8 ± 0.1	56 ± 3
		200	1.13	1.07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		400	1.14	-	1.25	-	1.23	-	-	-	-	-	-	-	-	-	-	-2.48	1.34	-	-	-	-	-
	14.5 Bé	**0**	-1.26	-1.22	-	-1.18	-1.63	-2.00	-1.47	-	-	-1.54	-	-	-2.25	2.13	-	-2.06	-	-3.36	-	-1.29	-2.10	-4.43
		200	-	-	1.31	-	-	-	1.13	3.46	-	-	-	1.31	1.29	-1.68	-	-	-	-	-	-	-	-
		400	1.07	-	1.36	-	1.62	-	-	2.57	-	-	-1.32	1.27	-	-2.22	-	-	-	-	-2.67	-	-	-
	13.5 Bé	**0**	-1.47	-1.57	-	-1.19	-1.36	-3.60	-1.53	-	-1.51	-1.59	-1.24	-	-2.46	-	-1.21	-	-	-	-3.54	-1.63	-3.41	-4.83
		200	-	-	-	-	-	-	1.15	-	-	-	-	1.29	-	-	-	-	-	-	-	-	-	-
		400	-	-	1.29	-	1.50	-	1.20	-	-	-	-	-	-	-	-	-3.58	-	-3.04	-	-	-	-
	12.5 Bé	**0**	-2.31	-2.08	-	-1.33	-1.44	-5.31	-1.68	-	-1.85	-	-1.48	-	-2.76	-	-1.24	-	-	-	-4.45	-1.93	-4.89	-8.96
		200	-	1.20	-	-1.28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		400	1.29	1.17	-	-1.17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

2

The effect of grape juice dilution on oenological fermentation.

Jennifer Margaret Gardner[1, §], Michelle Elisabeth Walker[1*], Paul Kenneth Boss,[2,3] and Vladimir Jiranek[1,3]*

Author Declaration:

All authors agree that this manuscript is original, has not been published before, and is not currently being considered for publication elsewhere.

We wish to confirm that there are no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

We confirm that the manuscript has been read and approved by all named authors, all have contributed significantly and that there are no other persons who satisfied the criteria for authorship but are not listed.

We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

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CRediT author statement

Jennifer Gardner: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - Original Draft. **Michelle Walker:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - Review & Editing. **Paul Boss:** Methodology, Investigation, Writing - Review & Editing. **Vladimir Jiranek:** Conceptualization, Resources, Writing - Review & Editing, Supervision, Funding acquisition.

The effect of grape juice dilution and complex nutrient addition on oenological fermentation and wine chemical composition

Jennifer Margaret Gardner[1, §*], *Michelle Elisabeth Walker*[1*], *Paul Kenneth Boss,*[2,3] *and Vladimir Jiranek*[1,3]

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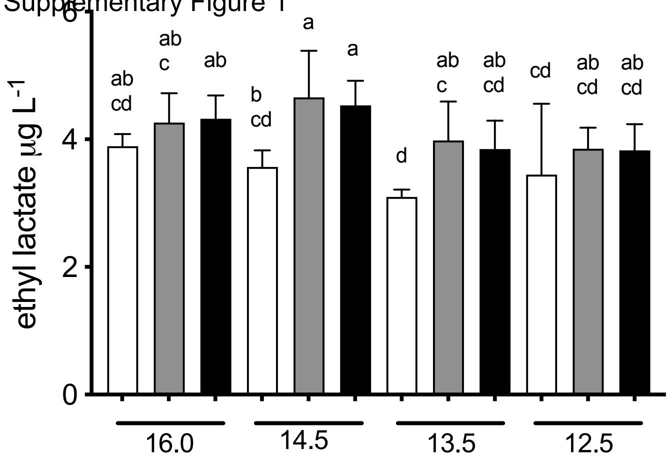
CRediT author statement

Jennifer Gardner: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - Original Draft. **Michelle Walker:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - Review & Editing. **Paul Boss:** Methodology, Investigation, Writing - Review & Editing. **Vladimir Jiranek:** Conceptualization, Resources, Writing - Review & Editing, Supervision, Funding acquisition.

Supplementary Figure 1



Supplementary Figure 1. Gardner et al.

Supplementary Table 1. Gardner et al.

(A)

	Nutrient (mg L^{-1})	-MLB		+MLB	
		Lalvin EC1118™	Lalvin R2™	Lalvin EC1118™	Lalvin R2™
16 Bé	**0**	298 ± 0 ^{a}	360 ± 0 ^{a}	306 ± 14 ^{a}	360 ± 0 ^{a}
	200	298 ± 0 ^{a}	355 ± 0 ^{a}	290 ± 28 ^{a}	357 ± 3 ^{a}
	400	309 ± 42 ^{a}	355 ± 0 ^{a}	306 ± 43 ^{a}	355 ± 0 ^{a}
14.5 Bé	**0**	238 ± 0 ^{b}	322 ± 0 ^{b}	238 ± 0 ^{b}	322 ± 0 ^{b}
	200	214 ± 0 ^{c}	274 ± 0 ^{d}	214 ± 0 ^{c}	274 ± 0 ^{d}
	400	214 ± 0 ^{c}	274 ± 0 ^{d}	214 ± 0 ^{c}	274 ± 0 ^{d}
13.5 Bé	**0**	198 ± 14 ^{cd}	298 ± 0 ^{c}	214 ± 0 ^{c}	290 ± 14 ^{c}
	200	190 ± 0 ^{d}	238 ± 0 ^{e}	214 ± 0 ^{c}	238 ± 0 ^{e}
	400	190 ± 0 ^{d}	222 ± 14 ^{ef}	214 ± 0 ^{c}	214 ± 0 ^{f}
12.5 Bé	**0**	190 ± 0 ^{d}	262 ± 21 ^{d}	152 ± 7 ^{d}	262 ± 21 ^{d}
	200	190 ± 0 ^{d}	214 ± 0 ^{f}	160 ± 0 ^{d}	214 ± 0 ^{f}
	400	198 ± 14 ^{cd}	190 ± 0 ^{g}	152 ± 7 ^{d}	214 ± 0 ^{f}

(B)

	Nutrient (mg L^{-1})	Lalvin EC1118™	Lalvin R2™	
16 Bé	**0**	2.3 ± 0.1	2.2 ± 0.1	Residual malic acid (g L^{-1})
	200	2.4 ± 0.1	2.2 ± 0.1	
	400	2.3 ± 0.1	2.2 ± 0.1	
14.5 Bé	**0**	2.1 ± 0.1	1.7 ± 0.2	
	200	1.4 ± 0.1*	1.4 ± 0.4	
	400	1.0 ± 0.9	1.4 ± 0.4	
13.5 Bé	**0**	1002 ± 0	1066 ± 96	Time (h)
	200	955 ± 0*	955 ± 0	
	400	955 ± 0*	955 ± 0	
12.5 Bé	**0**	917 ± 70	874 ± 70	
	200	834 ± 0	834 ± 0	
	400	834 ± 0	834 ± 0	

Supplementary Tables 2-4

Supplementary Table 2. Compounds (± standard deviations) detected in final wines for all treatments except for those inoculated with lactic acid bacteria for malolactic fermentation. The "no yeast controls" were also analysed, in most cases volatiles were below detection limits, where they were measured they were many magnitudes lower than fermented treatments and thus the data is not shown.

µg L^{-1}	EC1118 16Bé N0 MLF-	EC1118 16Bé N200 MLF-	EC1118 16Bé N400 MLF-	EC1118 14.5Bé N0 MLF-	EC1118 14.5Bé N200 MLF-	EC1118 14.5Bé N400 MLF-	EC1118 13.5Bé N0 MLF-	EC1118 13.5Bé N200 MLF-
Ethyl acetate	1768.95±228.13	1980.56±284.26	2043.34±146.59	1603.35±188.62	1744.16±70.35	1587.54±166.87	1379.49±61.82	1313.78±197.82
Ethyl propanoate	12.73±0.84	11.86±1.92	14.16±1.24	21.01±13.13	20.89±14.62	19.43±4.75	22.7±12.81	16.98±4.19
Ethyl isobutyrate	0.42±0.17	0.42±0.1	0.49±0.13	0.41±0.03	0.45±0.03	0.47±0.16	0.42±0.04	0.55±0.12
Isobutyl acetate	0.35±0.09	0.4±0.08	0.46±0.02	0.3±0.09	0.3±0.06	0.34±0.11	0.27±0.04	0.3±0.03
Ethyl butanoate	6.95±0.82	7.28±0.59	7.58±0.63	6.82±1.67	7.44±1.86	7.67±1.14	6.35±0.91	6.35±0.82
Ethyl 2-methylbutyrate	1.35±0.54	1.29±0.3	1.51±0.52	1.2±0.09	1.25±0.09	1.38±0.49	1.1±0.14	1.54±0.41
Ethyl isovalerate	0.1±0.03	0.09±0.02	0.11±0.03	0.08±0.01	0.08±0.01	0.09±0.03	0.08±0.01	0.1±0.03
Isobutanol	125698.23±53324.07	126726.05±69190.63	142155.4±70298.6	114285.41±52723.51	119149.25±79073.22	107815.65±58647.55	72809.37±5711.94	102496.48±55216.01
Isoamyl acetate	20.15±8.12	23.14±9.72	18.03±10.58	15.93±7.04	13.81±9.58	23.01±4.24	17.28±2.99	16.83±3.91
Methyl hexanoate	4.15±2.54	3.14±1.68	8.66±7.51	6.71±8.45	5.76±7.38	7.6±5.4	6.1±4.71	9.95±2.6
Hexyl acetate	0.34±0.07	0.44±0.17	0.38±0.1	0.3±0.11	0.35±0.09	0.29±0.04	0.28±0.09	0.26±0.09
4-Methyl-1-pentanol	649.6±21.64	722.02±37.93	790.12±14.93	447.2±9.04	473.06±9.96	495.03±23.85	363.61±5.55	368.98±16.36
3-Methyl-1-pentanol	217.43±16.3	237.48±22.86	268.57±17.62	121.68±5.33	115.04±3.38	130.52±9.03	89.73±3.43	90.07±15.27
Ethyl lactate	3.91±0.24	4.11±0.13	4.04±0.38	3.86±0.37	3.74±0.3	3.96±0.45	3.43±0.32	3.61±0.21
Methyl octanoate	5.9±3.54	5.11±2.51	10.7±7.96	5.39±4.55	4.87±4.03	7.89±5.24	7.22±7.1	11.95±2.74
Acetic acid	260.52±178.21	303.34±319.91	198.02±108.03	148.26±118.28	193.05±141.69	71.51±17.55	116.21±51.44	111.44±35.15
Ethyl nonanoate	0.01±0	0.01±0	0.01±0	0.01±0	0.01±0	0.01±0	0.01±0	0.01±0
Isobutyric acid	601.36±90.17	768.11±131.04	1010.84±207.19	700.81±192.6	798.26±189.21	764.09±261.11	698.22±70.03	741.69±153.17
Methyl decanoate	0.65±0.46	0.66±0.4	1.88±1.7	0.5±0.36	0.5±0.31	0.74±0.52	0.53±0.42	0.88±0.19
Butanoic acid	894.96±148.87	1024.72±176.33	1191.96±133.89	854.41±379.16	1032.4±66.82	997.52±153.55	784.94±65.09	919.23±64.29
Ethyl decanoate	20.38±3.33	27.18±7.16	37.51±17.06	15.01±5.77	19.04±9.05	15.13±3.2	12.05±4.42	12.2±3.04
Isoamyl octanoate	0.02±0	0.02±0	0.02±0.01	0.01±0	0.01±0.01	0.01±0	0.01±0	0.01±0
Isovaleric acid	2228.51±348.56	2438.44±432.24	3020.47±346.93	2327.57±1407.94	2293.53±717.66	2346.64±158.01	1809.91±608.65	2012.49±121.89
2-Methylbutanoic acid	2795.75±548.33	2961.35±592.74	3548.08±403.09	2832.92±1518.63	2864.7±831.53	2931.88±279.69	2106.96±628.69	2455.78±132.22
Methionol	1297.26±43.38	1546.55±238.25	1516.77±344.78	1166.77±343.26	1367.15±374.39	1157.83±137.46	1064.46±218.79	1251.15±179.04
2-Phenylethyl acetate	4.83±1.64	5.89±1.44	6.01±1.13	2.98±0.93	3.87±0.98	4.03±0.72	2.24±1.04	2.45±0.73
Ethyl dodecanoate	0.12±0.01	0.16±0.03	0.21±0.1	0.1±0.03	0.11±0.05	0.1±0.01	0.09±0.03	0.09±0.03
Hexanoic acid	6107.29±384.45	8509.74±587.18	8339.73±1358.46	6905.94±238.16	8682.74±1594.91	8617.89±547.51	6863.48±442.99	8360.89±518.06
Benzyl alcohol	37.02±4.86	38.51±4.93	38.88±5.6	29.38±6.98	32.3±8.07	31.37±3.79	29±5.99	29.38±2.53
2-Phenylethyl propanoate	0.19±0.04	0.2±0.04	0.22±0.01	0.22±0.06	0.24±0.07	0.29±0.03	0.22±0.02	0.22±0.04
Octanoic Acid	222.21±49.42	499.96±99.2	695.12±382.86	263.23±89.08	511.93±187.57	604.24±137.04	318.7±75.73	444.78±86.82
2-Methoxy-4-vinylphenol	2544.42±787.45	2228.15±348.6	1535.06±149.52	2028.07±591.07	1725.79±647.29	1431.93±492.53	2250.4±484.39	1898.33±242.44
4-vinylphenol	4102.24±1418.58	4055.88±919.13	3310.32±676.09	3522.71±1096.67	3773.27±1381.07	3608.69±867.58	4132.05±1083.36	4253.17±673.16
Isoamyl alcohol (1/100)	145139.95±13654.68	144590.34±14025.96	143960.98±11148.68	129787.17±19120.85	129635.03±18549.35	132704.76±19779.9	118268.24±16518.57	120678.32±17202.09
Ethyl hexanoate (1/100)	11.48±3.02	11.58±2.77	11.47±3.27	11.81±4.88	12.39±5.06	12.34±3.64	9.8±5.28	10.15±1.98
Ethyl octanoate (1/100)	3.28±1.89	3.45±1.66	3.23±1.35	2.4±0.57	2.82±0.56	2.34±1.08	1.91±0.74	2.33±1.03
Phenylethyl alcohol (1/100)	171614.28±17567.67	180885.68±23730.72	198504.71±33572.19	119530.39±7971.31	123171.42±23395.96	136900.72±6162.4	99590.5±17126.72	102239.13±17127.63

g L^{-1}	EC1118 16Bé N0 MLF-	EC1118 16Bé N200 MLF-	EC1118 16Bé N400 MLF-	EC1118 14.5Bé N0 MLF-	EC1118 14.5Bé N200 MLF-	EC1118 14.5Bé N400 MLF-	EC1118 13.5Bé N0 MLF-	EC1118 13.5Bé N200 MLF-
Malic Acid (HPLC)	3.38±0.05	3.37±0.07	3.36±0.07	3.11±0.05	3.1±0.01	3.16±0.05	2.88±0.03	2.92±0.04
Succinic Acid (HPLC)	2.68±0.11	2.72±0.02	2.8±0.02	2.2±0.1	2.25±0.13	2.31±0.06	1.97±0.1	2.07±0.04
Lactic Acid (HPLC)	1.1±0.14	0.67±0.6	1.13±0.04	0.72±0.63	0.65±0.56	0.74±0.65	0±0	0.36±0.62
Glycerol (HPLC)	7.77±0.01	7.77±0.01	7.79±0.1	6.73±0.05	6.76±0.04	6.81±0.07	6.16±0.15	6.15±0.12
Acetaldehyde (HPLC)	0.33±0.01	0.45±0.04	0.45±0.03	0.27±0.01	0.37±0.02	0.4±0.08	0.21±0.03	0.27±0.03
Ethanol (HPLC)	120.83±0.29	120.95±0.56	119.98±0.95	108.75±1.45	108.18±1.11	107.86±0.49	100.83±0.28	100.17±1.02

±

EC1118 13.5Bé N400 MLF-	EC1118 12.5Bé N0 MLF-	EC1118 12.5Bé N200 MLF-	EC1118 12.5Bé N400 MLF-	R2 16Bé N0 MLF-	R2 16Bé N200 MLF-	R2 16Bé N400 MLF-	R2 14.5Bé N0 MLF-	R2 14.5Bé N200 MLF-	R2 14.5Bé N400 MLF-
1568.33±133.61	1205.21±56.43	1250.56±52.63	1158.48±31.09	1942.12±53.77	2129.91±257.04	1994.87±151.87	1641.65±133.15	1708.91±260.32	1655.26±244.21
19.01±10.73	18.86±3.77	11.53±2.38	13.12±4.6	7.88±0.73	7.55±0.12	7.22±0.59	8.55±1.43	6.95±4.78	9.01±1.72
0.61±0.19	0.4±0.12	0.38±0.12	0.43±0.16	0.87±0.33	0.8±0.3	0.82±0.3	0.62±0.24	0.61±0.29	0.71±0.23
0.29±0.09	0.28±0.01	0.21±0.06	0.25±0.03	0.95±0.08	1.03±0.05	0.92±0.12	0.42±0.02	0.54±0.1	0.45±0.02
7.1±1.48	5.54±0.46	5±0.36	5.26±0.81	7.76±0.37	7.5±0.14	7.21±0.23	5.29±0.4	5.98±0.18	5.93±0.5
1.63±0.68	0.88±0.31	0.83±0.29	0.97±0.4	1.41±0.56	1.19±0.38	1.27±0.45	1.21±0.5	1.27±0.58	1.33±0.48
0.11±0.05	0.07±0.02	0.06±0.02	0.07±0.03	0.15±0.05	0.13±0.03	0.13±0.04	0.12±0.04	0.13±0.05	0.14±0.04
73651.95±8401.62	66295.94±7509.4	58513.27±4741.69	64253.44±7178.29	247540.7±142527.18	313769.05±118089.39	237953.24±130659.29	194559.8±84559.05	196773.96±78056.01	194353.67±82554.99
11.69±6.22	11.44±3.93	9.08±5.61	8.56±5.15	56.13±3.4	47.82±19.22	46.15±23.04	12.67±8.57	19.8±12.04	18.93±11.93
7.15±8.83	10.49±0.72	1.58±0.89	5.56±4.86	4.15±3.05	2.09±1.13	1.57±0.28	1.31±0.39	1.91±1.18	2.03±1.48
0.31±0.1	0.26±0.06	0.26±0.03	0.21±0.05	0.83±0.1	0.84±0.04	0.73±0.21	0.4±0.13	0.49±0.23	0.42±0.08
390.47±9.9	296.61±32.92	297.64±6.29	296.47±13.24	1647.66±49.25	1760.51±17.89	1720.51±116.86	1354.75±25.4	1385.13±12.14	1444.98±83.83
98.63±2.63	67.27±9.79	66.31±6.29	65.56±5.57	281.55±17.25	317.9±8.22	320.41±23.79	223.64±9.12	241.96±13.39	258.23±20.76
3.49±0.24	3.12±0.46	3±0.16	2.85±0.19	3.89±0.19	4.26±0.46	4.32±0.36	3.57±0.26	4.66±0.73	4.53±0.39
6.39±5.19	11.59±2.06	2.31±0.99	6.03±3.86	7.93±6.39	4.32±2.49	3.52±0.36	2.36±0.79	4.61±0.53	3.02±1.64
136.38±34.79	135.52±65.15	65.56±24.14	58.41±22.14	3577.54±467.55	3720.28±1332.34	4509.36±951.82	3376.53±2631.06	1802.53±1552.1	1262.97±527.08
0.01±0	0.01±0	0.01±0	0.01±0	0.02±0.01	0.01±0	0.02±0.01	0.02±0	0.02±0.01	0.02±0.01
1072.06±410.75	501.28±123.94	676.59±78.96	766.04±128.2	1401±297.78	1403.51±83.35	1880.81±231.93	1079.8±284.21	1149.45±146.28	1357.95±167.97
0.48±0.15	0.77±0.32	0.3±0.09	0.47±0.27	0.7±0.56	0.35±0.21	0.28±0.06	0.34±0.13	0.35±0.11	0.24±0.08
1300.59±404.4	710.18±54.43	859.23±119.3	960.62±80.55	1018.64±244.39	997±45.1	1248.35±187.2	624.25±64.14	797.81±27.46	1008.93±76.64
15.32±7.94	10.88±4.8	15.09±5.58	11.8±5.97	24.33±8.98	21.82±5.97	21.9±8.15	26.4±7.58	19.03±3.48	15.42±2.73
0.01±0.01	0.01±0	0.02±0.01	0.01±0	0.03±0.01	0.04±0.01	0.04±0.01	0.03±0	0.04±0	0.03±0.01
2598.28±1164.6	1390.76±83	1513.8±153.63	1806.54±270.41	2859.32±749.76	2859.86±240.62	3490.77±733.98	2477.73±341.56	2658.41±409.66	3436.71±380.5
3137.64±1446.78	1529.9±81.17	1674.71±144.82	1974.25±269.27	2640.71±679.41	2562.15±302.89	3199.1±762.2	2221.78±292.6	2358.32±481.97	3072.23±268.73
1120.01±191.07	723.3±157.03	776.11±153.28	745.96±231.42	1991.76±113.23	1379.25±240.34	1134.22±273.04	4247.53±1437.01	2528.13±1119.24	1916.6±739.24
2.99±1.03	1.69±0.59	2.07±0.43	1.82±0.44	7.9±0.84	9±2.18	8.92±1.02	3.94±1.28	5.81±1.46	5.72±1.97
0.11±0.05	0.09±0.02	0.13±0.02	0.1±0.03	0.14±0.03	0.13±0.02	0.13±0.03	0.15±0.05	0.14±0	0.12±0.02
9327.58±1629.67	6646.72±930.25	9370.06±522.92	9103.97±1827.25	5974.48±1888.83	6126.52±273.99	7453.07±1170	4736.21±78.19	6208.12±715.1	6460.09±138.62
30.36±4.77	24.6±4.44	26.57±6.65	25.25±5.73	47.35±3.07	46.62±7.32	46.4±4.57	36.79±5.4	37.32±3.1	38.04±5.44
0.21±0.05	0.17±0.06	0.14±0.05	0.15±0.04	0.13±0.02	0.1±0.01	0.11±0.02	0.1±0.02	0.13±0.04	0.14±0.04
509.41±320.5	264.74±73.69	538.08±59.44	568.55±152.45	258.25±117.97	298.72±62.54	399.42±40.03	156.35±69.83	541.51±355.49	401.99±84.13
1400.24±490.26	2253.32±431.19	1661.03±214.1	1311.9±352.09	1851.68±346	1761.63±444.81	1561.58±253.17	1202.87±95.23	1743.63±311.75	1177.93±398.07
3911.38±770.25	4057.1±1065.72	4091.55±1012.43	3905.82±671.62	3138±587.95	3559±1163.8	3427.97±761.39	2436.8±263.34	4344.01±1006.38	3582.77±656.98
118709.04±12145.76	112805.12±10786.68	109208.75±11316.3	107728.25±9857.3	177126.62±17640.29	178202.97±15471.91	179763.43±22098.7	160413.7±17426.88	159408.74±12613.05	163663.85±17344.57
9.98±4.75	10.57±3.97	7.89±2.22	6.58±1.46	10.17±0.89	9.42±1.08	8.9±2.31	9.51±2.33	8.89±1.99	8.49±1.04
2.03±0.8	2.45±1.76	2.31±1.13	1.64±0.79	3.41±0.64	3.71±1.08	3.1±1.38	3.37±1.62	2.97±1.03	2.49±0.74
111263.29±18489.68	84947.28±12437.26	92454.09±17194.55	94762.59±18071.24	181170.82±36204.95	184074.43±23929.88	193482.33±60301.19	156057.4±41463.33	155827.85±30786.41	173978.51±31969.46

EC1118 13.5Bé N400 MLF-	EC1118 12.5Bé N0 MLF-	EC1118 12.5Bé N200 MLF-	EC1118 12.5Bé N400 MLF-	R2 16Bé N0 MLF-	R2 16Bé N200 MLF-	R2 16Bé N400 MLF-	R2 14.5Bé N0 MLF-	R2 14.5Bé N200 MLF-	R2 14.5Bé N400 MLF-
2.93±0.04	2.64±0.04	2.66±0.04	2.67±0.05	2.84±0.04	2.81±0.03	2.87±0.05	2.63±0.03	2.6±0.03	2.63±0.02
2.1±0.14	1.8±0.03	1.96±0.02	1.96±0.07	3.22±0.03	3.79±0.58	3.54±0.12	3.03±0.1	2.9±0.01	3.02±0.07
0.7±0.61	0.69±0.6	0.63±0.55	0.29±0.5	1±0.02	0.3±0.51	0.27±0.47	0.65±0.56	0.82±0.03	0.53±0.46
6.22±0.05	5.75±0.03	5.77±0.01	5.73±0.06	7.87±0.04	7.98±0.04	7.98±0.08	7.12±0.06	7.08±0.09	7.11±0.05
0.26±0.02	0.16±0.04	0.23±0.02	0.22±0.03	0.12±0.01	0.15±0.06	0.11±0.01	0.24±0.07	0.27±0.03	0.24±0.01
101.14±0.59	92±0.21	92.65±0.27	91.95±0.31	122.54±0.27	122.35±0.49	122.24±0.46	109.37±0.47	109.05±0.7	109.07±0.3

R2 13.5Bé N0 MLF-	R2 13.5Bé N200 MLF-	R2 13.5Bé N400 MLF-	R2 12.5Bé N0 MLF-	R2 12.5Bé N200 MLF-	R2 12.5Bé N400 MLF-	NO Yeast 16 Be N0 MLF-	NO Yeast 14.5 Be N0 MLF-	NO Yeast 13.5 Be N0 MLF-	NO Yeast 12.5 Be N0 MLF-
1630.23±152.5	1531.15±74.51	1470.44±51.87	1459.07±67.13	1140.58±52.74	1246.44±52.2	89.37±66.58	93.58±87.88	119.35±92.89	114.08±81.59
14.52±4.98	15.2±6.3	15.11±5.73	15.53±9.75	12.45±0.98	13.16±2.52	0.72±0.67	1.27±0.61	0.88±0.72	0.89±0.74
0.76±0.33	0.72±0.28	0.74±0.15	0.89±0.29	0.67±0.17	0.7±0.17	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01
0.39±0.09	0.36±0.02	0.37±0.03	0.34±0.02	0.3±0.01	0.25±0.03	0.03±0.01	0.05±0.04	0.06±0.02	0.04±0
5.08±0.43	5.83±0.46	6.09±0.25	4.61±0.58	4.79±0.26	5.21±0.48	0.14±0.14	0.16±0.19	0.21±0.19	0.2±0.17
1.62±0.76	1.54±0.76	1.53±0.32	1.68±0.51	1.33±0.41	1.43±0.37	0±0	0±0	0±0	0.01±0
0.15±0.05	0.14±0.05	0.14±0.02	0.14±0.04	0.13±0.03	0.13±0.03	0±0	0±0	0±0	0±0
137977.49±21045.73	129447.78±14980.08	170366.03±65187.59	212565.18±147448.1	166882.56±91445.01	110906.56±5436.18	465.75±501.93	1327.85±1135.74	257.81±146.18	575.02±411.96
11.63±6.33	8.66±1.77	13.78±8.27	6.26±3.76	11.02±4.75	11.42±3.78	0.41±0.33	0.81±1.01	0.66±0.61	0.78±0.93
7.71±2	5.99±1.5	2.49±1.32	5.53±3.86	3.97±2.57	3.25±1.55	0.03±0.03	0.04±0.02	0.04±0.01	0.03±0.02
0.24±0.08	0.32±0.11	0.25±0.03	0.17±0.08	0.2±0.05	0.23±0.04	0±0	0±0	0±0	0.01±0
1051.02±36.01	1127±59.95	1027.66±86.25	791.25±86.51	952.06±50.04	929.42±56.41	8.24±6.16	9.47±9.14	9.48±5.83	9.96±6.24
191.93±22.66	193.49±6.77	178.27±17.08	121.69±15.4	144.15±3.51	157.01±6.9	1.21±0.92	1.68±1.35	1.5±0.79	1.4±1.09
3.1±0.11	3.98±0.61	3.85±0.44	3.45±1.11	3.85±0.33	3.83±0.41	0.28±0.04	0.27±0.01	0.3±0.03	0.31±0.03
8.92±2.4	7.52±1.6	2.93±1.23	5.48±4.83	4.46±2.93	3.72±1.58	0.01±0.01	0.02±0.01	0.02±0.01	0.01±0.01
1010.99±671.64	598.94±47.43	483.2±437.71	804.82±771.55	333.62±282.57	316.36±246.99	164.47±73	161.27±36.7	111.69±57.94	114.19±29.13
0.01±0	0.02±0	0.02±0	0.01±0	0.01±0	0.01±0	0±0	0±0	0±0	0±0
1237.68±200.91	1427.1±88.21	1533.87±308.74	1197.11±205.93	1249.74±50.5	1459.44±248.47	44.27±39.35	84.29±89.64	32.39±47.24	10.57±18.31
0.72±0.28	0.42±0.02	0.2±0.05	0.36±0.2	0.21±0.09	0.19±0.07	0±0	0.01±0.01	0.01±0	0±0
747.94±57.13	890.82±9.69	1120.08±131.19	709.2±171.36	746.94±62.43	902.99±212	159.71±66.08	149.65±44.42	104.42±37.06	151.23±66.11
13.71±1.2	10.88±2.92	9.47±0.91	9.19±1.51	6.42±2.28	6.52±1.58	0.13±0.07	0.21±0.1	0.16±0.03	0.11±0.04
0.02±0	0.02±0.01	0.02±0	0.02±0.01	0.02±0.01	0.02±0	0±0	0±0	0±0	0±0
3117.03±279.68	3611.5±165.57	4410.06±961.13	3137.77±1111.01	3239.95±53.66	3355.63±664.39	157.58±128.4	155.63±61.6	117.32±32.01	140.82±69.59
2889.96±239.7	3262.73±144.19	4027.75±814.98	2941.75±1020.9	2962.14±108.16	3120.5±580.25	151.68±59.08	151.94±61.36	131.8±59.18	135.62±62.76
3093.32±531.99	2340.43±1380.29	1677.52±333.29	2290.17±485.44	2345.07±743.54	1701.04±555.57	0±0	0.53±0.92	1.14±1.24	0.77±1.34
2.19±0.51	3.5±1.33	3.33±0.48	1.49±0.53	1.97±0.34	2.82±0.38	0±0	0.01±0	0.01±0	0±0
0.11±0.02	0.1±0.03	0.09±0.01	0.09±0.01	0.08±0.02	0.08±0.02	0±0	0±0	0±0	0±0
5260.05±180.79	5951.41±222.05	6769.6±606.66	5295.83±1086.18	6209.91±206.59	6530.56±524.3	1036.49±295.91	1235.69±383.19	1042.04±256.38	1231.58±769.61
28.97±4.23	29.19±7.03	28.74±4.16	24.57±4	26.58±5.69	26.88±4.81	12.71±1.22	11.88±1.66	11.83±1.93	10.56±2.34
0.1±0.02	0.14±0.05	0.14±0.03	0.09±0.02	0.12±0.01	0.14±0.02	0±0	0±0	0±0	0±0
225.52±53.41	294.2±89.56	320.85±29.79	200.68±127.54	238.91±69.09	270.85±40.5	92.78±30.67	128.42±55.71	142.85±52.31	131.72±129.49
1166.54±382.79	1362.73±365.68	1121.22±524.94	1303.75±259.1	1538.44±49.96	1104.14±438.61	24.32±15.93	27.1±8.54	35.88±23.49	31.66±20.82
2248.09±268.07	3589.73±591.13	3420.63±1031.65	2645.43±645.74	3956.86±835.27	3734.54±603.03	88.28±47.21	96.11±34.41	126±62.95	100.63±53.79
146802.48±15855.25	153754.09±19809.51	149094.16±17113.16	143323.63±13285.47	137558.51±10982.71	139608.15±15560.64	1720.28±1417.48	2164.38±1503.51	1610.65±1371.28	3057.66±861.93
7.66±2.64	8.28±1.86	7.39±1.69	8.33±1.32	6.3±2.05	7.37±1.9	0.2±0.02	0.16±0.05	0.19±0.09	0.17±0.08
1.84±0.96	1.99±0.96	1.47±0.33	1.45±0.85	1.23±0.73	1.4±0.58	0.11±0.16	0.01±0	0.04±0.01	0.02±0
119868.17±24876.06	138350.65±33418.35	144468.3±27287.21	97714.24±11684.31	114802.42±16415.29	131700.57±22601.3	2286.92±1926.89	1137.46±42.95	2066.1±352.81	2058.09±709.71

R2 13.5Bé N0 MLF-	R2 13.5Bé N200 MLF-	R2 13.5Bé N400 MLF-	R2 12.5Bé N0 MLF-	R2 12.5Bé N200 MLF-	R2 12.5Bé N400 MLF-
2.43±0.01	2.46±0.04	2.45±0.05	2.25±0.04	2.3±0.03	2.37±0.01
2.69±0.08	2.57±0.03	2.65±0.06	2.54±0.11	2.39±0.04	2.34±0
0.61±0.53	0.27±0.47	0.25±0.44	0.33±0.58	0.93±0.08	0.28±0.49
6.66±0.02	6.46±0.11	6.46±0.04	6.39±0.02	6.01±0.08	6.04±0.03
0.2±0.06	0.19±0.02	0.23±0.04	0.14±0.06	0.15±0.01	0.15±0.01
99.57±0.39	99.89±0.42	99.91±0.57	91.05±0.72	91.11±1.24	92.31±0.31

Supplementary Table 3. Compounds (measured by GC-MS: μg L^{-1} and HPLC: g L^{-1}) detected in final wines for all treatments except for those inoculated with lactic acid bacteria for malolactic fermentation. The "no yeast" controls were also analysed, in most cases volatiles were below detection limits, where they were measured they were many magnitudes lower than fermented treatments and thus the data is not shown. Blue highlighted cells indicate those measurements significantly different due to juice dilution (compared to the same yeast at 16 Bé) and orange highlighted cells indicate those significantly different due to nutrient addition (compared to the same yeast and same initial Bé juice). Significant differences were determined by ANOVA and significantly different data are indicated by different letters.

Significantly different to 16 Be (F0 only)
Significantly different to F0 (within Be)

Volatile compounds measured by GC-MS

ug L^{-1}	Ethyl acetate	Ethyl propanoate	Ethyl isobutyrate	Isobutyl acetate	Ethyl butanoate	Ethyl 2-methylbutyrate	Ethyl isovalerate	Isobutanol	Isoamyl acetate	Methyl hexanoate	Hexyl acetate	4-Methyl-1-pentanol	3-Methyl-1-pentanol	Ethyl lactate
EC1118 16Bé N0	1768.947 bc	12.727 a	0.420 ab	0.349 abc	6.947 abc	1.348 abc	0.096 abc	125698.228 ab	20.148 abc	4.149 a	0.340 abc	649.598 c	217.426 c	3.907 abc
EC1118 16Bé N200	1980.556 ab	11.857 a	0.425 ab	0.397 ab	7.277 ab	1.292 abc	0.092 abc	126726.052 ab	23.143 a	3.138 a	0.440 a	722.019 b	237.483 b	4.113 a
EC1118 16Bé N400	2043.336 a	14.160 a	0.489 ab	0.458 a	7.583 a	1.515 ab	0.107 ab	142155.403 a	18.030 abc	8.656 a	0.377 ab	790.116 a	268.574 a	4.042 a
EC1118 14.5Bé N0	1603.352 cd	21.009 a	0.409 ab	0.299 bcd	6.821 abc	1.200 abc	0.082 abc	114285.411 ab	15.930 abc	6.707 a	0.297 abc	447.196 e	121.685 d	3.861 abc
EC1118 14.5Bé N200	1744.157 bc	20.894 a	0.446 ab	0.304 bcd	7.440 a	1.248 abc	0.085 abc	119149.251 ab	13.811 abc	5.764 a	0.345 abc	473.063 de	115.042 de	3.744 abc
EC1118 14.5Bé N400	1587.542 cd	19.427 a	0.467 ab	0.340 bc	7.673 a	1.383 abc	0.093 abc	107815.645 ab	23.011 ab	7.603 a	0.289 abc	495.026 d	130.523 d	3.958 ab
EC1118 13.5Bé N0	1379.487 def	22.697 a	0.425 ab	0.268 cd	6.352 abcd	1.104 abc	0.079 abc	72809.370 ab	17.284 abc	6.098 a	0.284 bc	363.613 f	89.726 f	3.427 cde
EC1118 13.5Bé N200	1313.778 ef	16.980 a	0.550 ab	0.301 bcd	6.346 abcd	1.539 ab	0.102 abc	102496.484 ab	16.828 abc	9.946 a	0.260 bc	368.978 f	90.067 f	3.611 abcd
EC1118 13.5Bé N400	1568.332 cde	19.007 a	0.605 a	0.293 bcd	7.095 ab	1.627 a	0.112 a	73651.952 ab	11.690 abc	7.150 a	0.309 abc	390.471 f	98.629 ef	3.486 bcde
EC1118 12.5Bé N0	1205.213 f	18.859 a	0.396 ab	0.280 cd	5.541 bcd	0.883 bc	0.067 bc	66295.943 ab	11.435 bc	10.488 a	0.261 bc	296.611 g	67.265 g	3.122 def
EC1118 12.5Bé N200	1250.558 f	11.535 a	0.383 b	0.214 d	5.001 d	0.828 c	0.061 c	58513.274 b	9.075 c	1.580 a	0.263 bc	297.640 g	66.311 g	3.005 ef
EC1118 12.5Bé N400	1158.476 f	13.117 a	0.431 ab	0.249 cd	5.262 cd	0.969 abc	0.072 abc	64253.444 ab	8.563 c	5.557 a	0.212 c	296.473 g	65.565 g	2.853 f
Pr > F(Model)	< 0.0001	0.675	0.636	0.015	0.049	0.297	0.411	0.420	0.175	0.716	0.244	< 0.0001	< 0.0001	0.000
Significant	Yes	No	No	Yes	Yes	No	No	No	No	No	No	Yes	Yes	Yes

ug L^{-1}	Ethyl acetate	Ethyl propanoate	Ethyl isobutyrate	Isobutyl acetate	Ethyl butanoate	Ethyl 2-methylbutyrate	Ethyl isovalerate	Isobutanol	Isoamyl acetate	Methyl hexanoate	Hexyl acetate	4-Methyl-1-pentanol	3-Methyl-1-pentanol	Ethyl lactate
R2 16Bé N0	1942.120 ab	7.875 bcd	0.866 a	0.947 ab	7.757 a	1.412 a	0.149 a	247540.701 ab	56.127 a	4.153 bcd	0.831 a	1647.662 b	281.549 b	3.892 abcd
R2 16Bé N200	2129.914 a	7.552 cd	0.804 a	1.033 a	7.496 a	1.185 a	0.130 a	313769.050 a	47.817 a	2.091 d	0.840 a	1760.508 a	317.904 a	4.264 abc
R2 16Bé N400	1994.871 a	7.220 cd	0.818 a	0.920 b	7.209 a	1.267 a	0.135 a	237953.242 ab	46.150 a	1.566 d	0.730 a	1720.511 ab	320.411 a	4.323 ab
R2 14.5Bé N0	1641.650 c	8.552 abcd	0.619 a	0.422 d	5.286 cde	1.211 a	0.120 a	194559.799 ab	12.671 b	1.311 d	0.397 bc	1354.753 c	223.638 d	3.566 bcd
R2 14.5Bé N200	1708.911 bc	6.948 d	0.606 a	0.544 c	5.977 b	1.267 a	0.129 a	196773.961 ab	19.798 b	1.911 d	0.489 b	1385.129 c	241.955 cd	4.656 a
R2 14.5Bé N400	1655.257 c	9.013 abcd	0.709 a	0.447 cd	5.928 bc	1.328 a	0.135 a	194353.675 ab	18.931 b	2.027 d	0.415 bc	1444.979 c	258.234 bc	4.531 a
R2 13.5Bé N0	1630.226 c	14.518 abc	0.762 a	0.386 de	5.084 ef	1.621 a	0.146 a	137977.491 b	11.626 b	7.714 a	0.244 cd	1051.016 de	191.931 e	3.099 d
R2 13.5Bé N200	1531.145 c	15.201 ab	0.718 a	0.363 de	5.829 bcd	1.537 a	0.140 a	129447.783 b	8.658 b	5.989 ab	0.317 bcd	1126.997 d	193.490 e	3.984 abc
R2 13.5Bé N400	1470.441 cd	15.114 ab	0.736 a	0.366 de	6.089 b	1.533 a	0.145 a	170366.031 ab	13.783 b	2.493 cd	0.253 cd	1027.659 def	178.266 ef	3.850 abcd
R2 12.5Bé N0	1459.067 cd	15.534 a	0.890 a	0.343 def	4.612 f	1.679 a	0.144 a	212565.183 ab	6.262 b	5.530 abc	0.170 d	791.250 g	121.686 h	3.446 cd
R2 12.5Bé N200	1140.579 e	12.450 abcd	0.670 a	0.304 ef	4.788 ef	1.328 a	0.128 a	166882.558 ab	11.021 b	3.968 bcd	0.202 d	952.063 ef	144.151 gh	3.850 abcd
R2 12.5Bé N400	1246.438 de	13.162 abcd	0.702 a	0.251 f	5.206 def	1.431 a	0.133 a	110906.557 b	11.416 b	3.249 bcd	0.229 cd	929.416 f	157.008 fg	3.827 abcd
Pr > F(Model)	< 0.0001	0.082	0.963	< 0.0001	< 0.0001	0.982	0.999	0.419	< 0.0001	0.009	< 0.0001	< 0.0001	< 0.0001	0.046
Significant	Yes	No	No	Yes	Yes	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes

Compounds measured by HPLC

g L^{-1}	Malic Acid	Succinic Acid	Lactic Acid	Glycerol	Acetic acid	Acetaldehyde	Ethanol
EC1118 16Bé 0 FO MLF-	3.377 a	2.680 a	1.097 a	7.770 a	0.000 b	0.333 c	120.827 a
EC1118 16Bé 20 FO MLF-	3.367 a	2.717 a	0.673 ab	7.770 a	0.000 b	0.447 a	120.950 a
EC1118 16Bé 40 FO MLF-	3.357 a	2.797 a	1.133 a	7.790 a	0.033 a	0.453 a	119.983 a
EC1118 14.5Bé 0 FO MLF-	3.107 b	2.203 bc	0.717 ab	6.727 b	0.000 b	0.270 d	108.753 b
EC111814.5Bé 20 FO MLF-	3.103 b	2.253 b	0.650 ab	6.763 b	0.000 b	0.367 bc	108.177 b
EC1118 14.5Bé 40 FO MLF-	3.157 b	2.313 b	0.740 ab	6.813 b	0.000 b	0.403 ab	107.857 b
EC1118 13.5Bé 0 FO MLF-	2.877 c	1.967 de	0.000 b	6.163 c	0.000 b	0.207 ef	100.827 c
EC1118 13.5Bé 20 FO MLF-	2.917 c	2.073 cde	0.360 ab	6.153 c	0.000 b	0.273 d	100.170 c
EC1118 13.5Bé 40 FO MLF-	2.930 c	2.100 cd	0.700 ab	6.223 c	0.000 b	0.263 de	101.137 c
EC1118 12.5Bé 0 FO MLF-	2.640 d	1.803 f	0.687 ab	5.747 d	0.000 b	0.163 f	92.003 d
EC1118 12.5Bé 20 FO MLF-	2.663 d	1.960 e	0.633 ab	5.773 d	0.000 b	0.227 de	92.653 d
EC1118 12.5Bé 40 FO MLF-	2.667 d	1.960 e	0.290 ab	5.733 d	0.000 b	0.217 def	91.953 d
Pr > F(Model)	<0.0001	<0.0001	0.391	<0.0001	0.474	<0.0001	<0.0001
Significant	Yes	Yes	No	Yes	No	Yes	Yes

g L^{-1}	Malic Acid	Succinic Acid	Lactic Acid	Glycerol	Acetic acid	Acetaldehyde	Ethanol
R2 16Bé 0 FO MLF-	2.840 ab	3.217 b	1.003 a	7.873 b	0.333 a	0.120 d	122.537 a
R2 16Bé 20 FO MLF-	2.807 b	3.787 a	0.297 ab	7.980 a	0.313 a	0.147 cd	122.350 a
R2 16Bé 40 FO MLF-	2.867 a	3.537 a	0.273 ab	7.983 a	0.303 a	0.107 d	122.243 a
R2 14.5Bé 0 FO MLF-	2.627 c	3.033 bc	0.650 ab	7.123 c	0.277 a	0.237 ab	109.367 b
R2 14.5Bé 20 FO MLF-	2.600 c	2.903 cd	0.823 ab	7.077 c	0.137 b	0.267 a	109.050 b
R2 14.5Bé 40 FO MLF-	2.630 c	3.020 bc	0.527 ab	7.113 c	0.110 bcd	0.243 ab	109.070 b
R2 13.5Bé 0 FO MLF-	2.433 d	2.690 de	0.613 ab	6.663 d	0.123 bc	0.200 bc	99.573 c
R2 13.5Bé 20 FO MLF-	2.457 d	2.573 ef	0.273 ab	6.457 e	0.020 cd	0.193 bc	99.887 c
R2 13.5Bé 40 FO MLF-	2.447 d	2.647 de	0.253 b	6.463 e	0.023 cd	0.227 ab	99.910 c
R2 12.5Bé 0 FO MLF-	2.247 f	2.537 ef	0.333 ab	6.393 e	0.107 bcd	0.143 cd	91.047 e
R2 12.5Bé 20 FO MLF-	2.297 f	2.387 ef	0.933 ab	6.010 f	0.000 d	0.150 cd	91.107 e
R2 12.5Bé 40 FO MLF-	2.367 e	2.340 f	0.283 ab	6.040 f	0.017 cd	0.153 cd	92.310 d
Pr > F(Model)	<0.0001	<0.0001	0.328	<0.0001	<0.0001	0.000	<0.0001
Significant	Yes	Yes	No	Yes	Yes	Yes	Yes

Methyl octanoate	Acetic acid	Ethyl nonanoate	Isobutyric acid	Methyl decanoate	Butanoic acid	Ethyl decanoate	Isoamyl octanoate	Isovaleric acid	2-Methylbutanoic acid	Methionol	2-Phenylethyl acetate	Ethyl dodecanoate	Hexanoic acid	Benzyl alcohol	2-Phenylethyl propanoate	Octanoic Acid	2-Methoxy-4-vinylphenol	4-vinylphenol	Isoamyl alcohol	Ethyl hexanoate	Ethyl octanoate
5.899 ab	260.519 ab	0.009 b	601.359 c	0.652 b	894.955 bcd	20.383 bc	0.015 abc	2228.506 abc	2795.751 abc	1297.263 abc	4.830 ab	0.117 b	6107.293 e	37.019 abc	0.194 bcd	222.214 d	2544.424 a	4102.241 a	145139.951 a	11.483 a	3.284 a
5.111 ab	303.344 a	0.009 ab	768.112 abc	0.659 b	1024.724 abcd	27.175 ab	0.020 ab	2438.444 abc	2961.349 ab	1546.547 a	5.886 a	0.157 ab	8509.739 abc	38.509 ab	0.198 bcd	499.962 abcd	2228.150 ab	4055.882 a	144590.339 a	11.584 a	3.455 a
10.704 a	198.021 ab	0.012 a	1010.837 ab	1.877 a	1191.964 ab	37.508 a	0.021 a	3020.466 a	3548.075 a	1516.767 ab	6.007 a	0.211 a	8339.728 abcd	38.880 a	0.223 ab	695.116 a	1535.064 bc	3310.324 a	143960.979 a	11.474 a	3.231 a
5.393 ab	148.264 ab	0.009 ab	700.807 bc	0.503 b	854.408 cd	15.014 bc	0.012 c	2327.566 abc	2832.915 abc	1166.770 abcd	2.976 cde	0.097 b	6905.935 bcde	29.379 bcd	0.219 abc	263.233 cd	2028.072 abc	3522.712 a	129787.172 ab	11.813 a	2.398 a
4.867 ab	193.055 ab	0.010 ab	798.259 abc	0.498 b	1032.402 abc	19.039 bc	0.015 abc	2293.525 abc	2864.696 abc	1367.153 abc	3.866 bcd	0.113 b	8682.741 a	32.302 abcd	0.235 ab	511.926 abcd	1725.791 bc	3773.272 a	129635.030 ab	12.388 a	2.823 a
7.886 ab	71.506 b	0.009 ab	764.086 abc	0.740 b	997.523 abcd	15.133 bc	0.013 bc	2346.637 abc	2931.883 ab	1157.831 abcd	4.027 bc	0.096 b	8617.895 ab	31.372 abcd	0.289 a	604.238 ab	1431.928 c	3608.689 a	132704.757 ab	12.341 a	2.344 a
7.223 ab	116.210 ab	0.009 b	698.220 bc	0.526 b	784.940 cd	12.052 c	0.012 c	1809.915 bc	2106.958 bcd	1064.463 cde	2.237 de	0.088 b	6863.475 cde	28.996 cd	0.217 abc	318.697 bcd	2250.405 ab	4132.051 a	118268.242 b	9.801 a	1.915 a
11.945 a	111.442 ab	0.008 b	741.692 bc	0.878 b	919.228 bcd	12.197 c	0.012 c	2012.492 abc	2455.777 abcd	1251.152 abc	2.449 cde	0.093 b	8360.885 abcd	29.376 bcd	0.217 abc	444.784 abcd	1898.333 abc	4253.169 a	120678.321 ab	10.145 a	2.327 a
6.389 ab	136.379 ab	0.011 ab	1072.061 a	0.483 b	1300.594 a	15.322 bc	0.014 abc	2598.275 ab	3137.637 ab	1120.013 bcde	2.992 cde	0.110 b	9327.575 a	30.357 abcd	0.211 bcd	509.410 abcd	1400.244 c	3911.381 a	118709.043 b	9.979 a	2.031 a
11.587 a	135.523 ab	0.010 ab	501.282 c	0.767 b	710.177 d	10.877 c	0.012 c	1390.758 c	1529.896 d	723.303 e	1.689 e	0.094 b	6646.719 de	24.605 d	0.174 bcd	264.738 cd	2253.319 ab	4057.097 a	112805.124 b	10.567 a	2.447 a
2.312 b	65.562 b	0.012 a	676.589 c	0.299 b	859.231 cd	15.088 bc	0.015 abc	1513.797 c	1674.715 cd	776.108 de	2.072 e	0.127 b	9370.056 a	26.570 d	0.139 d	538.080 abc	1661.026 bc	4091.551 a	109208.752 b	7.891 a	2.307 a
6.030 ab	58.410 b	0.010 ab	766.036 abc	0.469 b	960.619 bcd	11.804 c	0.012 c	1806.538 bc	1974.250 bcd	745.961 e	1.823 e	0.103 b	9103.967 a	25.251 d	0.145 cd	568.554 ab	1311.901 c	3905.819 a	107728.255 b	6.582 a	1.639 a
0.317	0.396	0.250	0.079	0.234	0.047	0.007	0.202	0.156	0.058	0.002	< 0.0001	0.054	0.004	0.043	0.039	0.047	0.051	0.991	0.029	0.743	0.744
No	No	No	No	No	Yes	Yes	No	No	No	Yes	Yes	No	Yes	Yes	Yes	Yes	No	No	Yes	No	No

Methyl octanoate	Acetic acid	Ethyl nonanoate	Isobutyric acid	Methyl decanoate	Butanoic acid	Ethyl decanoate	Isoamyl octanoate	Isovaleric acid	2-Methylbutanoic acid	Methionol	2-Phenylethyl acetate	Ethyl dodecanoate	Hexanoic acid	Benzyl alcohol	2-Phenylethyl propanoate	Octanoic Acid	2-Methoxy-4-vinylphenol	4-vinylphenol	Isoamyl alcohol (1/100)	Ethyl hexanoate (1/100)	Ethyl octanoate (1/100)
7.929 ab	3577.538 ab	0.017 a	1401.002 bcd	0.703 ab	1018.644 bc	24.335 a	0.032 abc	2859.321 bc	2640.714 bc	1991.763 bcd	7.903 a	0.139 ab	5974.482 bcd	47.352 a	0.130 ab	258.247 bc	1851.679 a	3137.998 abcd	177126.622 ab	10.169 a	3.412 ab
4.321 abcd	3720.282 a	0.015 a	1403.506 bcd	0.348 bc	997.000 bc	21.816 abc	0.039 a	2859.855 bc	2562.149 bc	1379.255 cd	8.997 a	0.134 abc	6126.520 abc	46.616 a	0.105 ab	298.721 bc	1761.625 ab	3558.997 abc	178202.974 ab	9.421 ab	3.713 a
3.520 bcd	4509.363 a	0.017 a	1880.812 a	0.284 c	1248.355 a	21.895 ab	0.035 ab	3490.770 ab	3199.101 ab	1134.225 d	8.917 a	0.130 abcd	7453.072 a	46.403 ab	0.108 ab	399.425 ab	1561.584 abcd	3427.971 abcd	179763.434 a	8.902 ab	3.096 abc
2.359 d	3376.525 ab	0.018 a	1079.801 d	0.342 c	624.255 e	26.402 a	0.035 ab	2477.732 c	2221.780 c	4247.533 a	3.942 bc	0.155 a	4736.213 d	36.792 cde	0.100 ab	156.354 c	1202.873 bcd	2436.801 cd	160413.704 abcd	9.505 ab	3.366 ab
4.606 abcd	1802.530 bc	0.019 a	1149.448 cd	0.354 bc	797.814 cde	19.026 abcd	0.035 ab	2658.409 bc	2358.316 bc	2528.135 bc	5.809 b	0.136 ab	6208.116 abc	37.317 cd	0.129 ab	541.512 a	1743.625 abc	4344.011 a	159408.735 bcde	8.890 ab	2.967 abcd
3.019 cd	1262.973 c	0.017 a	1357.949 bcd	0.235 c	1008.933 bc	15.417 bcde	0.031 abc	3436.714 abc	3072.227 bc	1916.596 bcd	5.717 b	0.117 abcde	6460.088 abc	38.042 bc	0.144 a	401.989 ab	1177.932 bcd	3582.771 abc	163663.851 abc	8.487 abc	2.487 bcde
8.916 a	1010.992 c	0.014 a	1237.675 bcd	0.724 a	747.941 de	13.710 cdef	0.021 cd	3117.029 bc	2889.964 bc	3093.324 ab	2.194 cd	0.113 bcdef	5260.050 cd	28.973 def	0.103 ab	225.518 bc	1166.539 cd	2248.094 d	146802.482 cdef	7.655 bc	1.836 def
7.523 abc	598.938 c	0.018 a	1427.098 bcd	0.415 abc	890.820 cd	10.879 def	0.024 bcd	3611.503 ab	3262.732 ab	2340.426 bcd	3.501 c	0.100 bcdef	5951.407 bcd	29.189 def	0.144 a	294.202 bc	1362.726 abcd	3589.726 abc	153754.093 cdef	8.284 abc	1.993 cdef
2.931 cd	483.201 c	0.017 a	1533.871 ab	0.202 c	1120.084 ab	9.471 ef	0.021 cd	4410.062 a	4027.746 a	1677.523 cd	3.329 cd	0.090 def	6769.601 ab	28.736 ef	0.144 a	320.850 bc	1121.218 d	3420.632 abcd	149094.157 cdef	7.388 bc	1.469 ef
5.480 abcd	804.824 c	0.014 a	1197.112 bcd	0.355 bc	709.199 de	9.187 ef	0.017 d	3137.768 bc	2941.745 bc	2290.169 bcd	1.489 d	0.094 cdef	5295.833 cd	24.573 f	0.093 b	200.683 bc	1303.745 abcd	2645.434 bcd	143323.627 def	8.333 abc	1.448 ef
4.464 abcd	333.617 c	0.012 a	1249.742 bcd	0.214 c	746.944 de	6.418 f	0.017 d	3239.948 bc	2962.141 bc	2345.067 bcd	1.965 cd	0.076 f	6209.913 abc	26.577 f	0.122 ab	238.913 bc	1538.441 abcd	3956.861 a	137558.511 f	6.295 c	1.229 f
3.723 bcd	316.360 c	0.014 a	1459.444 bc	0.190 c	902.986 bcd	6.518 f	0.019 d	3355.628 bc	3120.496 abc	1701.042 cd	2.820 cd	0.081 ef	6530.561 abc	26.884 f	0.137 ab	270.847 bc	1104.140 d	3734.544 ab	139608.153 ef	7.367 bc	1.399 ef
0.126	0.000	0.778	0.011	0.059	0.000	< 0.0001	0.003	0.054	0.049	0.006	< 0.0001	0.007	0.026	< 0.0001	0.250	0.066	0.106	0.067	< 0.0001	< 0.0001	< 0.0001
No	Yes	No	Yes	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No	Yes	Yes	Yes

Phenylethyl alcohol
171614.276 a
180885.678 a
198504.714 a
119530.391 bc
123171.418 bc
136900.720 b
99590.497 cd
102239.133 cd
111263.286 bcd
84947.281 d
92454.094 cd
94762.586 cd
< 0.0001
Yes

Phenylethyl alcohol (1/100)
181170.823 ab
184074.426 a
193482.333 a
156057.404 abcd
155827.852 abcd
173978.507 abc
119868.174 def
138350.650 cde
144468.299 bcde
97714.239 f
114802.420 ef
131700.572 def
< 0.0001
Yes

Supplementary Table 4. Ratios of significantly different compounds measured by HPLC. Ratios of compounds detected in wines mad juices are in comparison to 16 Bé juices fermented by the same yeast, or in the case of where nutrient is added, to wines made from ju Bé. Increased ratios are highlighted in green and decreased in pink. Results for wines made from 16 Bé juices with no nutrient additio actual values (g L-1) ± SD. Significant differences were determined by ANOVA.

	Malic Acid	Succinic Acid	Lactic Acid	Glycerol	Acetaldehyde
EC1118 16Bé 0 FO MLF-	3.38±0.05	2.68±0.11	1.1±0.14	7.77±0.01	0.33±0.01
EC1118 16Bé 20 FO MLF-					1.34
EC1118 16Bé 40 FO MLF-					1.36
EC1118 14.5Bé 0 FO MLF-	0.92	0.82		0.87	0.81
EC111814.5Bé 20 FO MLF-					
EC1118 14.5Bé 40 FO MLF-					
EC1118 13.5Bé 0 FO MLF-	0.85	0.73	0.00	0.79	0.62
EC1118 13.5Bé 20 FO MLF-					1.32
EC1118 13.5Bé 40 FO MLF-					
EC1118 12.5Bé 0 FO MLF-	0.78	0.67		0.74	0.49
EC1118 12.5Bé 20 FO MLF-		1.09			1.39
EC1118 12.5Bé 40 FO MLF-		1.09			
Pr > F(Model)	<0.0001	<0.0001	0.391	<0.0001	<0.0001
Significant	Yes	Yes	No	Yes	Yes

	Malic Acid	Succinic Acid	Lactic Acid	Glycerol	Acetaldehyde
R2 16Bé 0 FO MLF-	2.84±0.04	3.22±0.03	1±0.02	7.87±0.04	0.12±0.01
R2 16Bé 20 FO MLF-		1.18		1.01	
R2 16Bé 40 FO MLF-		1.10		1.01	
R2 14.5Bé 0 FO MLF-	0.92			0.90	1.97
R2 14.5Bé 20 FO MLF-					
R2 14.5Bé 40 FO MLF-					
R2 13.5Bé 0 FO MLF-	0.86	0.84		0.85	1.67
R2 13.5Bé 20 FO MLF-				0.97	
R2 13.5Bé 40 FO MLF-				0.97	
R2 12.5Bé 0 FO MLF-	0.79	0.79		0.81	
R2 12.5Bé 20 FO MLF-				0.94	
R2 12.5Bé 40 FO MLF-	0.83			0.94	
Pr > F(Model)	<0.0001	<0.0001	0.328	<0.0001	0.000
Significant	Yes	Yes	No	Yes	Yes

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n are displayed as

Ethanol
120.83±0.29
0.90
0.83
0.76
<0.0001
Yes

Ethanol
122.54±0.27
0.89
0.81
0.74
1.01
<0.0001
Yes