The final publication is available at

http://dx.doi.org/ 10.1111/ajgw.12203

Additional Information

1 **Effect of acetaldehyde addition on the phenolic substances**

2 **and volatile compounds of red Tempranillo wines**

3

4 J. L. ALEIXANDRE-TUDO[1,3], V. LIZAMA[3], I. ÁLVAREZ[3], H.

5 NIEUWOUDT[2], M. J. GARCÍA[3], J. L. ALEIXANDRE[3] and W. J. DU TOIT[1,2]

6

7 [1] Department of Viticulture and Oenology and [2]Institute for Wine Biotechnology,

8 Stellenbosch University, Matieland, 7602, South Africa; [3] Departamento de Tecnología

9 de Alimentos, Universitat Politecnica de Valencia, Valencia, Spain

10

11 Corresponding author: Professor Wessel du Toit, email wdutoit@sun.ac,za

12

13 **Short title: Effect of acetaldehyde addition on red wines**

14

15

16

17

18

19

20

21

22   **Abstract**

23   **Background and Aims**: The introduction of controlled amounts of oxygen into red

24   wines influences the composition of the phenolic substances and volatiles, and therefore

25   thesensory properties of the wines. The main aim of this study was to evaluate the

26   impact of a simulation of the micro-oxygenation technique, through acetaldehyde

27   addition (AA), on colour, and the composition of phenolic substances and volatiles of

28   Tempranillo wines after 12 months of bottle storage.

29   **Methods and Results**: The analytical and sensory data were subjected to ANOVA,

30   principal component analysis (PCA) and orthogonal projection in latent structures

31   discriminant analysis (OPLS-DA). Addition of acetaldehyde led to an increase in the

32   anthocyanin fraction in coloured form, thus increasing wine colour, together with a

33   change in the composition of the volatiles. Acetaldehyde addition appeared to strongly

34   impact wine ester composition, with fatty acids and volatile phenols also being affected

35   by AA; furthermore AA positively influenced the concentration of volatiles.

36   **Conclusions**: Acetaldehyde addition to red wine caused an increase in the polymeric

37   fraction of the phenolic substances with its corresponding effect on wine colour density

38   and astringency index. Moreover the volatiles fraction has been better protected during

39   ageing in wines to which acetaldehyde has been added. The acetaldehyde treatments

40   therefore led to a clear difference in the chemical composition of the wines.

41   **Significance of the Study**: An increase in acetaldehyde in red wines can lead to

42   profound changes in the composition of phenolic substances and volatiles of the wines

43   and may serve as an alternative to oxygen addition in red wines where oxidation is

44   studied.

45

47

48 **Introduction**

49 The sensory properties of red wines depend mainly on the composition of phenolic

50 substances and volatiles. Anthocyanins, polymeric pigments, flavanols and their

51 polymers (tannins) are phenolic substances that have a considerable impact on the

52 sensory characteristics of red wine (Somers 1971). Wine aroma is one of the most

53 influential properties on consumer preference and is mostly determined by the

54 composition of the volatiles. The combination and concentration of volatile compounds

55 contribute to the overall wine aroma (Etievant 1991). Vine growing and winemaking

56 techniques may influence their concentration in wine (Perez-Prieto et al. 2003).

57 Micro-oxygenation (MOX) is a winemaking process in which small, controllable

58 amounts of oxygen are introduced into red wines (Gomez-Plaza and Cano-Lopez 2011).

59 The benefits claimed with this practice include improved wine colour and stability

60 (Mateus et al. 2002) and more complex sensory characteristics (Vidal et al. 2004,

61 Monagas et al. 2005). Moreover, the possible benefits of applying this technique include

62 an improvement of yeast performance during alcoholic fermentation (du Toit et al.

63 2006, Zoecklein 2007, Comfort 2008), a reduction of sulfur off-odours (Vidal and

64 Aagaard 2008, Cejudo-Bastante et al. 2011c) and also the ability to mimic the reactions

65 that occur during wine oak-aging (Cano-Lopez et al. 2007, Perez-Magariño et al. 2007,

66 du Toit 2010). .

67   Acetaldehyde in wine after fermentation is thought to be formed from the

68 oxidation of ethanol by peroxide or hydroxyl radicals (Singleton 1987, Waterhouse and

69 Laurie 2006, Danilewicz et al. 2008, Elias et al. 2009, Elias and Waterhouse 2010).

70 Micro-oxygenation also leads to acetaldehyde formation in wine, and its production has

71 been suggested as a means of monitoring this process. The addition of acetaldehyde

72 simulating MOX during fermentation (Sheridan and Elias 2015) and after bottling

73 (Aleixandre-Tudó et al. 2013) has been used in the past.

74   Micro-oxygenation has also been considered to influence wine aroma (Tao et al.

75 2007). To our knowledge the effect of acetaldehyde addition on the composition of

76 wine volatiles has never been investigated, being the main novelty of our study.

77 Contradictory results have been found in the literature regarding the effect of MOX.

78 Cejudo-Bastante et al. (2011a,b,c) reported varying effects depending on the volatile

79 group or the individual compound investigated. A slight difference was also found by

80 Hernandez-Orte et al. (2009), who remarked that the effect of MOX depends to a great

81 extent on the grape cultivar. In contrast, Ortega-Heras et al. (2008) did not observe

82 significant change in the concentration of volatile compounds, concluding that the effect

83 of MOX on wine volatiles depends on the main wine characteristics, such as cultivar,

84 origin or vintage.

85   Orthogonal projection in latent structures discriminant analysis (OPLS-DA) has

86 also been used in wine-related research. Pattern recognition methods, such as OPLS-DA

87 have been used together with ^{1}H NMR to deal with complexity in data and to improve

88 interpretation. Some of the practical applications of this technique include: the study of

89 fermentation behaviour of different wine yeast strains (Son et al. 2009a); lactic acid

90 bacteria alteration during fermentation (Lee et al. 2009a,b); and characterisation of

91   wines from grapes (Son et al. 2009b). The OPLS-DA method has also been successfully

92   used to deal with omics data derived from different sources (Boccard and Rutledge

93   2013). Finally, two more recently published reviews report the ability of OPLS-DA to

94   deal with large and complex metabolomics data (Hong 2011, Fotakis et al. 2013). In

95   contrast, as far as we know, OPLS-DA has not been employed to unravel the effect of

96   different winemaking treatments, such as AA, on wine colour, and the composition of

97   the phenolic substances and volatilesof wine .

98   The aim of this work was thus to evaluate the long-term impact of AA on the

99   colour, phenolic substances and volatiles of red Tempranillo wines from Valencia,

100   Spain 12 months after bottling. Discriminant techniques such as OPLS-DA were

101   applied to investigate further the changes originated in the elaborated wines.

102   **Materials and methods**

103   *Materials*

104   Acetaldehyde (ACS reagent $\geq$ 99.5%) and 2-octanol were purchased from Sigma-

105   Aldrich (St Louis, MO, USA), hypodermic syringes (Plastipak 1 mL) and needles

106   (Microlance3) from DB Medical (Drogheda, Ireland), and yeast *Saccharomyces*

107   *cerevisiae* strain EP 841 and lactic acid bacteria *Oenococcus oeni* strain OE 104 from

108   Agrovin (Ciudad Real, Spain).

109   *Wine samples*

110   Tempranillo grapes from 2008 were harvested in the Utiel-Requena region of Valencia,

111   Spain and wines were made at the experimental cellar at the Universitat Politècnica de

112   València (UPV). Manually harvested grapes were packed in 20 kg crates. Around 40 kg

113   of grapes were divided into closed 50-L stainless steel tanks after destemming, crushing

114   and mixing. Sulfur dioxide was added at 100 mg/kg as potassium metabisulfite after

115   malolactic fermentation (MLF). Traditional oenological practices were used throughout

116   winemaking, with inoculation of *S. cerevisiae* strain EP 841 yeast at 20 g/hL according

117   to the supplier's recommendations (). Fermentation temperature and sugar consumption

118   were monitored daily. Skins were punched down manually twice a day. After alcoholic

119   fermentation (residual sugar level lower than 2 g/L), the skins were pressed and 5 L of

120   pressed wine was combined with 20L of free-run wine. The wines were inoculated with

121   *O. oeni* strain OE 104 and held at room temperature (20 ºC). The progress of the MLF

122   was monitored by analysis of malic acid concentration with an anion exchange column

123   colorimetric method. Sulfur dioxide was added to all the wines at 50 mg/L after MLF.

124   Wines were bottled in 0.5-L bottles, closed under cork and stored at room temperature

125   (25ºC) for 3 months before acetaldehyde addition.

126   Three months after bottling and in order to reproduce the micro-oxygenation

127   effect in the bottle, additions of an acetaldehyde solution to the wines through the cork

128   were commenced with a hypodermic syringe (AA wines). Acetaldehyde was added

129   every 2 days, for a total of 22 additions over a period of one and half months. A total

130   amount of 11.3 µL acetaldehyde (equivalent of 4.5 mL of theoretical O_2/L of wine) was

131   added to the wines.

132   In the wine matrix oxygen can react with ethanol in an oxidation reaction which

133   yields acetaldehyde.

134        $$CH_3CH_2OH \text{ (l)} + \tfrac{1}{2}O_2 \text{ (g)} \rightarrow CH_3CHO \text{ (l)} + H_2O \text{ (l)} \qquad (1)$$

135   Where 4.5 mL of O_2 (g) corresponds to 2×10^{-4} moles of O_2 (1 mol of any gas

136   corresponds to 22.4 L at 0ºC and 101.325 Pa. From equation 1, theoretically 0.5 mol of

137   O_2 produces 1 mol of acetaldehyde, therefore 4×10^{-4} moles of CH_3CHO will be

138   produced in the reaction, considering a theoretical 100% reaction yield. A total volume

139   of 2.26×10^{-2} mL of CH_3CHO thus needs to be added (MW=44 and ρ=0.782 g/mL). As

140   0.5- L bottles were used in the study 0.514 µL CH_3CHO per sample were added at

141   every application (22 applications).

142   In order to limit additional oxygen diffusion through the syringes into the

143   bottles; needles were kept inserted in the corks with a sealed hypodermic syringe

144   attached to them. Sixty-six wines were elaborated, 33 of which were AA wines, while

145   the remainder were considered as control wines (Control) without any extra

146   acetaldehyde addition.

147   *Analytical methods*

148   Spectrophotometric and chromatographic analyses were undertaken with an UV-Visible

149   JASCO V-530 spectrophotometer and with a JASCO MD-2010 Plus HPLC instrument

150   coupled with a diode array detector (DAD) (JASCO LC-Net II/ADC, Tokyo, Japan).

151   The wines were analysed after 12 months of ageing in bottles. Colour intensity, hue,

152   gelatin (wine astringency) and EtOH indexes (tannin–polysaccharide interactions) were

153   analysed according to Glories (1984). Anthocyanins bleached by bisulfite were

154   quantified following the method described by Ribéreau-Gayon and Stonestreet (1965).

155   The monomeric flavan-3-ols catechins were determined according to Sun et al. (1998)

156   and proanthocyanindins by the methylcellulose tannin precipitation (MCP) assay

157   according to the modification reported by Mercurio et al. (2007) based on the method

158   developed by Sarneckis et al. (2006). The contribution of the copigmented

159   anthocyanins, non-copigmented free anthocyanins and polymeric anthocyanins to the

160   total wine colour was obtained according to Boulton (1996). Polyvinylpolypyrrolidone

161   (PVPP) (anthocyanin-tannin interactions) and DMACH indexes (mean degree of

162 proanthocyanidins polymerisation) were calculated using the method described by

163 Vivas and Glories (1995). The concentration of phenolic substances was determined

164 with the Folin-Ciocalteu method (Singleton and Rossi 1965). All the spectrophotometric

165 measurements were made in triplicate. Individual phenolic substances (phenolic acids,

166 flavan-3-ols, flavonols, main anthocyanidins and acylated anthocyanins) were estimated

167 with HPLC analysis according to Boido et al. (2006). The sum of anthocyanidins and

168 acylated anthocyanins was used to calculate the concentration of anthocyanins. Method

169 performance and data analysis are reported elsewhere (Aleixandre-Tudo et al. 2013).

170   The volatile composition of wines was analysed with an Agilent GC (Agilent

171 Technologies, Waldbronn, Germany) equipped with a split/splitless capillary injection

172 port and flame ionisation detector (FID). Volatiles were separated on a ZB-WAX Plus

173 column (50 m x 0.25 mm i.d., 0.25 μm film thickness) from Phenomenex

174 (Aschaffenburg, Germany). The following conditions were used: injector temperature,

175 250°C; detector temperature, 300°C; and carrier gas flow (N_2), 1 ml/min. Injections

176 were made in split mode (split ratio, 1/60; sample size, 1 μL). The oven temperature

177 was programed as follows: 40°C for 7 min, from 40 to 110°C at 4 °C/min, from 110 to

178 170°C at 10°C/min, and then held for 10 min. Injections were in duplicate. Volatile

179 compounds were identified by comparing retention time with that of standard

180 compounds and were quantified with 2-octanol as internal standard. Samples were

181 prepared following a liquid–liquid extraction method proposed by Cocito et al. (1995)

182 and further developed by Hernanz et al. (1999). Thirty-nine volatile compounds were

183 identified, including alcohols, esters, organic/volatile acids, aldehydes, ketones, lactones

184 and terpenes. Only the compounds that were detected in all the samples were selected

185 and used for statistical data treatment.

186   *Statistical analysis*

187   The data of the colour, phenolic substances and volatiles of the wines were analysed by

188   ANOVA with the Statgraphics Plus 5.1 software (Statpoint Technologies, Warrenton,

189   VA, USA) Principal component analysis and OPLS-DA (Trygg and Wold 2002)

190   analysis were also performed for the phenolic substances and volatile data using

191   SIMCA version 13.0.3 software (www.umetrics.com). Discriminant analysis is a

192   statistical treatment used to examine the set of variables associated with a given object

193   and assigns the object to a group or class based on similarities and differences between

194   variables.

195   Orthogonal projection in latent structures discriminant analysis provides a way

196   to remove systematic variation from a data set X (phenolic substances or volatile

197   compounds) not correlated to the response set Y (acetaldehyde addition or control

198   wines), that is to remove variability in the composition of phenolic substances and

199   volatiles that is orthogonal to acetaldehyde addition (information that does not explain

200   differences between techniques) (Trygg and Wold 2002). A bi-plot including

201   observations and variables was performed. Cross validated (CV) models were

202   performed in the study. In CV, segments of the data set are kept out of model

203   development in both X and Y. The segments kept out are compared with the references

204   values after being predicted by the model. Seven CV segments are considered by default

205   in SIMCA modelling. The process is repeated until all parts are kept out once.

206   Moreover, using volatile data, OPLS-DA classification has been studied with the aim of

207   identifying which group of volatile compounds would better classify Tempranillo wines

208   with AA .

209   To further investigate the compounds that influence the aromatic profile of the

210   elaborated wines an S-Plot was also performed. S-Plots provide visualisation of the

211   cross validated OPLS-DA predictive component loadings. X variables located far out of

212   the wings of the 'S' show high influence model reliability. Finally to further identify the

213   volatile compounds that strongly influence each vinification technique a coefficients

214   plot was also constructed. Coefficients plots rewrite as a regression model an OPLS

215   model. Scaled and centred coefficients, which pertain to the predictive components,

216   represent the change in the Y variable when the X variable varies one standard

217   deviation. Significant coefficients, indicated by the error bars confidence intervals,

218   show significant X variables when the coefficient does not contain 0.

219   **Results and discussion**

220   As previously mentioned the calculation of the total AA was done based on the

221   oxidation of ethanol to acetaldehyde. In reality any source of oxygen may be able to

222   oxidise ethanol and the reaction should be written as follows:

223            $CH_3CH_2OH + [O] \rightarrow CH_3CHO + H_2O$ (2)

224   Our approach in this regard is based on the oxidation ability of the oxygen

225   incorporated through the MOX process, without taking into account any other oxygen

226   sources (Ribéreau-Gayon et al. 2006b). In our approach we therefore assumed that

227   oxygen introduced in a MOX treatment (4.5 mL O_2/L of wine) theoretically reacts

228   exclusively with ethanol to form acetaldehyde. A total amount of 11.3 µL of

229   acetaldehyde was therefore added to each AA wine, as mentioned in the Materials and

230   methods section. As acetaldehyde is a volatile compound, 20 µL of a 2.6% acetaldehyde

231   solution was incorporated at every addition.

232   Acetaldehyde is also known to strongly bind sulfur dioxide (SO_2) in wine. As

233   SO_2 was added to the wines at the end of MLF, acetaldehyde was added only 3 months

234   after storage in bottles. Normally equilibrium is reached 4–5 days after the addition of

235   SO_2 to wine, therefore no more binding of this antioxidant occurs. A decrease in SO_2

236   occurring afterwards is due to oxidation reactions which are catalysed by iron and

237   copper ions. New combinations would happen only if the chemical composition of the

238   wine is modified (Ribéreau-Gayon et al. 2006a). Moreover the effect of variable

239   concentration of SO_2 in micro-oxygenated wines has been studied by Tao et al. (2007).

240   A strong effect of micro-oxygenation on the wine's phenolic fraction was still observed

241   where 50 mg/L of SO_2 was added. Moreover other studies also showed an important

242   micro-oxygenation effect in wines where free SO_2 concentration was maintained at

243   25–35 mg/L throughout the process (du Toit et al. 2006). Thus SO_2 was added as it is

244   considered a common practice in wine industry applications.

245

246   *Effect of acetaldehyde addition on wine colour and phenolic composition*

247   **ANOVA**. From the ANOVA analysis 17 out of 24 parameters showed a significant

248   difference between treatments, which clearly points out the great impact that this

249   technique has on the composition of wine phenolic substances, maintaining this effect

250   even after 12 months of bottle aging. These differences include parameters related to

251   wine colour, concentration of some individual phenolic substances and also with the

252   interactions among phenolic substances (Table 1).

253

254   Colour density and hue were significantly different in AA wines (Table 1). The

255   colour enhancing effect of acetaldehyde formation through micro-oxygenation on wine

256   has been extensively reported (Cano-López et al. 2006, 2007, 2008, 2010, Wirth et al.

257   2010, Rayne et al. 2011). In addition, the higher hue values observed in AA wines

258   suggests that a decrease in the free SO_2 concentration could be occurring. Cano-Lopez

259   et al. (2010) also observed an increase in wine hue of micro-oxygenated wines after 6

260   months of aging. The interaction between the acetaldehyde and SO_2 can have a large

261   influence on the development of wine hue. Acetaldehyde strongly binds the preservative

262   SO_2, thereby reducing its antioxidative effect. Wines with high acetaldehyde

263   concentration will require more SO_2 to achieve an adequate concentration of free or

264   active SO_2, since bound SO_2 does not have the same properties (Jackowetz et al. 2011).

265   The concentration of bisulfite-bleached anthocyanins, petunidin, malvidin,

266   anthocyanidins and anthocyanins was lower in the AA wines. The presence of

267   anthocyanins taking place in polymerisation reactions through ethyl-bridged linkages

268   might be the reason of the observed results (Cejudo-Bastante et al. 2010, Gonzalez-del

269   Pozo et al. 2010, Laurie et al. 2014,). This hypothesis is further supported by the higher

270   colour density and polymeric anthocyanins fraction observed in the AA wines (Table 1).

271   Tao et al. (2007) studied the effect of MOX at different SO_2 concentration and reported

272   a significant decrease in the monomeric anthocyanins and flavan-3-ol fractions in wines

273   with a lower SO_2 concentration, together with an increase in the polymeric pigments

274   (Geldenhuys et al. 2012) and in the concentration of tannins. After a MOX treatment a

275   decrease in the total red pigments occurred, but the proportion of pigments in red form

276   increased (Atanasova et al. 2002, Fourie 2005). It was hypothesised that this

277   transformation of colourless anthocyanins into the coloured form compensated for their

278   loss and leads to an increase in colour density (du Toit et al. 2006).

279   The AA wines also contained a lower concentration of copigmented and free

280   non-copigmented anthocyanin fractions . Acetaldehyde addition also led to a lower

281   concentration of phenolic acids, compounds which also can form pigments with the

282   anthocyanins (Schwarz et al. 2003), and of flavonols. In contrast tannins and catechin

283   (Tao et al. 2007) and their mean degree of polymerisation (DMACH index) showed a

284   significantly higher concentration and higher degree of polymerisation of tannins in AA

285   wines. Proanthocyanidins and flavan-3-ol monomers take part in polymerisation

286   reactions with anthocyanins (Cejudo-Bastante et al. 2010). Acetaldehyde addition could

287   have favoured these reactions, increasing the presence of these compounds at 12 months

288   of storage.

289   **Principal component analysis.** The PCA bi-plot (Figure 1a) [principal components 1

290   and 2 (PC1 and PC2)] induced 54.3% of the total variability), showed separation of the

291   wines samples into two groups. Control wines were located towards the positive part of

292   PC2, in contrast to the AA wines, which were positioned towards the negative side of

293   PC2. Although samples did not appear perfectly separated, the distribution of the data

294   suggests differences with the addition of acetaldehyde. Control wines were

295   characterised by a higher concentration of bisulfite-bleaching anthocyanins, which were

296   mainly in the free form, while having lower tannins polymerisation (higher DMACH

297   index values). Other parameters related with control wines include higher concentration

298   of phenolic substances (Folin index) together with more astringent wines (gelatin

299   index). In contrast, AA wines were classified as wines where phenolic substances are

300   taking part in polymerisation reactions, leading to more stable polymeric pigments after

301   12 months of aging.

302

303   **OPLS-DA**. The main aim when applying OPLS-DA lies in its ability to use only the

304 information in the data set which is related to acetaldehyde addition. The bi-plot allows

305 for the visual detection of the parameters highly related with each treatment. The bi-plot

306 also leaves apart the orthogonal information correlated only with the phenolic

307 substances analysed and non-correlated with the vinification treatments, helping thus in

308 the interpretation of the results observed.

309

310   Figure 1(b) shows the cross validated OPLS-DA bi-plot compiled with the

311 phenolic data. The analysis identified one predictive component which accounted for

312 14.7% of the variation. The predictive component summarises the systematic

313 information in X (phenolic substances) that is predictive to Y (treatments) (differences

314 between treatments). Furthermore four orthogonal components were also identified. The

315 first one accounts for 24.1% of the variability, the second one for 19.7% whilst the third

316 and fourth account for 9 and 6% of the total variation, respectively. The orthogonal

317 components express the systematic information that is unique to X, that is information

318 in X that is orthogonal to Y (information within treatment).

319   Cross validated OPLS-DA resulted in a better separation between control and

320 AA wines. Control wines appeared towards the negative-left side of PC1, while AA

321 wines were in the positive-right side of the plot. Again acetaldehyde addition led to

322 wines with more polymerised phenolic substances, highly influencing colour stability

323 and astringency (Monagas et al. 2005, du Toit et al. 2006, Gonzalez del Pozo et al.

324 2010, Arapitsas et al. 2012) after 12 months of aging.

325 *Effect of MOX on wine aroma composition*

326 **ANOVA**. As a first step, the concentration of the 20 quantified volatile compounds was

327 subjected to ANOVA (Table 2). More than two-thirds of the volatiles showed a

328   significant difference between treatments, including fatty acids, esters and volatile

329   phenols. Esters are responsible for fruity aromas in wines, and acids contribute to

330   freshness and fruity aroma (Rodriguez-Bencomo et al. 2008). Thus, it appears that AA

331   could strongly impact the volatile composition of red wine after 12 months of aging.

332   Table 2 also shows the average concentration of the identified groups of volatiles. The

333   compounds which showed higher concentration in wines were higher alcohols, followed

334   by esters, acids and volatile phenols. Of all the volatile groups AA wines showed the

335   higher concentration, although significant difference was observed only for total esters

336   and volatile phenols. The results are not in accordance with those observed by

337   Hernandez-Orte et al. (2009), who found a higher concentration of volatile phenols in

338   non-MOX wines after 8 months of barrel aging. The concentration of volatile phenols

339   might be increased by *Brettanomyces* as it has been shown that these microorganisms

340   can grow even in wines containing a low level of oxygen (du Toit et al. 2006). The

341   development of aerobic microorganisms such as acetic acid bacteria or *Brettanomyces*

342   has been identified as a possible disadvantage of MOX (Gomez-Plaza and Cano-Lopez

343   2011), although as an acetaldehyde solution was added to the wines, oxygen addition

344   could not further explain the observed increase. Moreover Cejudo-Bastante et al.

345   (2011b) cited a slight improvement of red wine aroma quality as a consequence of

346   oxygen addition after 5 months of storage. Other authors indicate an increase in

347   alcohols during MOX pre- and post-MLF (Schmarr et al. 2010). Finally, the

348   concentration of total volatiles was significantly higher in AA wines. The effect of AA

349   addition was clearly observed, but the compounds that are mainly responsible the

350   differences between AA and control wines remains unclear at this point.

351

352 **PCA**. The data set (20 aromatic compounds and 63 wine samples) was subjected to

353 PCA using the SIMCA software package in order to provide partial visualisation of the

354 data in a reduced dimension (Figure 2a). Three samples were detected as clear outliers

355 and were therefore not further included in the study. The first two principal components

356 accounted for 55.2% of the variance (35% and 20.2% for PC1 and PC2, respectively).

357 Samples appeared to be separated by the second PC. Control wines were located more

358 towards the positive part of PC2, while AA wines were located predominantly at the

359 negative side. Although such a separation of the wines was observed, almost half of the

360 control and AA wines were located in the negative side of PC1 and thus the association

361 between AA and their volatile profiles was not clear.

362 **OPLS-DA.** Orthogonal projection in latent structures discriminant analysis removes

363 variability in the volatiles data that is orthogonal to the volatiles composition of the AA

364 and control wines, that is it removes the variability in X that is not correlated to Y.

365 Variables considered in the study represent the volatile compounds while two categories

366 are tested corresponding to AA and control wines.

367

368 The analysis identified one predictive component (PC1) which accounts for

369 19.7% of the variation (Figure 2b). Furthermore two orthogonal components were also

370 identified. The first one accounts for 31.9% of the variability whilst the second one

371 accounts for the 8% of the total variation. Cross validated OPLS-DA bi-plot exhibited a

372 better separation among treatments, with AA wines located in the right side of the bi-

373 plot while control wines are located in the negative side.

374 Compounds, such as fatty acids (hexanoic, decanoic, butyric and isobutyric

375 acids), esters (isoamyl acetate, diethyl succinate, lactate and ethyl-3-hydroxybutyrate),

376   volatile phenols (2-methoxyphenol and 4-vinylphenol) and higher alcohols (2-

377   phenylethanol and *cis*-3-hexen-1-ol), were related with AA wines. Fatty acids,

378   compounds associated with lacteal and soapy notes, are formed during fermentation

379   from the hydrolysis of the corresponding esters by yeast and lactic acid bacteria

380   metabolism (Ortega-Heras et al. 2008). It appears, however, that these acids play a

381   positive role in wine aroma as long as they are present at low concentration (Etievant

382   1991, Ortega-Heras et al. 2008) and below the odour threshold level (Ferreira et al.

383   2000). Moreover, higher alcohols are formed as a result of amino acid metabolism of

384   the yeast during fermentation, and may also be formed from related aldehydes by

385   reduction during yeast fermentation (Ferreira et al. 1995). Esters and higher alcohols

386   particularly influence the aroma of the final wine. Factors, such as grape composition

387   and winemaking techniques, can also play an important role in their final concentration

388   (Rapp and Mandery 1986).

389   With the objective of identifying the volatile compounds mainly responsible for

390   the aroma of AA wines, a cross validated OPLS-DA bi-plot (Figure 2c) was again

391   performed, but in this case using only the statistically significant volatile compounds

392   extracted from the ANOVA. One predictive component (PC1) accounting for 25.3% of

393   the variance was identified. Moreover two orthogonal components were also identified,

394   representing 27 and 11.6% of the variance, respectively. Compounds, such as the fatty

395   acids, butyric, isobutyric, isopentanoic and decanoic acids, the esters, diethyl succinate,

396   ethyl lactate and isoamyl acetate, and the volatile phenol 2-methoxyphenol, were the

397   volatile compounds which might significantly characterise AA wines aroma.

398   Even though the main purpose of the OPLS-DA is the increased interpretation

399   clarity and simplicity, the results indicated the power of OPLS-DA analysis to augment

400   classification performance in cases where individual classes exhibit divergence in

401   within-class variation (Bylesjö et al. 2006). The analysis clearly showed that differences

402   between wines do exist and also which volatile compounds characterise AA wines.

403   **S-Plot.** To better understand the aroma of the AA wines a more individualised analysis

404   is required. Figure 3 represents the S-Plot of the aroma-significant volatile compounds

405   identified in the ANOVA. The volatile compounds, ethyl lactate and diethyl succinate,

406   are located at the upper right extreme of the X variables distribution, highly influencing

407   a change in the concentration of the volatile aromas due to acetaldehyde addition. In

408   contrast the compounds octanoic acid and 4-ethylphenol are related to the control wines

409   (lower extreme).

410   Ethyl lactate is an important aroma compound produced by yeast and acetic acid

411   bacteria (Matthews et al. 2004, Swiegers et al. 2005). The hydrolysis of an ester

412   substrate by esterase activity has been proposed as the pathway leading to these

413   products (Swiegers et al. 2005). Ethyl lactate has been described as having a fruity,

414   sweet and resembling pineapple aroma with candy brown nuances (Lloret et al. 2002).

415   Ethyl lactate also gives a broader and fuller taste to the wine (Henick-Kling 1993). A

416   detection threshold between 60–110 mg/L has been proposed by Dittrich (1987); AA

417   wines had an ethyl lactate concentration below the threshold level (30.6 mg/L), but

418   significantly higher than that found in the control wines.

419   Significant changes in wine aroma occur during maturation and aging. During

420   wine storage, esters are hydrolysed and their fresh and fruity aroma is decreased or

421   disappears (Perez-Coello et al. 2003). Concurrent with the degradation of esters,

422   synthesis of new esters occurs, such as the formation of isoamyl acetate and diethyl

423   succinate (Rapp and Mandery 1986). Diethyl succinate has been found in high

424   concentration in aged wines (Alves et al. 2005). An increase in the concentration of the

425   diethyl ester of succinic acid during storage in Riesling wines has also been observed

426   (Rapp and Marais 1993). While a decrease in the majority of esters was found as storage

427   time increases, in a study evaluating the differences in major volatile compounds of red

428   wines during storage, an increase in the concentration of ethyl lactate and diethyl

429   succinate was also observed. The authors suggest that this could be due to chemical

430   esterification during the course of aging (Perez Prieto et al. 2003). The aroma of diethyl

431   succinate has been described as mild, but fruity and reminiscent of watermelon (Jordan

432   et al. 2002). The higher values observed in the AA wines indicate that acetaldehyde

433   could play an important role in the evolution of this compound during ageing.

434   In contrast the C_8 fatty acid octanoic acid and the volatile phenol 4-ethylphenol

435   are compounds which could contribute to the aroma of the control wines. Fatty acids

436   are produced in the lipid metabolism of yeast (Schreier 1979) and also can be formed

437   due to a hydrolysis of the corresponding esters (Perez Prieto et al. 2003). The odour

438   threshold of octanoic acid has been established at 500 μg/L (Guth 1997, Ferreira et al.

439   2000). The fatty acids at a concentration of 4 to 10 mg/L impart a mild and pleasant

440   aroma to wine, however, at a concentration beyond 20 mg/L, their impact in wine

441   becomes negative (Shinohara 1985, Pozo-Bayon et al. 2005). The descriptors rancid,

442   harsh and cheesy have been proposed to describe the odour of this compound (Jiang and

443   Zhang 2010). In contrast, fatty acids contribute to a fresh flavour and also help to

444   modify the perception of other taste sensations (Ribéreau-Gayon et al. 2001). The

445   concentration of octanoic acid was above the odour threshold (>500 μg/L) only in the

446   control wines, and therefore this fatty acid could have an impact on aroma since its

447   concentration was far below 20 mg/L when it contributes negatively to the wine.

448   The volatile phenol 4-ethylphenol (4-EP) is produced by the spoilage yeast

449   *Brettanomyces* from the precursor *p*-coumaric acid (Chatonnet et al. 1992, 1995,

450   Singleton 1995). When present at a concentration above the odour threshold (140 µg/L)

451   the aroma of the wine is described as horsy, leather, animal, barnyard, medicinal, band-

452   aids and mousy (Chatonnet et al. 1992, Towey and Waterhouse 1996). Although the

453   concentration of this compound in the control wines was slightly higher than that in the

454   AA wines, both treatments had a 4-EP concentration lower than the odour threshold

455   and is therefore probably not contributing to the Brett aroma of the wines.

456   **Coefficients plot.** Figure 4 shows a coefficients plot for the comparison between AA

457   and control wines. The significant parameters that have a major impact on the

458   composition of the volatiules of the AA wines (significant) were, in this order, diethyl

459   succinate, ethyl lactate (identified also in the S-plot analysis), 2-methoxyphenol and

460   isopentanoic acid. In contrast the compounds octanoic acid (identified also in the S-plot)

461   and 2-phenylethyl acetate, ethyl decanoate and ethyl hexanoate were also significant for

462   the aroma of the control wines. Coefficients plot helped in the identification of the

463   parameters that significantly influence the differences between treatments, also

464   providing information on how strong its influence is on wine aroma composition.

465   The commonly known defect cork taint, which is attributed to the cork stopper,

466   is applicable to the contamination of wine expressing a serious off-odour (Alvarez-

467   Rodriguez et al. 2003). 2-Methoxyphenol (guaiacol) has been found in cork-tainted

468   wines and could be partially contributing to this fault. The sensory attributes associated

469   with guaiacol, a compound resulting from lignin degradation, are phenolic, medicinal,

470   wood, sweet, spicy and smoky. The perception threshold in red wine was established at

471   75 µg/L (Boidron et al. 1988). The compound is thought to be an intermediate in the

472   degradation of vanillic acid, via catechol (Li and Rosaza 2000), as a result of an

473   enzymatic non-oxidative decarboxylation reaction (Chow et al. 1999). Moreover this

474   compound has also been identified from red grape juice, formed from grape shikimic

475   acid derivates, at a concentration up to 50 μg/L. The concentration of 2-methoxyphenol

476   in AA wines was higher than the perception threshold and therefore this compound

477   might highly influence wine aroma. Simpson et al. (1986) reported guaiacol to be

478   responsible for an off-flavour when the concentration ranged from 0.07 and 2.63 mg/L.

479   The aroma composition and the interaction between volatile compounds would define if

480   the observed guaiacol concentration is conferring an unpleasant aroma to the wines,

481   although this can only be determined by a sensory analysis.

482   Finally, isopentanoic acid (valeric acid), a C_5 fatty acid, is thought to impart

483   cheesy and rancid aromas to the wine. Its perception threshold has been fixed at 3 mg/L

484   (Fazzalari 1978). As reported previously, at lower concentration, fatty acids can

485   contribute to wine volatile profile, imparting mild and pleasant attributes, but the wines

486   in this study showed a concentration far below the perception threshold, and it is highly

487   improbable that this compound influences wine aroma. It is important to mention here

488   that the statistical treatment helped in the interpretation of the treatment differences, but

489   further work is required when wine aroma wants to be defined.

490   Regarding control wines three esters were identified as potential important

491   compounds. Specifically 2-phenylethyl acetate, ethyl hexanoate and ethyl decanoate

492   were detected as significant compounds. 2-Phenylethyl acetate has been described as

493   floral, honey and rose with a perception threshold of 250 μg/L (Ferreira et al. 2000).

494   The concentration found in control wines was close to the perception threshold. In

495   contrast ethyl hexanoate has been mentioned as imparting apple peel, fruit, banana,

496 strawberry, violets, apple and anise notes and and decanoate soap, fruit, floral, grape,

497 fatty and pleasant, respectively. Their perception threshold has been established at 14

498 and 200 µg/L, respectively. The concentration in the control wines was much higher

499 than the perception threshold for both compounds and are they thus considered as

500 important impact odorants of the control wines. Based on these results it

501 seappearedemed that acetaldehyde addition had a strong impact on wine esters

502 modifying the profile of these compounds in the elaborated wines.

503

504 *OPLS-DA misclassification*

505 Cross validated OPLS-DA classification has been applied to the volatiles data set with

506 the aim to identify which group of volatile compounds would better classify

507 Tempranillo wines. When performing a classification, better ability to classify indirectly

508 explains larger differences between treatments. Acetaldehyde addition classification

509 appears in Table 3. Esters and fatty acids appeared as the groups of compounds with

510 higher accuracy since 90.91% of the samples were classified correctly. This led us to

511 consider that AA increases the presence of esters and fatty acids in Tempranillo wines.

512 In contrast volatile phenols, lactones and higher alcohols showed 74.24, 59.09 and

513 51.52% accuracy, respectively. Further classifications considering only the significant

514 volatile compounds as well as considering all the quantified volatile compounds were

515 also performed. The results show ahigh prediction accuracy for both models (95.45 and

516 96.97%, respectively) highlighting once more the strong impact that this technique has

517 on the composition of the volatiles of Tempranillo wines at 12 months of bottle storage.

518

519 **Conclusion**

520 Acetaldehyde addition can affect the colour and the composition of the phenolic

521 substances and volatiles of Tempranillo wines after 12 months of aging. Wines to which

522 acetaldehyde was added had better colour and a higher concentration of polymeric

523 pigments together with a decrease in astringency. Moreover changes in the volatile

524 fraction were also observed with esters and fatty acids being mainly affected. The

525 changes in aroma and flavour, however, that this technique induces need to be further

526 confirmed with sensory analysis, and therefore the conclusions presented in this study

527 must be carefully considered. Even though AA is not legal and this compound is

528 considered potentially a carcinogen the total amount added (17.6 mg/L) is far below the

529 concentration found in some commercial wines. Acetaldehyde additions to red wine

530 thus appear as a viable alternative to study MOX if MOX facilities are not available.

531   It has also been demonstrated in this study that discriminant techniques used as a

532 means of interpretation, by representing bi-plot graphs, can be used to identify the

533 chemical parameters that better characterise each individual treatment. After OPLS-DA,

534 the loadings of the predictive component (S-plot) also allowed the targeting of the

535 individual compounds that could potentially influence wine aroma; and finally, the

536 coefficients plot indicated the volatile compounds that are statistically significant.

537 **Acknowledgments**

540 **References**

541 Aleixandre-Tudo, J.L., Alvarez, I., Lizama, V., Garcia, M.J., Aleixandre, J.L. and du
542 Toit, W. (2013) Impact of caffeic acid addition on phenolic composition of Tempranillo
543 wines from different winemaking techniques. Journal of Agricultural and Food
544 Chemistry **61**, 11900-11912.

545

546 Alvarez-Rodriguez, M.A., Belloch, C., Villa, M., Uruburu, F., Larriba, G. and Coque,
547 J.J. (2003) Degradation of vanillic acid and production of guaiacol by microorganisms
548 isolated from cork samples. FEMS Microbiology Letters **220**, 49-55.

549

550 Alves, R.F., Nascimento, A.M.D. and Nogeira, J.M.F. (2005) Characterization of the
551 aroma profile of Madeira wine by sorptive extraction techniques. Analytical Chimica
552 Acta **546**, 11-21.

553

554 Arapitsas, P., Scholz, M., Vrhovsek, U., di Blasi, S., Bartolini, A.B., Masuero, D.,
555 Perenzoni, D., Rigo, A., Mattivi, F. (2012) A metabolomic approach to the study of
556 wine micro-oxygenation. PLoS One **7(5)**, e37783.

557

558 Atanasova, V., Fulcrand, H., Cheynier, V. and Moutounet, M. (2002) Effect of
559 oxygenation on polyphenol changes occurring in the course of winemaking. Analytical
560 Chimica Acta **458**, 15-27.

561

562 Boccard, J, Douglas, N. and Rutledge, D.N. (2013) A consensus orthogonal partial least
563 squares discriminant analysis (OPLSDA) strategy for multiblock Omics data fusion.
564 Analytical Chimica Acta **769**, 30–39.

565

566 Boido, E., Alcalde-Eon, C., Carrau, F., Dellacassa, E. and Rivas-Gonzalo, J.C. (2006)
567 Aging effect on the pigment composition and colour of *Vitis vinífera* L. cv. Tannat
568 wines. Contribution of the main pigment families to wine colour. Journal of
569 Agricultural and Food Chemistry **54**, 6692-6704.

570

571 Boidron, J.N., Chatonnet, P. and Pons, M. (1988) Influence du bois sur certaines
572 substances odorantes des vins. Connaissance Vigne Vin **22**, 275–294.

573

574 Boulton, R.B. (1996) Methods for the assessment of copigmentation in red wines.
575 Proceedings at the 47th annual meeting of the American Society for Enology and
576 Viticulture;26-28 June 1996;Reno, NV, USA (American Society for Enology and
577 Viticulture: Davis, CA, USA).

578

579 Bylesjo, M, Rantalainen, M, Cloarec, O, Nicholson, J.K., Holmes, E. and Trygg, J.
580 (2006) OPLS discriminant analysis: combining the strengths of PLS-DA and SIMCA
581 classification. Journal of Chemometrics **20**, 341–351.

582

583 Cano-López, M., López-Roca, J.M., Pardo-Minguez, F. and Gómez-Plaza, E. (2010)
584 Oak barrel maturation vs. micro-oxygenation: Effect on the formation of anthocyanin-
585 derived pigments and wine colour. Food Chemistry **119**, 191–195.

586 Cano-López, M., Pardo-Minguez, F., López-Roca, J.M. and Gómez-Plaza, E. (2006)
587 Effect of micro-oxygenation on anthocyanin and derived pigment content and chromatic
588 characteristics of red wines. American Journal of Enology and Viticulture **57**, 325–331.

589

590 Cano-López, M., Pardo-Minguez, F., López-Roca, J.M. and Gómez-Plaza, E. (2007)
591 Chromatic characteristics and anthocyanin profile of a micro-oxygenated red wine after
592 oak or bottle maturation. European Food Research and Technology **225**, 125–132.

593

594

595 Cano-López, M., Pardo-Minguez, F., Schmauch, G., Saucier, C., Teissedre, P.L.,
596 López-Roca, J.M. and Gomez-Plaza, E. (2008) Effect of micro-oxygenation on colour
597 and anthocyanin related compounds of wines with different phenolic contents. Journal
598 of Agricultural and Food Chemistry **56**, 5932–5941.

599

600 Cejudo-Bastante, M.J., Hermosin-Gutierrez, I. and Perez-Coello, M.S. (2011a) Micro-
601 oxygenation and oak chip treatments of red wines: Effects on colour-related phenolics,
602 volatile composition and sensory characteristics. Part II: Merlot wines. Food Chemistry
603 **124**, 738-748.

604

605 Cejudo-Bastante, M.J., Hermosin-Gutierrez, I. and Perez-Coello, M.S. (2011b) Micro-
606 oxygenation and oak chip treatments of red wines: Effects on colour-related phenolics,
607 volatile composition and sensory characteristics. Part I: Petit Verdot wines. Food
608 Chemistry **124**, 727-737.

609 Cejudo-Bastante, M.J., Perez-Coello, M.S. and Hermosin-Gutierrez, I. (2010) Effect of
610 wine micro-oxygenation treatment and storage period on colour-related phenolics,
611 volatile composition and sensory characteristics. Food Science and Technology **44**, 866-
612 874.

613

614 Cejudo-Bastante, M.J., Perez-Coello, M.S. and Hermosin-Gutierrez, I. (2011c) Effect of
615 wine micro-oxygenation treatment and storage period on colour-related phenolics,
616 volatile composition and sensory characteristics. Food Science and Technology **44**, 866-
617 874.

618

619 Chatonnet, P., Dubourdieu, D. and Boidron, J.N. (1995) The Influence of
620 *Brettanomyces/Dekkera sp.* yeasts and lactic acid bacteria on the ethylphenol content of
621 red wines. American Journal of Enology and Viticulture **46**, 463-468.

622

623 Chatonnet, P., Dubourdieu, D., Boidron, J.N. and Pons, M.J. (1992) The origin of
624 ethylphenols in wines. Journal of the Science of Food and Agriculture **60**, 165-178.

625

626 Chow, K.T., Pope, M.K. and Davies, J. (1999) Characterization of a vanillic acid non-
627 oxidative decarboxylation gene cluster from Streptomyces sp. D7. Microbiology **145,**
628 2393-2403.

629

630 Cocito, C., Gaetano, G. and Delfini, C. (1995) Rapid extraction of aroma compounds in
631 must and wines by means of ultrasounds. Food Chemistry **52**, 311-320.

632

633 Comfort, S. (2008) An introduction to understanding oxygen and fermentation. The
634 YeastWhisperer. <http://www.yeastwhisperer.com>.

635

636 Danilewicz, J.C., Seccombe, J.T. and Whelan, J. (2008) Mechanism of interaction of
637 polyphenols, oxygen, and sulphur dioxide in model wine and wine. American Journal of
638 Enology and Viticulture **59**, 128-136.

639

640 Dittrich, H.H. (1987) Mikrobiologie des Weines (Ulmer, Stuttgart, Germany).

641

642 Du Toit, W.J. (2010) Chapter 8: Micro-oxygenation, oak alternatives and added tannins
643 and wine quality. Reynolds, A.G., ed. Managing wine quality: oenology and wine
644 quality. Volume 2 (Woodhead Publishing: Cambridge, England) pp. 226-254.

645

646 Du Toit, W.J., Lisjak, K., Marais, J. and Du Toit, M. (2006) The effect of micro-
647 oxygenation on the phenolic composition, quality and aerobic wine-spoilage
648 microorganisms of different South African red wines. South African Journal of Enology
649 and Viticulture **27**, 57−67.

650

651 Elias, R.J. and Waterhouse, A.L. (2010) Controlling the Fenton reaction in wine.
652 Journal of Agricultural and Food Chemistry **58**, 1699-1707.

653

654 Elias, R.J., Andersen, M.L., Skibsted, L.H. and Waterhouse, A.L. (2009) Identification
655 of free radical intermediates in oxidized wine using electron paramagnetic resonance
656 spin trapping. Journal of Agricultural and Food Chemistry **57,** 4359–4365.

657

658 Etievant, P.X. (1991) Wine. Maarse, H., ed. Volatile compounds in food and beverages.
659 (Marcel Dekker, New York, NY, USA).

660

661 Fazzalari, F.A. (1978) Compilation of odor and taste threshold data. ASTM Data Series
662 DS 48A (American Society for Testing and Materials: West Conshocken, PA, USA) .

663

664 Ferreira, V., Fernandez, P., Pena, C., Escudero, A. and Cacho, J.F. (1995) Investigation
665 on the role played by fermentation esters in the aroma of young Spanish wines by
666 multivariate analysis. Journal of the Science of Food and Agriculture **67**, 381–392.

667

668 Ferreira, V., Lopez, R. and Cacho, J.F. (2000) Quantitative determination of the
669 odorants of young red wines from different grape varieties. Journal of the Science of
670 Food and Agriculture **80**, 1659-1667.

671

672 Fourie, B.P. (2005) The influence of different barrels and oak derived products on the
673 colour evolution and quality of red wines. MSc Agric. thesis, Stellenbosch University,
674 Stellenbosch, South Africa.

675

676 Fotakis, C., Kokkotou, K., Zoumpoulakis, P. and Zervou, M. (2013) NMR metabolite
677 fingerprinting in grape derived products: an overview. Food Research International **54**,
678 1184–1194.

679

680 Geldenhuys, L, Oberholster, A. and du Toit, W.J. (2012) Monitoring the effect of
681 micro-oxygenation before malolactic fermentation on South African Pinotage red wine
682 with different colour and phenolic analyses. South African Journal of Enology and
683 Viticulture **33**, 150-160.

684

685 Glories, Y. (1984) La couleur des vins rouges. Les equilibres des anthocyanes et des
686 tannins. Connaissance de la Vigne et du Vin **18,** 195-217.

687

688 Gomez-Plaza, E. and Cano-Lopez, M.A. (2011) A review on micro-oxygenation of red
689 wines: claims, benefits and the underlying chemistry. Food Chemistry **125**, 1131–1140.

690

691 González-Del Pozo, A., Arozarena, I., Noriega, M.J., Navarro, M. and Casp, A. (2010)
692 Short- and long-term effects of micro-oxygenation treatments on the colour and
693 phenolic composition of a Cabernet Sauvignon wine aged in barrels and/or bottles.
694 European Food Research and Technology **231**, 589–601.

695

696 Guth, H. (1997) Quantitation and sensory studies of character impact odorants of
697 different white wine varieties. Journal of Agricultural and Food Chemistry **45**, 3027–
698 3032.

699

700 Henick-Kling, T. (1993) Malolactic fermentation. Fleet, G. H., ed. Wine microbiology
701 and biotechnology (Harwood Academic Publishers: Chur, Switzerland) pp. 289–326.

702

703 Hernández-Orte, P., Lapeña, A.C., Escudero, A., Astrain, J., Baron, C., Pardo, I., Polo,
704 L., Ferrer, S., Cacho, J. and Ferreira, V. (2009) Effect of micro-oxygenation on the
705 evolution of aromatic compounds in wines: malolactic fermentation and ageing in
706 wood. LWT-Food Science and Technology **42**, 391–401.

707

708 Hernanz, D., Heredia, F.J., Beltran, R. and Recamales, M.A.F. (1999) Optimization of
709 an extraction method of aroma compounds in white wine using ultrasound. Talanta **50**,
710 413–421.

711

712 Hong, Y.S. (2011) NMR-based metabolomics in wine science. Magnetic Resonance
713 Chemistry **49**, S13–S21.

714

715 Jackowetz, J.N., Dierschke, S.E. and Mira de Orduña, R. (2011) Multifactorial analysis
716 of acetaldehyde kinetics during alcoholic fermentation by *Saccharomyces cerevisiae*.
717 Food Research International **44**, 310-316.

718

719 Jiang, B. and Zhang, Z. (2010) Volatile compounds of young wines from Cabernet
720 Sauvignon, Cabernet Gernischet and Chardonnay varieties grown in the Loess Plateau
721 region of China. Molecules **15**, 9184-9196.

722

723 Jordan, M.J., Margaria, C.A., Shaw, P.E. and Goodner, K.L. (2002) Aroma active
724 components in aqueous kiwi fruit essence and kiwi fruit puree by GC-MS and
725 multidimentional GC-GC-O. Journal of Agricultural and Food Chemistry **50**, 5386-
726 5390.

727

728 Laurie, V.F., Salazar, S., Campos, M.I., Cáceres-Mella, A. and Peña-Neira, A. (2014)
729 Periodic aeration of red wine compared to microoxygenation at production scale.
730 American Journal of Enology and Viticulture. **65,** 254-260

731

732 Lee, J.E., Hong, Y.S. and Lee, C.H. (2009a) Characterization of fermentative behaviors
733 of lactic acid bacteria in grape wines through 1H NMR- and GC-based metabolic
734 profiling. Journal of Agricultural and Food Chemistry **57**, 4810–4817.

735

736 Lee, J.E., Hwang, G.S., Lee, C.H. and Hong, Y.S. (2009b) Metabolomics reveals
737 alterations in both primary and secondary metabolites by wine bacteria. Journal of
738 Agricultural and Food Chemistry **57**, 10772–10783.

739

740 Li, T. and Rosaza, J.P.N. (2000) Biocatalytic synthesis of vanillin. Applied and
741 Environmental Microbiology **66**, 684-687.

742

743 Lloret, A., Boido, E., Lorenzo, D., Medina, K., Carrau, F., Dellacassa, E. and Versini,
744 G. (2002) Aroma variation in Tannat wines: effect of malolactic fermentation on ethyl
745 lactate level and its enantiomeric distribution. Italian Journal of Food Science **14,** 175–
746 180.

747

748 Mateus, N., De Pascual-Teresa, S., Rivas-Gonzalo, J., Santos-Buelga, C. and de Freitas,
749 V. (2002) Structural diversity of anthocyanin derived pigments in port wines. Food
750 Chemistry **76**, 335–342.

751

752 Matthews, A., Grimaldi, A., Walker, M., Bartowsky, E., Grbin, P. and Jiranek, V.
753 (2004) Lactic acid bacteria as a potential source of enzymes for use in vinification.
754 Applied and Environmental Microbiology **70**, 5715–5731.

755

756 Mercurio, M.D., Dambergs, R.G., Herderich, M.J. and Smith, P.A. (2007) High
757 throughput analysis of red wine and grape phenolics: adaptation and validation of
758 methyl cellulose precipitable tannin assay and modified Somers colour assay to a rapid
759 96 well plate format. Journal of Agricultural and Food Chemistry **55,** 4651-4657.

760

761 Monagas, M., Bartolome, B. and Gomez-Cordoves, C. (2005) Updated knowledge
762 about the presence of phenolic compounds in wine. Critical Reviews in Food Science
763 and Nutrition **45**, 85–118.

764

765 Ortega-Heras, M., Rivero-Pérez, M.D., Pérez-Magariño, S., González-Huerta, C. and
766 González-Sanjosé, M.L. (2008) Changes in the volatile composition of red wines during
767 aging in oak barrels due to microoxygenation treatment applied before malolactic
768 fermentation. European Food Research and Technology **226**, 1485–1493.

769

770 Perez-Coello, M.S., Gonzalez-Vinas, M.A., Garcia-Romero, E., Diaz-Maroto, M.C. and
771 Cabezudo, M.D. (2003) Influence of storage temperature on the volatile compounds of
772 young white wines. Food Control **14**, 301-306.

773

774 Pérez-Magariño, S., Sánchez-Iglesias, M., Ortega-Heras, M., González-Huerta, C. and
775 González-Sanjosé, M.L. (2007) Colour stabilization of red wines by microoxygenation
776 treatment before malolactic fermentation. Food Chemistry **101**, 881–893.

777

778 Perez-Prieto, L.J., Lopez-Roca, J.M. and Gomez-Plaza, E. (2003) Differences in major
779 volatile compounds of red wines according to storage length and storage conditions.
780 Journal of Food Composition and Analysis **16**, 697–705.

781

782 Pozo-Bayón, M.A., Alegría, E.G., Polo, M.C., Tenorio, C., Martín-Álvarez, P.J., Calvo
783 de la Banda, M.T., Ruiz-Larrea, F. and Moreno-Arrivas, M.V. (2005) Wine volatile and

784 amino acid composition after malolactic fermentation: effect of *Oenococcus oeni* and
785 *Lactobacillus plantarum* starter cultures. Journal of Agricultural and Food Chemistry
786 **53**, 8729–8735.

787

788 Rapp, A. and Mandery, H. (1986) Wine aroma. Experientia, **42**, 873–883.

789

790 Rapp, A. and Marais, J. (1993) The shelf life of wine: changes in aroma
791 substancesduring storage and ageing of white wines. Charalambous, G., ed. Shelf Iife
792 studies of foods and beverages. (Elsevier: Amsterdam, The Netherlands).

793

794 Rayne, S., Sheppard, S., Di Bello, T. and Eggers, N.J. (2011) Chromatic characteristics
795 and optically derived compositional descriptors of micro-oxygenated wines from *Vitis*
796 *vinifera* cv. Merlot and Cabernet Sauvignon. Food and Bioprocess Technology **4**, 254-
797 265.

798

799 Ribéreau-Gayon, P. and Stronestreet E. (1965) Le dossage des anthocyanes dans le vin
800 rouge. Bulletine de la Societe Chimique de France **9**, 119-142.

801

802 Ribéreau-Gayon, P., Glories, Y., Maujean, A. and Dubourdieu, D. (2001) Handbook of
803 enology. The chemistry of wine stabilization and treatments. Volume 2 (John Wiley:
804 Chichester, England).

805

806 Ribéreau-Gayon, P., Dubordieu, D., Doneche, B. and Lonvaud, A. (2006a). Handbook
807 of enology. Volume 1. The microbiology of wine and vinification. (John Wiley:
808 Chichester, England).

809

810 Ribéreau-Gayon, P., Dubordieu, D., Doneche, B. and Lonvaud, A. (2006b). Handbook
811 of enology. Volume 2. The chemistry of wine: stabilization and treatments (John Wiley:
812 Chichester, England).

813

814 Rodrıguez-Bencomo, J.J., Mendez-Siverio, J.J., Perez-Trujillo, J.P. and Cacho, J.
815 (2008) Effect of skin contact on bound aroma and free volatiles of Listan blanco wine.
816 Food Chemistry **110**, 214–225.

817

818 Sarneckis, C.J., Dambergs, R.G., Jones, P., Mercurio M., Herderich, M.J. and Smith.
819 P.A. (2006) Quantification of condensed tannins by precipitation with methyl cellulose:
820 Development and validation of an optimised tool for grape and wine analysis.
821 Australian Journal of Grape and Wine Research **12**, 1-11.

822

823 Schmarr, H.G., Bernhardt, J., Fischer, U., Stephan, A., Müller, P. and Durner, D. (2010)
824 Two dimensional gas chromatographic profiling as a tool for a rapid screening of the
825 changes in volatile composition occurring due to microoxygenation of red wines.
826 Analytica Chimica Acta **672**,114-123.

827

828 Schreier, P. (1979) Flavour composition of wines: a review. Critical Reviews in Food
829 Science and Nutrition **12**, 59-111.

830

831 Schwarz, M., Wabnitz, T.C.P. and Winterhalter, P. (2003) Pathway leading to the
832 formation of anthocyanin-vinylphenol adducts and related pigments in red wines.
833 Journal of Agricultural and Food Chemistry **51**, 3682− 3687.

834

835 Sheridan, M.K. and Elias, R.J. (2015) Exogenous acetaldehyde as a tool for modulating
836 wine colour and astringency during fermentation. Food Chemistry **17**, 17–22

837 Shinohara, T. (1985) Gas chromatographic analysis of volatile fatty acids in wines.
838 Agricultural and Biological Chemistry **49**, 2211–2212.

839

840 Simpson, R.F., Amon, J.M. and Daw, A.J. (1986) Off-flavour in wine caused by
841 guaiacol. Food Technology Australia **38(1)**, 31–33.

842

843 Singleton, V.L. (1987) Oxygen with phenols and related reactions in musts, wines, and
844 model systems: observations and practical implications. American Journal of Enology
845 and Viticulture **38**, 69-77.

846

847 Singleton, V.L. (1995) Maturation of wines and spirits: comparisons, facts, and
848 hypotheses. American Journal of Enology and Viticulture **46**, 98-115.

849

850 Singleton, V.L. and Rossi, J.A. (1965) Colorimetry of total phenolics with
851 phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and
852 Viticulture **16**, 144-158.

853

854 Somers, T.C. (1971) Polymeric nature of wine pigments. Phytochemistry **10**,
855 2175−2186.

856

857 Sun, B., Ricardo Da Silva, J.M. and Spranger, I. (1998) Critical factors of vanillin assay
858 for catechins and proanthocyanidins. Journal of Agricultural and Food Chemistry **46**,
859 4267-4274.

860 Son, H.S., Hwang, G.S., Ahn, H.J., Park, W.M., Lee, C.H. and Hong, Y.S. (2009a)
861 Characterization of wines from grape varieties through multivariate statistical analysis
862 of 1 H NMR spectroscopic data. Food Research International **42**, 1483−1491

863

864 Son, H.S., Hwang, G.S., Kim, K.M., Kim, E.Y., van der Berg, F., Park, W.M., Lee,
865 C.H. and Hong, Y.S. (2009b) 1 H NMR-based metabolomics approach for
866 understanding the fermentation behaviours of wine yeast strains. Analytical Chemistry
867 **81**, 1137–1145.

868

869

870 Swiegers, J.H., Bartowsky, E.J., Henschke, P.A. and Pretorius, I.S. (2005) Yeast and
871 bacterial modulation of wine aroma and flavour. Australian Journal of Grape and Wine
872 Research **11**,139–173.

873

874 Tao, J., Dykes, S. and Kilmartin, P. (2007) Effect of SO2 concentration on polyphenol
875 development during red wine micro oxygenation. Journal of Agricultural and Food
876 Chemistry **55**, 6104-6109.

877

878 Towey, J.P. and Waterhouse, A.L. (1996) Barrel-to-barrel variation of volatile oak
879 extractives in barrel-fermented Chardonnay. American Journal of Enology and
880 Viticulture **47**, 17-20.

881

882 Trygg, J. and Wold, S. (2002) Orthogonal projections to latent structures (O-PLS).
883 Journal of Chemometrics **16**, 119–128.

884

885 Vidal, S. and Aagaard, O. (2008) Oxygen management during vinification and storage
886 of Shiraz wine. The Australian and New Zealand Wine Industry Journal **23**,56, 58,63.
887 http://www.winebiz.com.au.

888

889 Vidal, S., Francis, L., Noble, A.C., Kwiatkowski, M., Cheynier, V. and Waters, E.J.
890 (2004) Taste and mouth-feel properties and different types of tannin-like polyphenolic
891 compounds and anthocyanins in wine. Analytica Chimica Acta **513**, 57–65.

892

893 Vivas, N. and Glories, Y. (1995) Racking of red wines matured in barrels. A tentative
894 classification of racking techniques. The Australian & New Zealand Wine Industry
895 Journal **10**, 241-243.

896

897 Waterhouse, A.L. and Laurie, V.F. (2006) Oxidation of wine phenolics: a critical
898 evaluation and hypotheses. American Journal of Enology and Viticulture **57**, 306-313.

899

900 Wirth, J., Morel-Salmi, C., Souquet, J.M., Dieval, J.B., Aagaard, O., Vidal, S.,
901 Fulcrand, H. and Cheynier, V. (2010) The impact of oxygen exposure before and after
902 bottling on the polyphenolic composition of red wines. Food Chemistry **123**, 107–116.

903 Zoecklein, B. (2007) Factors impacting sulphur-like off odours in wine and winery
904 options. Proceedings of the 8th annual enology and viticulture British Columbia wine
905 grape council conference; 23-24July 2007; Penticton, BC, Canada.
906 http://www.apps.fst.vt.edu/extension/enology/downloads/SLOFactorsFinal.pdf

907

908

909 **Figure 1**. Colour and phenolic (a) principal component analysis (PCA) bi-plot (PC:
910 principal component) and (b) cross validated orthogonal projection on latent structures
911 discriminant analysis (OPLS-DA) bi-plot (PC: predictive component; OC: orthogonal
912 component) of control and acetaldehyde added wines. Control wines without
913 acetaldehyde added (●) and wines with acetaldehyde added(♦). CDe: color density;
914 BBA: bisulfite bleached anthocyanins (mg/L); Del: delfinidine (mg/L); Cya: cyanidine
915 (mg/L); Pet: petunidine (mg/L); Peo: peonidine (mg/L); Mal: malvidine (mg/L); And:
916 anthocyanidins (mg/L); ADe: anthocyanin derivates (mg/L); Ant: anthocyanins (mg/L);
917 %CA: % copigmented anthocyanins; %FA: % free anthocyanins; %PA: % polymerized
918 anthocyanins; PVI: PVPP index; FoI: Folin index; PhA: phenolic acids (mg/L); Flo:
919 flavonols (mg/L); Catechins (mg/L): Fla: flavan-3-ols (mg/L); Tan: tannins (g/L); DMI:
920 DMACH index; EtI: ethanol index; GeI: gelatin index.

921

922 **Figure 2**. Volatile data acetaldehyde addition based (a) PCA bi-plot (PC:
923 principal component), (b) cross validated OPLS-DA bi-plot and (c) CV OPLS-DA bi-
924 plot using only the significant compounds identified in the ANOVA (PC: predictive
925 component; OC: orthogonal component). Control wines without acetaldehyde added (●)
926 and wines with acetaldehyde added (♦). IsA: isoamyl acetate; EHe: ethyl hexanoate;
927 ELa: ethyl lactate; C3H: *cis*-3-hexenol; EhB: ethyl-3-hydroxybutyrate, IbA: isobutyric
928 acid; 4Vp: 4-vinylphenol; BtA: butiric acid; EDe: ethyl decanoate; Btl: γ-butyrolactone;
929 IpA: isopentanoic acid; DeS: diethyl succinate; 2PA: 2-phenylethyl acetate; HeA:
930 hexanoic acid; 2Mp: 2-methoxyphenol; 2Pe: 2-phenylethanol; 4Eg: 4-ethylguaiacol;
931 OcA: octanoic acid; 4Ep: 4-ethylphenol; DeA: decanoic acid.

932

933 **Figure 3**. S-Plot of the ANOVA significant aroma compounds (X variables) of wines
934 with different acetaldehyde treatments. DeS, diethyl succinate; ELa, ethyl lactate; 2MP,
935 2-methoxyphenol; IpA, isopentanoic acid; IsA, isoamyl acetate; 4Ep, 4-ethylphenol;
936 BtA, butyric acid; IbA, isobutyric acid; DeA, decanoic acid; EHe, ethyl hexanoate; Ede,
937 ethyl decanoate; 2PA, 2-phenylethyl acetate; OcA, octanoic acid.

938

939 **Figure 4**. Coefficients plot considering the significant volatile compounds identified in
940 the ANOVA of wines elaborated with and without acetaldehyde addition. DeS, diethyl
941 succinate; ELa, ethyl lactate; 2MP, 2-methoxyphenol; IpA, isopentanoic acid; IsA,
942 isoamyl acetate; 4Ep, 4-ethylphenol; BtA, butyric acid; IbA, isobutyric acid; DeA,
943 decanoic acid; EHe, ethyl hexanoate; Ede, ethyl decanoate; 2PA, 2-phenylethyl acetate;
944 OcA, octanoic acid.