The effect of grape juice dilution on oenological fermentation.

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Highlights: Gardner et al. The effect of grape juice dilution on fermentation

- Grape juice dilution shortened both alcoholic and malolactic fermentation
- In some conditions addition of commercial nutrient decreased fermentation duration
- In general wine volatiles decrease with grape juice dilution
- Isoamyl acetate can be decreased in wine by grape juice dilution

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Abstract

The impact of water addition to grape juice in winemaking, on both alcoholic and malolactic fermentation duration and outcome has been examined using commercial wine yeasts, Lalvin EC1118TM and Lalvin R2TM and malolactic bacteria Lalvin VP41TM. 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 R2TM and in some conditions also reduced the duration of malolactic fermentation. In general, volatile compounds and some major yeast metabolites 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 compounds in 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, dilution, yeast, nutrient, volatile compounds*

1. Introduction

The addition of water pre-fermentation is legal and commonplace in many countries, including more recently, Australia. The wording of legislature around water additions varies between countries, for instance additions are allowed “to facilitate fermentation” (USA, Australia), “pre-fermentation and not to below 13.5 °Bé” (Australia) or “where required on account of a specific technical necessity” (European Union). 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 specification. 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 (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, presumably this 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, as reported by (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 EC1118TM (aromatically neutral) and Lalvin R2TM (aromatic) over a range of juice dilutions (16 Bé juice diluted to 14.5, 13.5 and 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 (Schelezi et al., 2020; Teng et al., 2020). Nitrogen was ameliorated to be equal across all juices to ensure this was not impacting fermentation dynamics. Yeast nutrient (FERMAID® O, an organic complex nutrient; Lallemant) 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 (Torrea, Fraile, Garde, & Ancin, 2003; Bell & Henschke, 2005), 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™ and Lalvin R2™ (Lallemant, Canada) were chosen due to their common commercial use, variation in nutrient requirements and contribution to wine aroma (Lalvin EC1118™ regarded as neutral and Lalvin R2™ as aromatic). Yeast were grown from single colonies in 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 2.5×10^6 cells mL⁻¹ in diluted juice (45%, sterile (0.22 µm), 2018 Viognier-Marsanne blend, 10% YPD, 45% water) and grown overnight. This culture was used to inoculate experimental fermentations.

Oenococcus oeni malolactic bacteria strain VP41™ (Lallemant) was chosen as it is widely used in the Australian wine industry. 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) supplemented with 20% (v/v) apple juice and 0.1% cyclohexamide (v/v)) for 4 days at

97 30 °C and 20% CO₂. The culture was centrifuged (10 min, 4600 x g), and the pellet was washed in
98 phosphate buffered saline (PBS) and re-centrifuged. The cell pellet was washed with 50 mL
99 Viognier-Marsanne juice (13.5 Bé, 0.22 µm), spun down and resuspended in 50 mL Viognier-
100 Marsanne juice. Light microscopy confirmed the culture as viable *O. oeni*. The culture was grown
101 overnight at 30 °C and 20% CO₂ prior to inoculation at 1:100 (0.25 mL) in the experimental
102 fermentations. Culture viability was analysed using spot plating (10 uL of serially diluted cultures
103 were grown on MRSAJ with 2% agar, and colonies enumerated post growth).

104 A filter sterile (0.22 µm) 2018 Viognier-Marsanne (~75% Viognier) blended juice was used for
105 experimental fermentations (16 Bé, pH 3.2, 3.13 g L⁻¹ malic acid, 18:63 mg L⁻¹ free:total SO₂, 60
106 mg L⁻¹ ammonia, 196 mg L⁻¹ alpha amino nitrogen, 245 mg L⁻¹ yeast assimilable nitrogen (YAN)).
107 Juices were diluted with ultrapure water (pH 7.0) to 14.5, 13.5 and 12.5 Bé (10.5, 17.2 and 24%
108 water). Density after addition of water was calculated instead of measured as, based on experience,
109 measurement at that stage is inaccurate. Nitrogen was also adjusted in diluted juices to 245 mg
110 YAN L⁻¹ with diammonium phosphate and complex organic nutrient (FERMAID® O, Lallemand)
111 was added where indicated at either 0 (control), 200 or 400 mg L⁻¹.

112 2.2 Experimental fermentation

113 Fermentations were conducted with a custom-made high throughput robotic platform ‘Tee-bot
114 v.2.0’ built on an EVO freedom workdeck (Tecan, Männedorf, Switzerland) that can accommodate
115 384 discreet fermentations and sample automatically at programmable intervals. Fermentation
116 vessels had custom-made airlocks that allowed sampling through a silicon septum and were mixed
117 at inoculation and briefly before sampling with magnetic stir bars. Viognier-Marsanne juice was
118 pressed from solids to alleviate the complexity of changing the juice:solid ratio by dilution in
119 fermentations typically undertaken with most red wines. Temperature was regulated to 17 °C by a
120 water bath that housed the fermentation flasks. 25 mL of juice (ameliorated with dilution or addition
121 of FERMAID® O) was inoculated with 5 x 10⁶ yeast cells mL⁻¹ (either EC1118™ or Lalvin R2™).
122 After 24 hours, experimental fermentations treated with lactic acid bacteria (LAB+) were inoculated

with 0.25 mL of cultured VP41. Control (LAB-) fermentations had 0.25 mL sterile Viognier-Marsanne juice added. Fermentations were performed in triplicate. This combination of treatments of yeast (Lalvin EC1118TM, Lalvin R2TM or none), dilution (16, 14.5, 13.5 or 12.5 Bé), nutrient addition (0, 200 or 400 mg L⁻¹) and LAB (+/-) resulted in 216 unique fermentations. During fermentation, sugars (glucose, fructose) and malic acid consumption were monitored using modified commercial enzymatic kits (Megazyme, Bray, Ireland) as described in (Walker et al., 2014) and (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 terminal fermentation samples by HPLC according to (Lin, Boss, Walker, Sumby, Grbin, & Jiranek, 2020).

Terminal fermentation samples were also analysed by solid phase micro-extraction (SPME)-GC/MS according to Hranilovic et al. (2018) with extraction and chromatographic condition as outlined by Boss and coworkers (2015). 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 Statistical 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) and utilised standard scores derived from the volatile compound concentrations measured in each wine sample.

149 3. Results and Discussion

150 3.1 Dilution and addition of nutrients reduced alcoholic and malolactic fermentation duration

151 Both alcoholic (AF) and malolactic fermentation (MLF) duration were shortened in diluted juices,
 152 as is expected where initial sugar concentrations were reduced (AF, Fig. 1 and 2; MF, data not
 153 shown). For instance, AF of juice diluted from 16 Bé to 12.5 Bé was reduced in duration with the
 154 use of Lalvin EC1118TM by 126 hours, or 42% (298 vs 172 h). AF of undiluted juices by Lalvin
 155 R2TM was slightly longer (in comparison to Lalvin EC1118TM), however, dilution resulted in a
 156 similar reduction of fermentation duration of 121 hours or 34% (355 v 234 h). Dilution to 13.5 Bé
 157 or below also allowed the completion of MLF within 45 days in wines fermented by either yeast,
 158 whereas MLF of higher Baumé juices failed to complete in this time frame, leaving residual malic
 159 acid ($>1.0 \text{ g L}^{-1}$) (Supp. Tab. 1). The very slow MLF in these conditions is thought to be particular
 160 to this juice, perhaps due to limitation of a micronutrient or presence of an inhibitor.

161 The effect on fermentation dynamics of diluted juices with nutrient addition was also evaluated, this
 162 was in an effort to alleviate effects, if any, of micronutrient dilution. Additions were made at an
 163 industry standard rate, as recommended by the manufacturer (200 mg L^{-1}) and also at a higher rate
 164 (400 mg L^{-1}). The addition of nutrient had no significant impact on AF duration by Lalvin
 165 EC1118TM in any juice (data not shown), however, fermentations by Lalvin R2TM, were up to 76
 166 hours (26%) shorter (Fig. 3). No difference in fermentation dynamics between juices supplemented
 167 at either nutrient rate was observed, except in 13.5 Bé juice. Moreover, when either 200 or 400 mg
 168 L^{-1} of nutrient was added to 16 Bé juice, fermentation was reduced by 5 hours or 48 hours in 14.5 or
 169 12.5 Bé juices, respectively (data not shown). Whereas addition of 400 mg L^{-1} of nutrient added to
 170 13.5 Bé juices reduced fermentation by 76 hours in comparison to 52 hours when only 200 mg L^{-1} of
 171 nutrient was added and malolactic acid bacteria (MLB) were present (Fig. 3). The presence of MLB
 172 also had no effect on AF duration (Fig. 3).

173 The addition of nutrient shortened MLF. Juice diluted to 13.5 Bé with 200 or 400 mg L⁻¹ of nutrient
174 and with AF undertaken by Lalvin EC1118TM completed MLF in 47 hours less (4.7% reduction)
175 than when no nutrient was added (Supplementary Table 1).

176 **3.2 Dilution and nutrient addition changed the chemical composition of wines**

177 The effect of juice dilution on volatile compounds and major yeast metabolites of the resulting
178 wines was examined. Similar to other studies (Schelezkki et al., 2020; Schelezkki, Suklje, et al.,
179 2018), the dilution of juices modified the final concentrations of many compounds. Of the 38
180 volatile compounds analysed, 22 were significantly different to the control in at least three
181 treatments (Table 1) with 19 influenced by dilution and 17 by nutrient addition. In almost all cases,
182 dilution of juices resulted in a reduction in the concentration of volatiles, presumably by dilution of
183 juice-derived precursors that arise from major metabolic pathways such as glycolysis. This is
184 outcome is similar to that seen by Schelezkki et al. (2020) where many compounds were reported to
185 decrease upon addition of water to Shiraz juice prior to fermentation.

186 2-Phenylethanol, described as contributing aromas of rose, honey and spice, was detected in all
187 fermentations above its aroma threshold, 14 mg L⁻¹ (Ferreira, Lopez, & Cacho, 2000), and with
188 almost all dilutions was significantly reduced (70 - 49% or -1.44 to -2.02 Fold change (FC)). Its
189 ester, 2-phenylethyl acetate was also reduced with dilution (62 - 19% or -1.62 to -5.31 FC). In
190 undiluted fermentations, isoamyl alcohol and, in fermentations conducted with Lalvin R2TM, its
191 ester, isoamyl acetate were also detected above their aroma thresholds. Isoamyl acetate could be
192 reduced to below its aroma threshold with any of the trialled dilutions (Fig. 4). Typically, isoamyl
193 acetate was reduced to around 20% of the control (6 +/-3 to 13 +/- 9 vs 56 +/- 3 µg L⁻¹, Supp. Tab.
194 2). This ester can contribute an overpowering banana aroma, especially in white wines, and as such,
195 a dilution strategy (where grapes have an elevated Bé) may represent an option for desirable flavour
196 modification during winemaking.

Acetic acid was also reduced in diluted fermentations conducted with Lalvin R2TM, 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 impact wine quality. The primary alcohols 3 and 4-methyl-1-pentanol were significantly reduced in almost all diluted juices. For instance, with the use of Lalvin EC1118TM, 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). 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 R2TM 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 R2TM.

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 R2TM (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). 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 R2TM, 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 (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 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 (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 R2TM*, dilution did result in 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 (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) 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; 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

275 with plentiful oxygen, and thus saturated fatty acids should be far exceed the concentration of non-
 276 saturated fatty acids thus reducing the possibility of feedback inhibition of the FAS complex.
 277 Changes to the redox status of yeast have also been shown to affect volatiles produced by yeast
 278 (Bloem et al., 2016). Of particular relevance to this study, Bloem and colleagues (2016) report
 279 decreased accumulation of medium chain fatty acids (hexanoic and octanoic) during increased
 280 demand for NADPH or NADH. As hexanoic, octanoic and butanoic acids were detected many
 281 magnitudes above their aroma threshold (hexanoic: 420 $\mu\text{g L}^{-1}$ (Guth, 1997), octanoic: 500 $\mu\text{g L}^{-1}$
 282 and butanoic: 173 $\mu\text{g L}^{-1}$ (Ferreira et al., 2000)), we expect these difference would translate to a
 283 sensorial difference. This group of compounds is commonly described as sweaty and cheesy. The
 284 presence of fatty acids have also been shown to translate to increases in their fatty acid ethyl ester,
 285 for instance ethyl hexanoate and ethyl butanoate (Saerens et al., 2008; Saerens et al., 2006). In this
 286 study, the sensorially desirable esters, ethyl lactate and ethyl butanoate, increased with the addition
 287 of nutrient (up to 131 and 120%, respectively in wines fermented by Lalvin R2™). Ethyl lactate is
 288 described as fruity and ethyl butanoate as floral, fruity, and strawberry like, but in this study they
 289 occurred below their aroma thresholds. Exogenously added amino acids have been suggested to be
 290 direct precursors of esters, however studies report variable effects of changes in nutrition upon the
 291 ester composition of wine, which may be a reflection of changes in juice composition, yeast strain
 292 and amount and timing of additions (Hernández-Orte, Ibarz, Cacho, & Ferreira, 2005; Torrea et al.,
 293 2011).

294

295 Both 3 and 4-methyl-1-pentanol increase with the addition of nutrient in the order of 9-29%, in
 296 comparison to wine made from the juice of the same Bé. The most consistent increases of these
 297 compounds occurred with addition of 400 mg L^{-1} nutrient, however the magnitude of increases was
 298 always well below that of the effect of the initial dilution, thus not resulting in full recovery of
 299 reduced volatiles caused by dilution. An increase in these compounds may simply reflect an

300 increase in flux through glycolysis and/or fatty acid synthesis as the 2-keto acid precursors for their
 301 formation might be originally derived from pyruvate, as has been shown possible with synthetic
 302 pathways built from a combination of yeast and bacterial genes with the goal of efficient production
 303 of these compounds for biofuel (Zhang, Sawaya, Eisenberg, & Liao, 2008). This is supported since
 304 the *Saccharomyces cerevisiae* enzyme Adh6p, an alcohol dehydrogenase with a strict specificity for
 305 NADPH, is capable of completing the final step in formation of methyl pentanols (Zhang et al.,
 306 2008). Other aliphatic alcohols can be formed by yeast via the Ehrlich pathway, however these are
 307 limited to 5-carbon chains as determined by amino acid precursors (Hazelwood, Daran, van Maris,
 308 Pronk, & Dickinson, 2008). 2-Methyl-4-vinylphenol, methyl octanoate, methyl decanoate, acetic
 309 acid and ethyl acetate and methionol were found to decrease with the addition of nutrient in selected
 310 conditions (Table 1), with methionol being previously reported to decrease with exogenous
 311 additions of nitrogen (ca. 70%; Hernández-Orte et al., 2005).

312 Few differences in major metabolites were detected with added nutrient (Supp. Tab. 4). The most
 313 prominent, as with dilution, was an increase of acetaldehyde. Whether this is simply a reflection of
 314 increased flux through the glycolytic pathway or accumulation due to reduced availability of
 315 cofactors to drive the activity of alcohol dehydrogenases remains to be proven. Acetaldehyde is
 316 generally regarded as undesirable when occurring above 100-125 mg L⁻¹, as occurred in this study
 317 (120-330 mg L⁻¹).

318 Principal Component Analysis of measured volatiles highlighted the interaction between dilution
 319 and yeast strain, with the effect of both of these variables on wine volatile compound composition
 320 being explained by both of the first 2 principal components (Fig. 5). Wines made with either yeast
 321 and from diluted juices were clearly separated, with wines made with Lalvin EC1118TM located
 322 more towards the right and the top of the score plot (i.e. they had more positive PC1 and PC2
 323 values) compared to the comparable juices fermented with Lalvin R2TM. The clear separation of
 324 these yeasts supports what is widely known in the winemaking industry as Lalvin R2TM can make a

significant contribution to wine aroma, thereby it is marketed as “aromatic”, whereas Lalvin EC1118TM is considered “neutral”. 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. 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 R2TM by up to 26%. 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. Also the addition of complex organic nutrient, irrespective of dilution rate resulted in increased medium chain fatty acids (hexanoic, butanoic and octanoic characterised by sweaty, cheesy and rancid aromas). The concentrations of 22 volatile compounds were significantly different in three or more treatments. In fact a single treatment resulted in modifications to as many as 15 different volatile compounds and even if these are not above their aroma threshold values, they may act in concert to result in global changes to wine aroma (Escudero et al., 2007). Sensory studies would be of great benefit to determine which of these impacts result in wines that are detectably different to consumers. 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 on both processing efficiencies as well as effect on the aroma and flavour of wine.

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361 **Conflicts of Interest**

362 We wish to confirm that there are no known conflicts of interest associated with this publication and
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504

505 **Figure Legends**

506 **Figure 1.** Sugar catabolism by Lalvin EC1118TM (A) or Lalvin R2TM (B) of 16 Bé juice (●) and
507 juices diluted with water to 14.5 (■), 13.5 (▲) and 12.5 (◆) Bé without the addition of malolactic
508 bacteria or complex organic nutrient. Data presented are the average of triplicate fermentations,
509 with the error bars representing the standard deviation.

510 **Figure 2.** Alcoholic fermentation duration of Lalvin EC1118TM (EC) and Lalvin R2TM (R2), with
511 (MLB+) and without (MLB-) malolactic bacteria. 16 Bé juice was either fermented neat (■), or
512 diluted with water to 14.5 (■), 13.5 (■) or 12.5 (■) Bé. Data presented is the average of triplicate
513 fermentations and includes standard deviations. *Significantly different to fermentation duration of
514 16 Bé juice within the set (same yeast and MLB treatment), one way ANOVA, $p < 0.05$.

515 **Figure 3.** Alcoholic fermentation duration of Lalvin R2TM, in juice diluted to 13.5 Bé with (MLB+) and
516 without (MLB-) malolactic bacteria and with the addition of complex organic nutrient at 0 (□),
517 200 (■) or 400 (■) mg L⁻¹. Data presented is the average of triplicate fermentations and includes
518 standard deviations. Values with different letters are significantly different, one way ANOVA, $p <$
519 0.05.

520 **Figure 4.** Isoamyl acetate measured in wines (GC-MS) fermented by Lalvin EC1118TM or Lalvin
521 R2TM 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 plot of volatiles (based on 39 compounds measured by GC-MS) in wines fermented by Lalvin EC1118TM (EC) or Lalvin R2TM (R2). 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 axes 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 EC1118TM or Lalvin R2TM 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. – no significant difference. Actual values (µg L⁻¹) are shown for undiluted juices. Aroma threshold as reported in ^aMoreno et al. (2005), ^bGuth (1997), ^cFerreira et al. (2000), ^dPeinado, Moreno, Bueno, Moreno, & Mauricio (2004); Salo (1970), ^eEtievant (1991), ^fTakeoka et al. (1989), ^gGomez-Miguez, Cacho, Ferreira, Vicario, & Heredia (2007). *Volatiles detected above the aroma threshold in at least one treatment.

Supplementary legends

Supplementary Table 1. Residual malic acid after 1122 hours or hours taken to metabolise all malic acid (< 0.2 g L⁻¹) by VP 41 when co-inoculated with either EC1118TM or Lalvin R2TM 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.

546 **Supplementary Table 2.** Volatile and major yeast metabolic compounds ($\mu\text{g L}^{-1}$ +/- standard
547 deviations) detected in final wines for all treatments except for those inoculated with lactic acid
548 bacteria for malolactic fermentation. No yeast controls were also analysed, in most cases volatiles
549 were below detection limits, where they were measured they were many magnitudes lower than
550 fermented treatments and thus the data is not shown.

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

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

566 **Supplementary Figure 1.** Ethyl lactate measured in wines (GC-MS) fermented by Lalvin R2TM
567 with no malolactic bacteria added. Wines were made from juice with initial Bé of 16 or diluted with
568 water to 14.5, 13.5 or 12.5. Nutrient was also added to juices; 0 ($\square$), 200 ($\blacksquare$) or 400 ($\blacksquare$) mg L^{-1} .
569 Dashed line represents aroma threshold of isoamyl acetate (30 $\mu\text{g L}^{-1}$ (Guth, 1997)). Significantly

570 different values are labelled with different letters or letter groups (a-d), one way ANOVA, $p <$
571 0.05).

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1 Table 1. Gardner et al.

2

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 ^{5b}	2x10 ^{5g}	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
	14.5 Bé	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	-	-	-	-	-
		0	-1.79	-1.45	-	-	-	-1.62	-	-	-1.44	-	-	-	-	-	-	-	-	-	-	-	-	-
	13.5 Bé	200	-	-	1.26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		400	-	1.11	-	-	-	-	-	2.30	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		0	-2.42	-1.79	-	-1.28	-	-2.16	-	-	-1.72	-	-	-	-	-	-1.23	-	-	-	-	-	-	-
	12.5 Bé	200	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		400	-	-	1.36	-	1.66	-	-	-	-	-1.61	-	-	-	-	-	-	1.54	-	-	-	-	-
		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
	14.5 Bé	200	1.13	1.07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		400	1.14	-	1.25	-	1.23	-	-	-	-	-	-	-	-	-	-	-2.48	1.34	-	-	-	-	-
		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
	13.5 Bé	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	-	-	-
		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
	12.5 Bé	200	-	-	-	-	-	-	1.15	-	-	-	-	1.29	-	-	-	-	-	-	-	-	-	-
		400	-	-	1.29	-	1.50	-	1.20	-	-	-	-	-	-	-	-	-3.58	-	-3.04	-	-	-	-
		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	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Figure 1. Gardner et al

Figure 2. Gardner et al

Figure 3. Gardner et al.

Figure 4. Gardner et al.

Figure 5. Gardner et al.

Supplementary Table 1. Gardner et al.

	Nutrient (mg L ⁻¹)	Lalvin EC1118 TM	Lalvin R2 TM	
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	Residual malic acid (g L ⁻¹)
	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 Table 2. Volatile and major yeast metabolic compounds ($\mu\text{g L}^{-1}$ +/- 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.

$\mu\text{g L}^{-1}$	EC1118 16Bé NO MLF-	EC1118 16Bé N200 MLF-	EC1118 16Bé N400 MLF-	EC1118 14.5Bé NO MLF-	EC1118 14.5Bé N200 MLF-	EC1118 14.5Bé N400 MLF-	EC1118 13.5Bé NO 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 NO MLF-	EC1118 16Bé N200 MLF-	EC1118 16Bé N400 MLF-	EC1118 14.5Bé NO MLF-	EC1118 14.5Bé N200 MLF-	EC1118 14.5Bé N400 MLF-	EC1118 13.5Bé NO 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 NO MLF-	EC1118 12.5Bé N200 MLF-	EC1118 12.5Bé N400 MLF-	R2 16Bé NO MLF-	R2 16Bé N200 MLF-	R2 16Bé N400 MLF-	R2 14.5Bé NO 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 NO MLF-	EC1118 12.5Bé N200 MLF-	EC1118 12.5Bé N400 MLF-	R2 16Bé NO MLF-	R2 16Bé N200 MLF-	R2 16Bé N400 MLF-	R2 14.5Bé NO 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	0.31+/-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. Volatile and major yeast metabolic compounds (volatiles: $\mu\text{g L}^{-1}$ and major metabolites g L^{-1}) detected in final wines for all treatments except for those inoculated with lactic acid bacteria for malolactic fermentation. The "no yeast" controls were also analyzed, 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 yeast dilution (compared to the same yeast at 16 h) and orange highlighted cells indicate those significantly different due to nutrient addition (compared to the same yeast and same initial (h) juice). Significant differences were determined by ANOVA and significantly different data are indicated by different letters.

Significantly different to 16 h (FO only)														
Significantly different to FO (within h)														
Volatile compounds														
$\mu\text{g L}^{-1}$	Ethyl acetate	Ethyl propanoate	Ethyl isobutyrate	Isobutyl acetate	Ethyl butanoate	Ethyl 2-methylbutyrate	Ethyl isovalerate	Isobutanol	Isomyl acetate	Methyl hexanoate	Hexyl acetate	4-Methyl-1-pentanol	3-Methyl-1-pentanol	Ethyl lactate
EC1118 168h NO	1768.947 bc	12.727 a	0.420 ab	0.349 abc	6.947 abc	1.348 abc	0.096 abc	125696.228 ab	20.148 abc	4.149 a	0.340 abc	649.598 c	217.426 c	3.907 abc
EC1118 168h N200	1580.556 ab	11.857 a	0.452 ab	0.397 ab	7.277 ab	1.252 abc	0.092 abc	126756.052 ab	21.143 a	3.138 a	0.440 a	722.039 b	237.489 b	4.113 a
EC1118 168h 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 14.58h NO	1603.352 cd	21.089 a	0.409 ab	0.299 bcd	8.821 abc	1.200 abc	0.082 abc	114285.411 ab	15.390 abc	6.707 a	0.257 abc	647.139 bc	141.085 d	1.861 abc
EC1118 14.58h N200	1744.127 bc	20.881 a	0.446 ab	0.304 bcd	7.460 a	1.248 abc	0.085 abc	115149.251 ab	13.811 abc	5.764 a	0.345 abc	473.963 de	115.942 de	1.744 abc
EC1118 14.58h N400	1587.542 cd	19.427 a	0.467 ab	0.340 bcd	7.673 a	1.383 abc	0.093 abc	107815.645 ab	23.011 ab	7.603 a	0.289 abc	495.026 de	130.523 d	3.958 ab
EC1118 12.58h NO	1279.487 abc	22.697 a	0.425 ab	0.268 cd	6.932 abcd	1.106 abc	0.079 abc	73809.370 ab	12.236 abc	6.288 a	0.294 bc	363.632 f	109.248 f	2.427 cde
EC1118 12.58h N200	1313.778 ef	16.580 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 12.58h N400	1568.332 cde	18.007 a	0.605 a	0.293 bcd	7.095 ab	1.627 a	0.112 a	7851.952 ab	11.690 abc	7.150 a	0.309 abc	390.471 f	98.629 ef	3.486 bcde
EC1118 12.58h NO	1205.213 f	18.859 a	0.396 ab	0.280 cd	5.541 bcd	0.883 bc	0.067 bc	66295.941 ab	11.455 bc	10.488 a	0.261 bc	296.931 g	67.265 g	1.122 def
EC1118 12.58h N200	1250.558 f	11.538 a	0.383 b	0.114 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 12.58h N400	1158.476 f	11.117 a	0.431 ab	0.249 cd	5.262 cd	0.969 abc	0.072 abc	64252.444 ab	8.563 c	5.507 a	0.212 c	296.473 g	65.565 g	2.853 f
Pr + F(MAGe)	<0.0001	0.675	0.636	0.015	0.049	0.297	0.411	0.420	0.175	0.716	0.344	<0.0001	<0.0001	0.000
Significant	Yes	No	No	Yes	Yes	No	No	No	No	Yes	No	Yes	Yes	Yes

$\mu\text{g L}^{-1}$	Ethyl acetate	Ethyl propanoate	Ethyl isobutyrate	Isobutyl acetate	Ethyl butanoate	Ethyl 2-methylbutyrate	Ethyl isovalerate	Isobutanol	Isomyl acetate	Methyl hexanoate	Hexyl acetate	4-Methyl-1-pentanol	3-Methyl-1-pentanol	Ethyl lactate
R2 168h NO	1942.120 ab	7.875 bcd	0.866 a	0.947 ab	7.757 a	1.412 a	0.149 a	247450.701 ab	56.127 a	4.153 bcd	0.831 a	1647.662 b	281.549 b	3.892 abcd
R2 168h N200	2129.914 a	7.552 cd	0.804 a	1.033 a	7.496 a	1.185 a	0.130 a	113769.050 a	47.817 a	2.091 d	0.840 a	1760.508 a	317.904 a	4.264 abc
R2 168h N400	1994.871 a	7.220 cd	0.818 a	0.920 a	7.209 a	1.267 a	0.135 a	217951.242 ab	46.150 a	1.566 d	0.739 a	1720.511 a	320.413 a	4.321 ab
R2 14.58h NO	1641.650 e	8.552 abcd	0.619 a	0.822 d	5.286 cde	1.211 a	0.120 a	194559.799 ab	12.873 b	1.311 d	0.397 bc	1354.793 c	223.638 d	3.146 bcd
R2 14.58h N200	1708.811 bc	8.944 d	0.606 a	0.844 c	5.977 b	1.267 a	0.129 a	198773.961 ab	19.798 b	1.911 d	0.480 b	1381.129 c	241.955 d	4.686 a
R2 14.58h N400	1655.257 c	9.013 abcd	0.709 a	0.447 cd	5.928 bc	1.328 a	0.135 a	194351.675 ab	18.931 b	2.027 d	0.415 bc	1444.979 c	258.234 bc	4.531 a
R2 12.58h NO	1630.226 f	14.518 abc	0.762 a	0.386 cde	5.084 ef	1.623 a	0.146 a	17977.491 b	11.628 bc	7.734 a	0.244 cd	1051.016 de	191.933 e	3.091 d
R2 12.58h N200	1531.145 c	15.101 ab	0.718 a	0.361 cd	5.829 bcd	1.537 a	0.140 a	120407.781 b	8.628 b	5.980 ab	0.117 bcd	1124.997 d	191.490 e	1.968 abc
R2 12.58h N400	1470.441 cd	15.114 ab	0.736 a	0.366 cd	6.089 b	1.533 a	0.145 a	170366.031 ab	13.781 b	2.493 cde	0.253 cd	1027.659 def	178.266 f	3.850 abcd
R2 12.58h NO	1459.967 cd	15.514 ab	0.890 a	0.341 cde	4.832 f	1.679 a	0.144 a	212565.181 ab	6.262 bc	5.530 abc	0.170 b	791.250 e	121.886 f	1.446 cd
R2 12.58h N200	1340.796 e	12.400 abcd	0.870 a	0.304 ef	4.788 ef	1.328 a	0.138 a	166882.558 ab	11.021 b	1.968 bcd	0.202 d	952.268 ef	144.511 g	3.890 abcd
R2 12.58h 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	11.418 b	3.249 bcd	0.229 cd	929.416 f	157.008 fg	3.827 abcd
Pr + F(MAGe)	<0.0001	0.082	0.963	<0.0001	<0.0001	0.382	0.999	0.419	<0.0001	0.009	<0.0001	<0.0001	<0.0001	0.046
Significant	Yes	No	No	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Major Yeast metabolic compounds								
	Malic Acid	Succinic Acid	Lactic Acid	Glycerol	Acetic acid	Acetaldehyde	Ethanol	
EC1118 168h FO MLF ⁺	3.377 a	2.680 a	1.097 a	7.770 a	0.000 b	0.333 c	120.827 a	
EC1118 168h FO FO MLF ⁻	3.367 a	2.717 a	0.473 ab	7.770 a	0.000 b	0.447 a	120.980 a	
EC1118 168h FO MLF ⁻	3.357 a	2.787 a	1.133 a	7.780 a	0.033 a	0.453 a	115.950 a	
EC1118 14.58h FO MLF ⁻	3.107 b	2.203 bc	0.717 ab	6.272 b	0.000 b	0.276 d	108.793 b	
EC1118 14.58h FO FO MLF ⁻	3.103 b	2.253 b	0.650 ab	6.761 b	0.000 b	0.367 bc	108.177 b	
EC1118 14.58h FO MLF ⁻	3.157 b	2.313 b	0.740 ab	6.813 b	0.000 b	0.403 ab	107.857 b	
EC1118 12.58h FO MLF ⁻	2.877 c	1.967 de	0.000 b	6.163 c	0.000 b	0.307 ef	100.827 c	
EC1118 12.58h FO FO MLF ⁻	2.917 c	2.073 cde	0.360 ab	6.153 c	0.000 b	0.275 bc	100.120 c	
EC1118 12.58h FO MLF ⁻	2.930 c	2.100 cd	0.700 ab	6.223 c	0.000 b	0.263 de	101.137 c	
EC1118 12.58h FO MLF ⁻	2.649 d	1.805 f	0.687 ab	5.747 d	0.000 b	0.385 f	92.009 d	
EC1118 12.58h FO MLF ⁻	2.663 d	1.968 e	0.633 ab	5.773 d	0.000 b	0.227 de	94.653 d	
EC1118 12.58h FO MLF ⁻	2.667 d	1.968 e	0.290 ab	5.733 d	0.000 b	0.217 def	91.953 d	
Pr + F(MAGe)	<0.0001	<0.0001	0.391	<0.0001	0.474	<0.0001	<0.0001	
Significant	Yes	Yes	No	Yes	No	Yes	Yes	

	Malic Acid	Succinic Acid	Lactic Acid	Glycerol	Acetic acid	Acetaldehyde	Ethanol	
R2 168h FO MLF ⁻	2.840 ab	3.217 b	1.003 a	7.873 b	0.333 a	0.130 d	122.537 a	
R2 168h FO FO MLF ⁻	2.807 b	3.787 a	0.297 ab	7.980 a	0.313 a	0.147 cd	122.350 a	
R2 168h FO MLF ⁻	2.807 a	3.157 b	0.273 ab	7.988 a	0.303 a	0.107 d	122.243 a	
R2 14.58h FO MLF ⁻	2.627 c	3.013 bc	0.650 ab	7.133 c	0.277 a	0.237 ab	108.367 b	
R2 14.58h FO FO MLF ⁻	2.600 c	2.903 cd	0.821 ab	7.077 c	0.137 b	0.247 a	109.050 b	
R2 14.58h FO MLF ⁻	2.630 c	3.000 bc	0.827 ab	7.131 c	0.110 bcd	0.243 ab	109.070 b	
R2 12.58h FO FO MLF ⁻	2.433 d	2.690 de	0.613 ab	6.663 d	0.133 bc	0.200 bc	99.573 c	
R2 12.58h FO MLF ⁻	2.457 d	2.571 ef	0.279 ab	6.487 d	0.020 cd	0.193 bc	99.887 c	
R2 12.58h FO FO MLF ⁻	2.447 d	2.647 de	0.253 b	6.463 d	0.023 cd	0.227 ab	99.910 c	
R2 12.58h FO MLF ⁻	2.247 f	2.537 ef	0.131 ab	6.393 e	0.107 bcd	0.143 cd	91.047 e	
R2 12.58h FO MLF ⁻	2.297 f	2.187 ef	0.913 ab	6.001 f	0.000 d	0.150 cd	91.107 e	
R2 12.58h FO MLF ⁻	2.367 e	2.340 f	0.283 ab	6.040 f	0.017 cd	0.153 cd	92.310 e	
Pr + F(MAGe)	<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	Isomyl octanoate	Isovaleric acid	2-Methylbutanoic acid	Methionol	2-Phenylethyl acetate	Ethyl dodecanoate	Hexanoic acid	Benzyl alcohol	2-Phenylethyl propionate	Octanoic Acid	2-Methoxy-4-vinylphenol	4-vinylphenol	Isomyl alcohol	Ethyl hexanoate	Ethyl octanoate	Phenylethyl alcohol
5.111 ab	260.519 ab	0.009 b	601.399 c	0.652 b	894.959 bcd	20.383 bc	0.013 abc	2228.566 abc	2795.751 abc	1297.263 abc	4.830 ab	0.177 b	6.107 293 e	37.019 abc	0.194 bcd	222.214 d	2544.424 a	4102.241 a	145139.993 a	11.483 a	3.284 a	171814.276 e
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	0.213 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	138504.714 a
3.593 ab	148.284 ab	0.009 ab	700.807 bc	0.591 b	854.468 cd	15.014 bc	0.012 c	2167.746 abc	2612.915 abc	1166.791 abcd	2.976 abc	0.091 b	6905.325 cdef	29.279 bcd	0.219 abc	263.233 cd	2018.072 abc	3522.712 a	129787.172 ab	11.813 a	2.288 a	128960.981 bc
4.867 ab	193.053 ab	0.010 ab	798.379 abc	0.498 b	1052.462 abc	19.039 bc	0.015 abc	2293.555 abc	2864.696 abc	1367.133 abc	3.466 bcd	0.113 b	8682.743 b	31.302 abcd	0.235 ab	511.709 abcd	1725.791 bc	3773.273 a	129635.036 ab	12.388 a	2.823 a	123171.418 bc
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	31.577 abcd	0.289 a	604.238 ab	1433.928 c	3668.689 a	132704.757 ab	12.341 a	2.344 a	136900.720 d
7.223 ab	116.210 ab	0.009 b	698.220 bc	0.526 b	784.940 cd	12.022 c	0.012 c	1809.915 bc	2106.958 bcd	1064.463 cde	2.227 abc	0.080 b	6862.475 cde	28.995 cd	0.217 abc	218.697 bcd	2550.405 ab	4122.051 a	148268.242 b	9.801 a	1.915 a	99599.497 cd
11.945 a	111.442 ab	0.008 b	741.692 bc	0.878 b	915.228 bcd	12.197 c	0.012 c	2012.452 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	4263.169 a	120678.321 ab	10.145 a	2.327 a	102239.133 cd
6.389 ab	136.379 ab	0.011 ab	1072.063 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	1480.244 c	3911.383 a	118709.043 b	9.979 a	2.031 a	111263.286 bcd
11.587 a	135.523 ab	0.010 ab	901.282 c	0.797 b	710.177 d	10.877 c	0.012 c	1390.758 c	1828.898 d	723.303 e	1.689 ef	0.094 b	6646.718 ef	24.605 d	0.174 bcd	364.758 cde	2253.319 ab	4027.097 a	118655.124 b	10.567 a	2.447 a	86847.231 ef
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	92454.094 cd
6.038 ab	58.403 b	0.010 ab	766.036 abc	0.469 b	960.613 bcd	11.804 c	0.012 c	1806.538 bc	1974.250 bcd	745.961 e	1.823 e	0.101 b	9318.967 a	25.251 d	0.145 cd	568.554 ab	1311.903 c	3903.819 a	107728.252 b	6.582 a	1.639 a	94764.586 cd
6.317	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	0.091	0.028	0.743	0.744	<0.0001
No	No	No	No	No	Yes	Yes	No	No	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No	No	Yes	No	Yes

Methyl octanoate	Acetic acid	Ethyl nonanoate	Isobutyric acid	Methyl decanoate	Butanoic acid	Ethyl decanoate	Isomyl octanoate	Isovaleric acid	2-Methylbutanoic acid	Methionol	2-Phenylethyl acetate	Ethyl dodecanoate	Hexanoic acid	Benzyl alcohol	2-Phenylethyl propionate	Octanoic Acid	2-Methoxy-4-vinylphenol	4-vinylphenol	Isomyl alcohol	Ethyl hexanoate	Ethyl octanoate	Phenylethyl alcohol
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	3.432 ab	181170.823 ab
4.321 abcd	3720.282 a	0.015 a	1403.506 bcd	0.348 bc	997.020 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	179202.974 ab	9.421 ab	3.713 a	184074.426 a
1.520 bcd	4500.361 a	0.017 a	1880.832 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 b	46.403 ab	0.108 ab	399.625 ab	1561.584 abcd	3637.971 abcd	179701.434 a	8.902 ab	3.096 abc	139482.313 a
2.359 b	3376.525 ab	0.018 a	1079.801 d	0.342 c	624.255 b	26.402 a	0.035 ab	2477.732 c	2221.780 c	4247.533 a	3.942 bcd	0.155 a	4736.213 d	36.792 cde	0.100 ab	116.354 a	1202.873 bcd	2436.803 cd	160413.704 abcd	9.505 ab	3.361 abc	156057.404 abcd
4.606 abcd	1802.530 bc	0.019 a	1149.448 cd	0.334 bc	797.814 cde	19.026 abcd	0.059 ab	2656.409 bc	2158.316 bc	2528.135 bc	5.809 b	0.136 ab	6208.116 abc	37.317 cd	0.129 ab	541.522 a	1743.625 abc	4384.021 a	159408.735 bcde	8.890 abc	2.967 abcd	151627.852 abcd
8.019 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 abcd	6440.088 abc	38.042 bc	0.144 a	401.989 ab	1177.932 bcd	3582.771 abc	161663.951 abc	8.487 abc	2.487 bcd	173975.507 abc
8.916 a	1010.992 c	0.014 a	1237.675 bcd	0.724 a	747.941 bde	13.710 cdef	0.021 cd	3117.029 bc	2889.964 bc	3091.324 ab	2.194 cde	0.113 bcdef	5260.050 cd	28.973 cde	0.103 ab	225.518 bc	1166.539 cde	2248.094 d	146802.482 cdef	7.653 bc	1.836 cde	119868.174 cde
7.521 abc	506.938 c	0.018 a	1427.096 bcd	0.413 abc	690.820 cd	10.879 cde	0.024 bcd	3611.503 ab	3262.732 ab	2140.426 bcd	1.501 c	0.100 bcdef	5951.407 bcd	29.189 cde	0.144 a	294.302 bc	1362.729 abcd	1809.708 abc	151734.031 cdef	8.284 abc	1.993 cde	138150.650 cde
2.933 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 cde	6769.601 abc	28.736 ef	0.144 a	130.850 bc	1321.218 d	3420.632 abcd	149094.157 cdef	7.388 bc	1.469 ef	144446.299 bcde
1.480 abcd	804.824 c	0.014 a	1197.112 bcd	0.333 bc	799.189 cde	9.187 ef	0.037 cde	3117.768 bc	2741.745 bc	2220.169 bcd	1.489 f	0.094 cde	5275.831 cd	28.373 f	0.093 b	200.683 bc	1301.745 abcd	2645.414 bcd	143232.827 cde	8.111 abc	1.448 ef	97714.297
4.464 abcd	131.617 c	0.012 a	1249.742 bcd	0.214 c	746.944 cde	6.418 f	0.017 d	3238.948 bc	2962.141 bc	2145.097 bcd	1.960 cd	0.076 f	6209.161 abc	26.377 f	0.122 ab	238.913 bc	1338.441 abcd	3960.860 c	1375.561 f	6.796 c	1.239 f	114802.420 ef
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 abc	270.847 bc	1104.140 d	3734.544 abc	139608.151 ef	7.367 bc	1.399 ef	131700.572 cde
0.128	0.000	0.778	0.011	0.059	0.000	<0.0001	0.003	0.054	0.049	0.006	<0.0001	0.007	0.028	<0.0001	0.260	0.066	0.036	0.067	<0.0001	<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	No	Yes	Yes	Yes

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 pink. 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.

	Malic Acid	Succinic Acid	Lactic Acid	Glycerol	Acetaldehyde	Ethanol
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	120.83+/-0.29
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	0.90
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	0.83
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	0.76
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	<0.0001
Significant	Yes	Yes	No	Yes	Yes	Yes

	Malic Acid	Succinic Acid	Lactic Acid	Glycerol	Acetaldehyde	Ethanol
R2 16Bé 0 FO MLF-	2.84+/-0.04	3.22+/-0.03	1+/-0.02	7.87+/-0.04	0.12+/-0.01	122.54+/-0.27
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	0.89
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	0.81
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		0.74
R2 12.5Bé 20 FO MLF-				0.94		
R2 12.5Bé 40 FO MLF-	0.83			0.94		1.01
Pr > F(Model)	<0.0001	<0.0001	0.328	<0.0001	0.000	<0.0001
Significant	Yes	Yes	No	Yes	Yes	Yes

Supplementary Figure 1. Gardner et al.