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1 **H, C and O stable isotope ratios of *Passito* wine**

2

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33 **Abstract:**

34 In this study we investigated the effect of the grape withering process occurring during the
35 production of Italian *Passito* wines on the variability of the $(D/H)_I$, $(D/H)_{II}$, $\delta^{13}C$ and $\delta^{18}O$ of wine
36 ethanol and the $\delta^{18}O$ of wine water. The production of PDO *Erbaluce di Caluso Passito* in five
37 different cellars in Piedmont (Italy) was considered in two successive years. Moreover, samples of
38 17 different traditional Italian *Passito* wines taken at different stages of maturation were taken into
39 account. We found that the $\delta^{18}O$ of must and wine water and the $\delta^{18}O$ of ethanol decrease in the
40 case of *Passiti* produced in northern and central Italy using postharvest drying of the grapes in
41 dedicated ventilated or unventilated fruit drying rooms (*fruttaio*), during autumn-winter. For *Passiti*
42 produced in southern Italy, where the main technique involves withering on the plant (*en plein air*),
43 $\delta^{18}O$ tends to increase. The $(DH)_I$ of wine ethanol did not change during withering, whereas the
44 $(DH)_{II}$ and $\delta^{13}C$ values changed slightly, but without any clear trend. Particular attention must be
45 therefore paid when evaluating the $\delta^{18}O$ data of *Passito* wines for fraud detection.

46

47 **Keywords:** Isotope Ratio Mass Spectrometry; *Passito* wine; $\delta^{18}O$; Withering process

48

49 **Introduction**

50

51 The term *Passito* generally refers to different Italian wines (such as Zibibbo, Vin Santo, Sagrantino
52 or Recioto) made from withered grapes using ancient, traditional and artisan winemaking
53 procedures.¹⁻³ Grape variety, the degree of grape drying, vinification technology and the length and
54 method of ageing can result in varying characteristics for different types of *Passito*.

55 The *Passito* wines produced in Italy have in common a key winemaking procedure, involving
56 postharvest drying of grapes, which can last several weeks. The drying can be performed in a
57 dedicated ventilated or unventilated fruit drying room, (called *fruttaio*), during autumn-winter, this
58 method being more widespread in northern Italy, whereas in southern Italy the main technology is
59 withering on the plant (*en plein-air*).⁴ In both cases, during this period the grapes lose water, thus
60 reducing total weight, whereas the concentration of sugars and other substances increases.⁵

61 Progressive grape dehydration can encounter extremely variable trends according to the weather
62 conditions (e.g. average daily humidity or temperature) in the case of *plein air* withering or
63 withering in the *fruttaio*, but it can be kept under control in ventilated rooms. The dehydration
64 process and infection with *Botritis cinerea* Pers. can influence the secondary metabolites of grapes,
65 in particular in relation to colour, volatile compounds and phenolic composition.⁶⁻⁹

66 Most *Passito* production is still carried out manually. The cost of manual procedures and the low
67 yield/hectare mean that the price of this product is very high and for this reason *Passito* wines can
68 often fall victim to counterfeiting.¹⁰ Since the introduction of EU regulations controlling wine
69 production in 1990,¹¹⁻¹² isotopic methods have been recognised as a good weapon for combating
70 some types of wine fraud.¹³ These methods are based on analysis of the isotopic ratios of hydrogen
71 (D/H) and carbon (¹³C/¹²C) in ethanol and of oxygen (¹⁸O/¹⁶O) in water. They are used officially
72 (OIV MA-AS-311-05, MA-AS-312-06 and MA-AS2-12) to detect wine chaptalization and watering
73 down and to guarantee the geographical origin of the product by means of a specific European wine
74 data bank of reference.¹⁴⁻¹⁵ As shown by Perini and Camin (2013)¹⁶ unofficial analysis of the
75 ¹⁸O/¹⁶O ratio of ethanol is in some cases able to improve detection of watering down of wine.

76 Since the 1990s, many authors have investigated the variability of isotopic ratios in wines on a
77 worldwide scale,¹⁷⁻²⁰ but to the best of our knowledge, the effect of withering on the stable isotope
78 ratios of *Passito* wines has not been investigated in the literature to date, although it could be a very
79 useful tool for characterising and protecting this particular type of wine.

80 Five wine cellars of the Controlled Denomination Origin (DOC) *Erbaluce di Caluso Passito* in
81 Piedmont (Italy), applying different withering techniques (in boxes, on wicker trellises, or with
82 hanging of the grapes) was considered in two successive years. Moreover, numerous samples of

83 different traditional Italian *Passito* wines from grapes taken at different stages of withering and
84 withered in different conditions were taken into account focusing on the $\delta^{18}\text{O}$ of water.
85 The final aim was to define the isotopic characteristics of Italian *Passito* wines and to check
86 whether isotopic methods can be used for authenticity verification for this type of wine.
87 The novelty of this study lies in the fact that for the first time, the effect of grape withering
88 processes (up to 5 months) on the variability of the $(\text{D}/\text{H})_{\text{I}}$, $(\text{D}/\text{H})_{\text{II}}$, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of wine ethanol
89 and the $\delta^{18}\text{O}$ of wine and must water was monitored during the production of *Passito* wines.
90 Considering different types of withering and different years, the monitoring carried out becomes
91 very important, because variations in the isotopic values with withering can lead to wrong
92 interpretation of the data and therefore to false conclusions about the authenticity of the samples.

93

94 **Materials and Methods**

95

96 *Grape samples*

97 The study focused on *Erbaluce di Caluso Passito* DOC a wine produced with Erbaluce white
98 grapes grown in the province of Turin (Piedmont, north-west Italy) during 2011 and 2012.²¹ The
99 grape samples were withered in five different cellars with fruit-drying rooms (*fruttaio*) located in
100 Caluso (N45.301045, E7.894921), identified as ER-A, ER-B, ER-C, in San Giorgio Canavese
101 (N45.333054, E7.798944) ER-D and in Piverone ER-E (N45.445302, E8.006088) and the
102 producers guaranteed respect of the procedural guidelines (G.U n. 248). ER-A withered the grapes
103 by hanging them, ER-B put the product on wicker trellises, ER-C and ER-D withered the grapes in
104 small boxes (holding about 5 kg) and ER-E used big boxes (holding about 20 kg), both in single
105 layers. Two tons of Erbaluce grapes were withered for all cellars and years. During withering,
106 relative humidity and temperature were monitored in each cellar using a Hobo data logger (Onset,
107 MA, USA). In 2011, the withering process was faster due to specific climatic conditions. In
108 particular, the RH% (Relative Humidity Percentage) in the first period of withering was low (60-
109 65%) and the sugar increment achieved was about 10°Brix. In 2012, the RH% in the first period of
110 dehydration was 70-82%, with a low sugar concentration (+3°Brix) in some cellars (ER-A, ER-D,
111 ER-D). In this case, a longer processing time was necessary in order to obtain the minim sugar
112 content for the production of *Erbaluce di Caluso Passito* wine in accordance with its Production
113 Disciplinary. In 2012 one sample of fresh grapes from ER-C was also withered in controlled
114 climatic conditions (25°C; 55 RH%) in a controlled chamber (ER-F).
115 Moreover, 78 grape samples used to produce 17 different traditional Italian *Passito* wines (Table 1)
116 were sampled in 2011, 2012 and 2013 by inspectors of the Italian Ministry of Agriculture. For each
117 product, the grapes were collected immediately after harvesting and at the end of withering. In some

118 cases an intermediate sample was also collected. We can identify two macro groups (samples
119 withered in the *fruttajo* and *en plein air*) and within these, particular types of withering (e.g. on
120 trellises, in boxes or hung).

121

122 *Vinification*

123 For the study focused on *Erbaluce di Caluso*, a sample of about 10 kg of fresh and dried berries
124 were used for must and wine production and analysis, with an interval of 4-5 weeks during the
125 withering processes.

126 For the other traditional *Passito* wines (Table 1), about 10 kg of fresh and dried berries were used
127 for the must and sometimes wine production.

128 All samples of fresh and dried grapes were manually crushed and pressed and the must was
129 clarified with static decantation for 24 hours, without adjuvants. In the must sample fermentation
130 was arrested with sodium metabisulphite (800 mg/L).

131 For wine production each sample of must was inoculated with *Saccharomyces cerevisiae* (Uvaferm
132 BC strain, Lallemand, Italy) rehydrated in each must and 1 g/L of yeast nutrient (Fermaid,
133 Lallemand, Italy). Alcoholic fermentation took place at 25°C in glass bottles with a bubbler valve in
134 the cap.

135

136 *Analysis*

137 The D/H and $^{13}\text{C}/^{12}\text{C}$ isotope ratios of the alcohol obtained by distillation using Cadiot columns
138 were determined following the official methods established by the International Organization of
139 Vine and Wine (OIV), MA-AS-311-05 MA-AS-312-06, using SNIF-NMR (Site-specific Natural
140 Isotope Fractionation-Nuclear Magnetic Resonance) (FT-NMR AVANCE III 400, Bruker BioSpin
141 GmbH, Karlsruhe, Germany) and IRMS (Isotope Ratio Mass Spectrometry) (SIRA II-VG ISOGAS,
142 FISIONS, Rodano, Milano, Italy) interfaced with an Elemental Analyser (Flash 1112, Carlo Erba,
143 Milano, Italy). The D/H values were measured site-specifically in the methyl and methylene
144 positions of ethanol [(D/H)_I and (D/H)_{II}].

145 $^{18}\text{O}/^{16}\text{O}$ ratio analysis of wine and must water was performed using a SIRA II, (VG Fisons,
146 Middlewich, United Kingdom) connected to a water/CO₂ equilibration system Isoprep 1, (VG
147 Fisons, Middlewich, United Kingdom) The procedure is described in the OIV-MA-AS2-12 method
148 (2009).

149 A DeltaPlus-XP isotope ratio mass spectrometer (IRMS) coupled to a Thermal
150 Conversion/Elemental Analyser (TC/EA) (both Thermo Scientific, Bremen, Germany) was used for
151 $^{18}\text{O}/^{16}\text{O}$ analysis of ethanol samples following the procedure reported by Perini and Camin (2013).¹⁶

152 The $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$ values are denoted in delta in relation to the international V-PDB (Vienna-
 153 Pee Dee Belemnite) and V-SMOW (Vienna-Standard Mean Ocean Water) standards according to
 154 the following general equation:

$$155 \quad \delta_i E = \frac{(i \text{ RSA} - i \text{ RREF})}{i \text{ RREF}}$$

156
 157 where i is the mass number of the heavier isotope of element E (for example, ^{13}C);
 158 RSA is the respective isotope number ratio of a sample (such as for C : number of ^{13}C atoms/number
 159 of ^{12}C atoms or as $^{13}\text{C}/^{12}\text{C}$ approximation).²²⁻²³
 160 RREF is that of internationally recognised reference materials (see above).

161 The delta values are multiplied by 1000 and are expressed in units “per mil” (‰).

162 The working reference materials are specified in the OIV methods. A sample of wine was used to
 163 validate the whole analysis (distillation + measurement). With one sample of must we also allowed
 164 for fermentation. The final value was reported in a control chart and if it was within the tolerance
 165 range the whole session was validated.

166 The analytical uncertainty (estimated by combining within-laboratory reproducibility standard
 167 deviation with estimates of method and laboratory bias using proficiency test data)²⁴ of the
 168 measurements was 0.3 ‰, 0.6 ‰, 0.8 ppm and 1.2 ppm for $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, $(\text{D}/\text{H})_{\text{I}}$ and $(\text{D}/\text{H})_{\text{II}}$ in
 169 ethanol respectively. For the $\delta^{18}\text{O}$ of water, the value was 0.3 ‰.

170 The alcohol content and residual sugar content were analysed following the OIV-MA-AS312-01A
 171 and OIV MA-AS311-02 official methods.

172 Total soluble solid concentration (°Brix, as SSC) was measured with an Atago 0–32 °Brix
 173 temperature compensating refractometer (Atago Corporation, Tokyo, Japan), and pH was
 174 determined with potentiometry, using a Crison electrode (Carpi, Italy). Titratable acidity (TA),
 175 expressed as grams of tartaric acid per litre, and ethanol expressed in % (v/v) were estimated using
 176 the OIV method. Glucose, fructose and glycerol were quantified (as g/L) using a high performance
 177 liquid chromatography system.⁴

178

179 *Statistical analysis*

180 All statistical analysis was performed in R [R]. Linear modelling was applied to identify significant
 181 relations between the $\delta^{18}\text{O}$ and temperature/humidity profiles recorded during withering. To
 182 account for differences in the initial conditions in the five different fruit-drying rooms, the initial
 183 conditions were subtracted from the $\delta^{18}\text{O}$, temperature and humidity profiles before modelling.
 184 Modelling was then performed on the variation in temperature, humidity and oxygen shift. Stepwise

185 model selection was performed using the MASS R package, freely available at the CRAN website
186 (<http://cran.r-project.org/>),²⁵ and the best model was identified with the Akaike Information
187 Criterion (AIC).

188

189 **Results and Discussion**

190

191 *Effect of withering in fruit rooms (Erbaluce di Caluso Passito)*

192 In Table 2 the $\delta^{18}\text{O}$ values of must and wine water and the $(\text{D}/\text{H})_{\text{I}}$, $(\text{D}/\text{H})_{\text{II}}$, R, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of wine
193 ethanol are shown, together with some quality data for must and wine. Samples are grouped by
194 cellar and year.

195 As expected, the, sugar content in the grape increased with the degree of withering. The acidity of
196 Erbaluce in the grape was normally very high. During the withering process the decrease in
197 titratable acidity could be attributed to the concentration of cations, in particular K^+ , caused by
198 higher weight loss with salification of part of the tartaric acid, and to malic and citric acid
199 depletion.⁷ The must for *Passito* production has a high sugar content and complete fermentation
200 with *Saccharomyces cerevisiae* is not possible. The alcohol content and yield generally depend on
201 the initial sugar content in must, which influences yeast metabolism such as the production of
202 glycerol.²⁶

203 Of the isotopic parameters, $\delta^{18}\text{O}$ was most influenced by the degree of withering. In each of the five
204 cellars, $\delta^{18}\text{O}$ decreased significantly from the starting value (from grapes immediately after
205 harvesting) to the final value (grapes with a higher sugar content), with differences from -2 to -6 ‰
206 in must and wine water and from -2 to -10 ‰ in wine ethanol. As expected, the $\delta^{18}\text{O}$ of must was
207 not different to that of wine.

208 The O stable isotope composition of grape juice is initially controlled by that of the source water
209 and, as the grape berries mature and approach harvest, by environmental conditions such as
210 temperature and relative humidity, which control the degree of evapotranspiration, tending to enrich
211 the grape water in ^{18}O .^{20,27-28} Moreover the $\delta^{18}\text{O}$ of grape water is affected by precipitation during
212 the final development of the grape, with a relatively rapid response, due to stable isotope exchange
213 with water vapour.¹⁷

214 Hermann et al. (2008) assumed that isotope fractionation takes place at leaf level and that
215 isotopically modified water is transported into the grapes, where its isotopic signature should be
216 preserved.²⁸ Other authors state that this fractionation takes place particularly in the leaves but also
217 in the berry.^{17, 29} In a more recent paper,³⁰ isotope fractionation was also observed in a grape juice
218 exposed to the atmosphere for several days, but protected from rain and snow.

219 In our study it is evident that grape water is also subject to isotopic fractionation after harvesting in
220 grapes separated from the vines, when dehydration occurs. The decrease in $\delta^{18}\text{O}$ was coherent with
221 the decrease in temperature recorded automatically every hour in the different fruit-drying rooms
222 (Figure 1), but not with the variation in relative humidity (supplementary material figure B).

223 In order to statistically evaluate the significance of relations between $\delta^{18}\text{O}$ and temperature and
224 humidity, a statistical modelling approach was applied. The rationale behind this is that the actual
225 value for oxygen shift is determined by the preceding average environmental conditions. By taking
226 into account relative data (see Material and Methods) the five different fruit drying rooms can be
227 considered as independent replicates of the same phenomenon.

228 The modelling was performed by considering the average temperature and humidity values in the
229 period before each $\delta^{18}\text{O}$ analysis. To better characterise the process, three scenarios were examined,
230 calculating the average temperature and humidity one, two and three weeks before each $\delta^{18}\text{O}$
231 analysis. Each scenario was then modelled separately. In all cases a general linear model of the
232 form $\delta^{18}\text{O} = A*\text{Temperature} + B*\text{Humidity} + C*(\text{Temperature}*\text{Humidity}) + D + \varepsilon$ was considered.

233 This form is able to capture the separate effect of changes in temperature and humidity, the implicit
234 contribution of time, and also the possible presence of an interaction effect between the two
235 predictors. This separate modelling approach was preferred to a more complex linear model taking
236 into account all the possible factors and their interactions for two reasons: a) the very small number
237 of experimental points (only five time-resolved measurements of the isotopic ratios), b) high
238 correlations between the averages of temperature and humidity at the one, two and three week
239 intervals. In all three cases, the best model was found by stepwise model selection. For all three
240 scenarios, stepwise selection indicated that the interaction term is not necessary to model the $\delta^{18}\text{O}$
241 shift during withering. In all cases, the optimal model had the form $\delta^{18}\text{O} = A*\text{Temperature}$
242 $+ B*\text{Humidity} + C + \varepsilon$, the level of significance for the two predictors in the three models being
243 summarised in Table 3.

244 The results confirmed that temperature is the most significant parameter in influencing $\delta^{18}\text{O}$
245 variations in *Passito* wine. Comparing the adjusted R^2 for the three models (0.71 for week 1, 0.62
246 for week 2 and 0.57 for week 3, see Table 3) it is also possible to identify the model which shows
247 the best fit with the experimental data. Considering that the one week model shows the highest
248 adjusted R^2 , it is possible to conclude that the average temperature and humidity in that period
249 were most influential on the isotopic shifts observed.

250 It is interesting to note that by withering one of the grape samples (ER-F, 2012) in a temperature-
251 controlled room with specific and constant temperature (25°C) and humidity (55.5%), the grapes

252 were dehydrated to about 49°Brix in a short time (22 days) and the $\delta^{18}\text{O}$ value of wine increased
253 from 4.9‰ to 9.6‰ (Table 2).

254 One reason for this is that in this case kinetic isotope fractionation takes place, as described by
255 Ingraham et al. (1999) for wines obtained from grapes subject to extreme evapotranspiration
256 processes due to high daily temperatures and low humidity.¹⁷ To simplify, kinetic or equilibrium
257 isotope fractionation can occur in nature. Kinetic fractionation is a process that separates stable
258 isotopes from each other by their mass during unidirectional processes. One naturally occurring
259 example of kinetic fractionation is the evaporation of seawater to form clouds or the process of
260 evapotranspiration observed in plants. Isotopically lighter water molecules (i.e. those with ^{16}O)
261 evaporate more easily than the isotopically heavier ones, with ^{18}O . During this process the vapour
262 becomes enriched with ^{16}O , whereas water becomes enriched in ^{18}O . Equilibrium
263 isotope fractionation is the partial separation of isotopes between two or more compounds or phases
264 in the thermodynamic equilibrium. Most equilibrium fractionation results from the reduction in
265 vibrational energy (especially zero-point energy) when a heavier isotope is substituted for a lighter
266 one.³¹ An example of equilibrium isotope fractionation is the concentration of heavy isotopes
267 of oxygen in liquid water compared with water vapour at low temperature (see Raleigh
268 Fractionation).³²

269 It is possible to surmise that in the case of withering in the *fruttaio*, in the particular environmental
270 conditions in the drying room, with low temperature and high humidity causing slow withering (up
271 to 4 months and over), there is competition between at least two different processes: diffusive
272 transport of water from the grape, but also isotopic exchange between the grape water and air
273 humidity in the boundary layer at the interface between the skin and atmosphere, until an isotopic
274 equilibrium is reached. The final result in this type of samples is a decrease in the $\delta^{18}\text{O}$ of grape
275 water.

276 The $\delta^{18}\text{O}$ of ethanol obtained after distillation of *Passito* samples showed good correlation with the
277 $\delta^{18}\text{O}$ of *Passito* wine water ($R^2=0.78$), as already observed.¹⁶

278 In contrast with $\delta^{18}\text{O}$, the $(\text{DH})_{\text{I}}$ values of wine ethanol did not change during the experiment in
279 either of the years considered ($p<0.05$), also in the temperature-controlled room, whereas the $(\text{DH})_{\text{II}}$
280 and $\delta^{13}\text{C}$ values could did change, but without any clear trend (Table 2). At all events, in most
281 cases, both $(\text{DH})_{\text{II}}$ and $\delta^{13}\text{C}$ tended to decrease with withering time (up to 1 ‰ for $\delta^{13}\text{C}$). This may
282 be due to the fact that *Passito* wines have a high sugar content which can obstruct fermentation, in a
283 similar way to sweet wines subject to the stopping of fermentation, with a subsequent decrease in
284 $(\text{DH})_{\text{II}}$ and $\delta^{13}\text{C}$.³³

285

286 **SURVEY OF ITALIAN PASSITO WINES**

287 In this section we considered $\delta^{18}\text{O}$ of must water for 78 samples of 17 different traditional Italian
288 *Passito* wines (Table 1), including *Erbaluce di Caluso*. For each sample we measured only the $\delta^{18}\text{O}$
289 of must water, as this is the official parameter most influenced by withering, as described above.

290 The samples from northern Italian regions Piedmont, Lombardy, Emilia Romagna, Veneto and
291 Friuli Venezia Giulia (Figure 2a), subjected to postharvest drying of grapes in dedicated fruit rooms
292 (*fruttaio*), showed a change in the $\delta^{18}\text{O}$ of water by -6.5 ‰, as observed above. In two cases
293 (Figure 2a), Ramandolo 2013 (withering on trellises with forced ventilation) and Picolit 2011 (in
294 boxes with controlled temperature) the $\delta^{18}\text{O}$ value increased by around 1‰, probably because
295 withering took place in a shorter time (less than one month), which caused kinetic isotopic
296 fractionation.

297 For the *Passito* samples of regions in central Italy (Figure 2b): Tuscany, Marche and Umbria, which
298 also adopted withering in the *fruttaio*, $\delta^{18}\text{O}$ decreased up to -6‰.

299 For *Passito* produced *en plein air* in the northern regions (Friuli Venezia Giulia and Emilia
300 Romagna) (Figure 3a), climatic conditions caused equilibrium isotope fractionation, as in the
301 *fruttaio*. Indeed a decrease in $\delta^{18}\text{O}$ was observed. In the southern regions of Apulia, Sicily and
302 Sardinia, in most cases $\delta^{18}\text{O}$ increased with withering (Figure 3b). Indeed, normally in these regions
303 the grapes overripen on the plants in autumn, and due to the relatively higher temperature in these
304 areas, kinetic evapo-transpiration takes place. In two cases (*Passito di Manduria* and *Moscato di*
305 *Trani* both 2012) $\delta^{18}\text{O}$ decreased up to -4.6‰, probably because of precipitation, which caused an
306 exchange with water atmospheric vapour.

307 The significant variation in the $\delta^{18}\text{O}$ of must and wine water and wine ethanol observed during
308 withering have never previously been reported in the literature. This variation can lead to problems
309 when interpreting data for detecting authenticity, if referring to an official wine databank based only
310 on fresh grape wines. Indeed, decreases or increases of up to 6 ‰, can make the $\delta^{18}\text{O}$ values fall
311 outside the limits defined by the European wine databank for an authentic wine for the declared
312 origin,³⁴ with consequent false declaration of watering down or mislabelling. Furthermore, the
313 ethanol $\delta^{18}\text{O}$ value of final samples were sometimes (ER-B, ER-C and ER-E) outside the range
314 proposed for authentic wine samples (from +24‰ to +36‰).¹⁶ Particular attention must be
315 therefore paid when evaluating the $\delta^{18}\text{O}$ data of *Passito* wines for fraud detection.

316

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FIGURE CAPTIONS

Figure 1: Variation in $\delta^{18}\text{O}$ with temperature in the '*fruttaio*'.

Figure 2: Difference between $\delta^{18}\text{O}$ of must water at the end of withering ($\delta^{18}\text{O}_{\text{passito}}$) and that at harvest ($\delta^{18}\text{O}_{\text{harvest}}$). Withering in northern (2a) and central (2b) Italy using '*fruttaio*'.

Figure 3: Difference between $\delta^{18}\text{O}$ of must water at the end of withering ($\delta^{18}\text{O}_{\text{passito}}$) and that at harvest ($\delta^{18}\text{O}_{\text{harvest}}$). Withering in northern (3a) and in southern Italy (3b) '*en plein air*'.

TABLES

Table 1: Withering procedures for *Passito* wine samples.

DOCdenomination	Geographical origin	Type of withering	Grape variety	Year
<i>in a fruit drying room (fruttaio)</i>				
<i>Erbaluce di Caluso Passito</i>	North, Piedmont	on trellises, controlled temperature	Erbaluce	2011, 2012
<i>Oltrepò Pavese Moscato Passito</i>	North, Lombardy	on trellises, controlled temperature	Moscato	2012
<i>Loazzolo Passito</i>	North, Piedmont	on trellises, natural	Moscato	2011, 2012, 2013
<i>Ramandolo Passito *</i>	North, Friuli Venezia Giulia	on trellises, forced ventilation	Verduzzo	2013
<i>Colli Orientali del Friuli</i>	North, Friuli Venezia Giulia	in boxes, controlled temperature	Verduzzo	2011
<i>Picolit</i>	North, Friuli Venezia Giulia	in boxes, controlled temperature	Picolit	2011, 2012
<i>Strevi Passito</i>	North, Piedmont	in boxes, controlled temperature	Moscato	2013
<i>Recioto di Gambellara</i>	North, Veneto	hung, controlled temperature	Garganega	2011, 2012
<i>Montefalco Sagrantino Passito</i>	Centre, Umbria	on trellises, forced ventilation	Sagrantino	2011, 2012, 2013
<i>Verdicchio dei Castelli di Jesi Passito</i>	Centre, Marche	on trellises, controlled temperature	Verdicchio	2012, 2013
<i>Vin Santo del Chianti</i>	Centre, Tuscany	on trellises, controlled temperature	Trebbiano	2011, 2012, 2013
<i>Vin Santo del Chianti Classico</i>	Centre, Tuscany	in boxes, controlled temperature	Trebbiano	2012, 2013
<i>En plein air</i>				
<i>Albana di Romagna Passito</i>	North, Emilia Romagna	over-ripening on the plants	Albana	2012
<i>Ramandolo Passito *</i>	North, Friuli Venezia Giulia	over-ripening on the plants	Verduzzo	2011
<i>Ramandolo Passito*</i>	North, Friuli Venezia Giulia	on trellises, covered	Verduzzo	2012
<i>Moscato di Noto passito</i>	South, Sicily	over-ripening on the plants	Moscato	2012, 2013
<i>Passito di Manduria</i>	South, Apulia	over-ripening on the plants	Primitivo	2012, 2013
<i>Moscato di Trani</i>	South, Apulia	over-ripening on the plants	Moscato	2012, 2013
<i>Alghero passito</i>	South, Sardinia	on trellises, covered	Nasco	2011, 2012, 2013

*For the *Ramandolo Passito* different withering procedures were adopted in the 3 years

Table 2: Variation in isotopic values and quality parameters of must and wine with the withering period from the harvest date.

Chain	Date of sampling	$\delta^{18}\text{O}_{\text{VSMOW}} / \text{‰}$, must water	*Brix	pH	Acidity g/L tartaric acid	$\delta^{18}\text{O}_{\text{VSMOW}} / \text{‰}$, wine water	$\delta^{18}\text{O}_{\text{VSMOW}} / \text{‰}$, ethanol	(D/H)I ppm ethanol	(D/H)II ppm ethanol	R ethanol	$\delta^{13}\text{C}_{\text{VPDB}} / \text{‰}$, ethanol	pH	Acidity g/L tartaric acid	glucose g/L	tose g/L	glycerol g/L	ethanol % vol
ER-A 1	Sept 26, 2011	5.0	22.2	3.12	8.70	4.6	30.2	103.2	128.9	2.5	-26.6	3.29	8.1	nr*	nr	nd**	12.65
ER-A 2	Nov 7, 2011	3.2	28.8	3.49	6.70	3.0	28.3	103.3	132.5	2.6	-27.2	3.55	6.90	8	17	nd	14.1
ER-A 3	Dec 23, 2011	1.8	34.6	3.66	6.20	2.1	27.8	104.0	135.2	2.6	-27.7	3.75	6.35	23	78	nd	13.6
ER-B 1	Sept 26, 2011	6.0	30.4	3.38	8.30	5.5	31.1	103.3	134.2	2.6	-26.5	3.5	7.4	4	52	nd	14.05
ER-B 2	Nov 7, 2011	4.6	41.5	3.52	7.90	4.9	25.8	103.6	129.6	2.5	-27.5	3.58	6.80	124	159	nd	7.1
ER-B 3	Dec 23, 2011	-0.1	48.2	3.73	6.00	-0.2	24.3	103.1	124.5	2.4	-27.5	3.8	7.05	97	248	nd	7.3
ER-C 1	Sept 26, 2011	5.4	35.6	3.58	7.90	5.7	30.8	102.9	134.3	2.6	-27.0	3.63	6.8	33	81	nd	13.45
ER-C 2	Nov 7, 2011	0.7	45.8	3.62	5.70	1.0	25.2	101.9	129.6	2.5	-27.6	3.72	6.05	167	195	nd	6.5
ER-C 3	Dec 23, 2011	-0.3	47.4	3.87	6.70	0.1	22.9	102.4	131.9	2.6	-27.5	3.85	7.20	121	238	nd	5.9
ER-E 1	Sept 26, 2011	4.6	27.7	3.08	8.15	4.5	29.5	102.1	131.0	2.6	-28.0	3.22	7.75	2	15	nd	14.8
ER-E 2	Nov 7, 2011	3.2	38.8	3.33	6.70	3.3	28.5	102.5	131.7	2.6	-28.5	3.42	7.10	81	125	nd	10.2
ER-E 3	Dec 23, 2011	2.0	44.4	3.54	5.70	2.5	25.8	102.8	130.0	2.5	-28.4	3.59	6.80	93	201	nd	8.1
ER-A 1	Sept 25, 2012	4.3	25.2	3.17	8.40	4.0	29.5	101.7	131.1	2.6	-27.2	3.31	7.73	nr	nr	12.9	14.7
ER-A 2	Oct 17, 2012	2.7	27.1	3.25	7.09	2.5	27.1	101.8	130.8	2.6	-26.8	3.26	7.50	1	1	13.1	15.8
ER-A 3	Nov 21, 2012	1.7	30.2	3.26	7.80	1.7	27.1	101.8	129.1	2.5	-26.7	3.40	8.55	21	54	15.5	14.2
ER-A 4	Dec 18, 2012	0.4	33.8	3.29	8.48	1.0	24.8	100.4	125.9	2.5	-26.9	3.47	9.00	63	103	17.5	12.2
ER-A 5	Feb 15, 2013	1.1	40.5	3.58	6.45	1.6	25.6	102.8	129.4	2.5	-26.6	3.80	9.15	101	161	17.7	11.2
ER-B 1	Sept 25, 2012	5.3	27.3	3.34	7.46	5.3	30.6	102.0	133.5	2.6	-27.1	3.62	7.13	nr	nr	12.1	16.5
ER-B 2	Oct 17, 2012	2.1	32.2	3.49	6.75	1.8	28.4	100.2	130.2	2.6	-27.4	3.66	7.91	18	59	14.8	15.2
ER-B 3	Nov 21, 2012	-0.2	34.3	3.45	7.50	0.0	26.4	101.9	128.9	2.5	-26.5	3.65	8.85	55	92	17.2	13.4
ER-B 4	Dec 18, 2012	-1.3	40.0	3.65	7.01	-0.6	21.2	101.4	126.5	2.5	-26.5	3.90	8.78	118	156	19.3	10.4
ER-C 1	Sept 25, 2012	5.0	30.7	3.31	6.68	4.9	30.8	101.7	131.9	2.6	-26.1	3.50	6.90	7	36	13.6	15.9

ER-C 2	Oct 17, 2012	3.1	36.0	3.57	5.44	2.8	28.2	101.5	131.0	2.6	-26.7	3.67	7.05	54	108	16.4	13.3
ER-C 3	Nov 21, 2012	0.3	39.3	3.27	7.91	0.5	25.5	101.1	129.7	2.6	-27.3	3.44	9.23	113	149	18.3	10.9
ER-C 4	Dec 18, 2012	-0.7	43.4	3.41	6.45	-0.1	21.1	100.8	124.0	2.5	-27.2	3.59	8.18	169	191	18.6	7.2
ER-D 1	Sept 25, 2012	5.3	27.7	3.35	8.03	4.7	30.7	102.0	133.1	2.6	-26.8	3.59	7.65	nr	4	13.3	16.0
ER-D 2	Oct 17, 2012	4.1	30.0	3.34	7.24	3.7	29.9	102.0	131.5	2.6	-26.6	3.42	8.10	1	8	12.8	16.3
ER-D 3	Nov 21, 2012	2.5	35.5	3.28	8.63	3.0	28.1	102.2	130.1	2.5	-27.0	3.49	10.50	67	104	17.9	12.5
ER-D 4	Dec 18, 2012	2.4	36.5	3.46	8.03	2.8	26.4	102.8	129.1	2.5	-27.0	3.68	8.63	68	120	18.4	12.5
ER-D 5	Feb 15, 2013	3.3	46.6	3.51	8.63	3.0	26.5	103.0	133.1	2.6	-27.3	3.75	11.18	250	220	17.4	5.8
ER-E 1	Sept 25, 2012	4.6	25.1	3.26	7.13	4.0	29.0	101.3	130.0	2.6	-27.7	3.38	6.90	nr	nr	11.7	14.7
ER-E 2	Oct 17, 2012	3.6	28.1	3.33	6.30	3.2	28.0	101.4	131.2	2.6	-27.3	3.30	6.75	8	33	14.3	15.9
ER-E 3	Nov 21, 2012	1.8	31.8	3.44	6.00	1.4	26.4	101.1	128.3	2.5	-27.6	3.58	7.43	34	76	14.6	13.4
ER-E 4	Dec 18, 2012	0.3	34.9	3.43	5.93	0.6	24.2	101.5	127.8	2.5	-27.7	3.61	7.09	63	115	16.1	12.3
ER-E 5	Feb 15, 2013	0.0	39.9	3.6	5.81	0.3	23.5	101.7	129.0	2.5	-27.9	3.83	8.48	98	160	17.9	11.5
ER-C	Sept 25, 2012	5.0	30.7	3.31	6.68	4.9	30.8	101.70	131.90	2.60	-26.10	3.50	6.90	7	36	13.6	15.9
ER-F	Oct 17, 2012	9.7	48.8	3.57	6.00	9.6	33.8	102.20	122.70	2.40	-26.10	3.92	7.01	283	170	14.1	6.7

*nr: not detectable; **nd: not determined

Table 3: Levels of significance for the modelling of $\delta^{18}\text{O}$ on the basis of temperature and humidity..

	Estimate	Std. Error	P value	Signif. Level ¹
Week 1 (Adjusted R² = 0.71)				
C	-1.4	0.4	0.005	**
A (Temperature)	0.27	0.04	8E-6	***
B (Humidity)	-0.05	0.01	0.007	**
Week 2 (Adjusted R² = 0.62)				
C	-1.7	0.4	0.001	**
A (Temperature)	0.3	0.06	1E-4	***
B (Humidity)	-0.05	0.02	0.06	*
Week 3 (Adjusted R² = 0.57)				
C	-1.7	0.4	0.001	**
A (Temperature)	0.27	0.05	2E-4	***
B (Humidity)	-0.04	0.02	0.1	NS

¹ ***: < 0.0001; **: < 0.001; *: < 0.1; NS: not significant

FIGURES

Figure 1

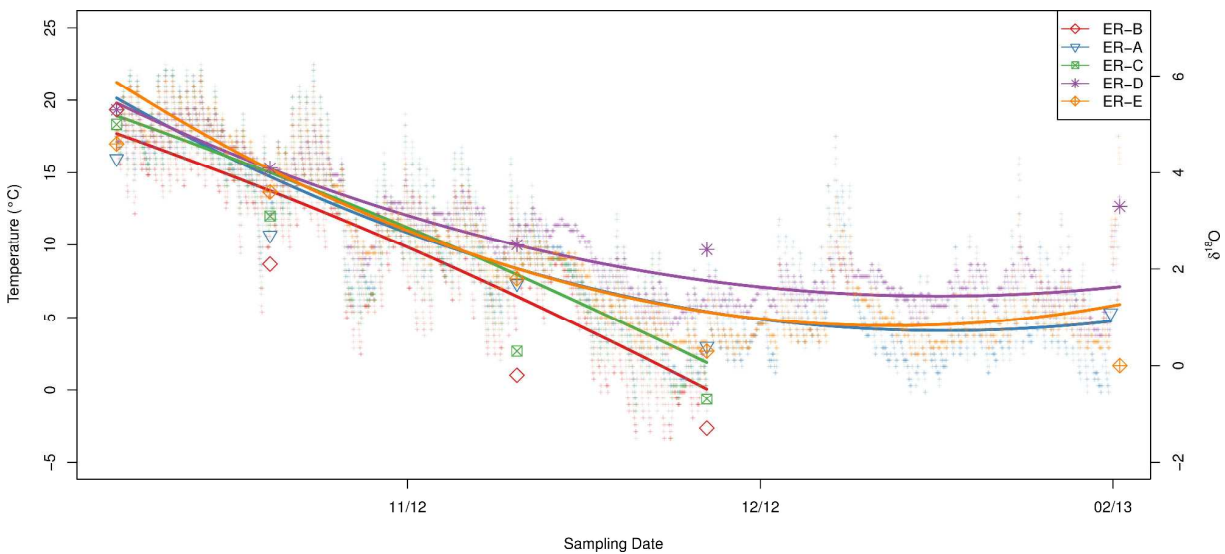


Figure 2

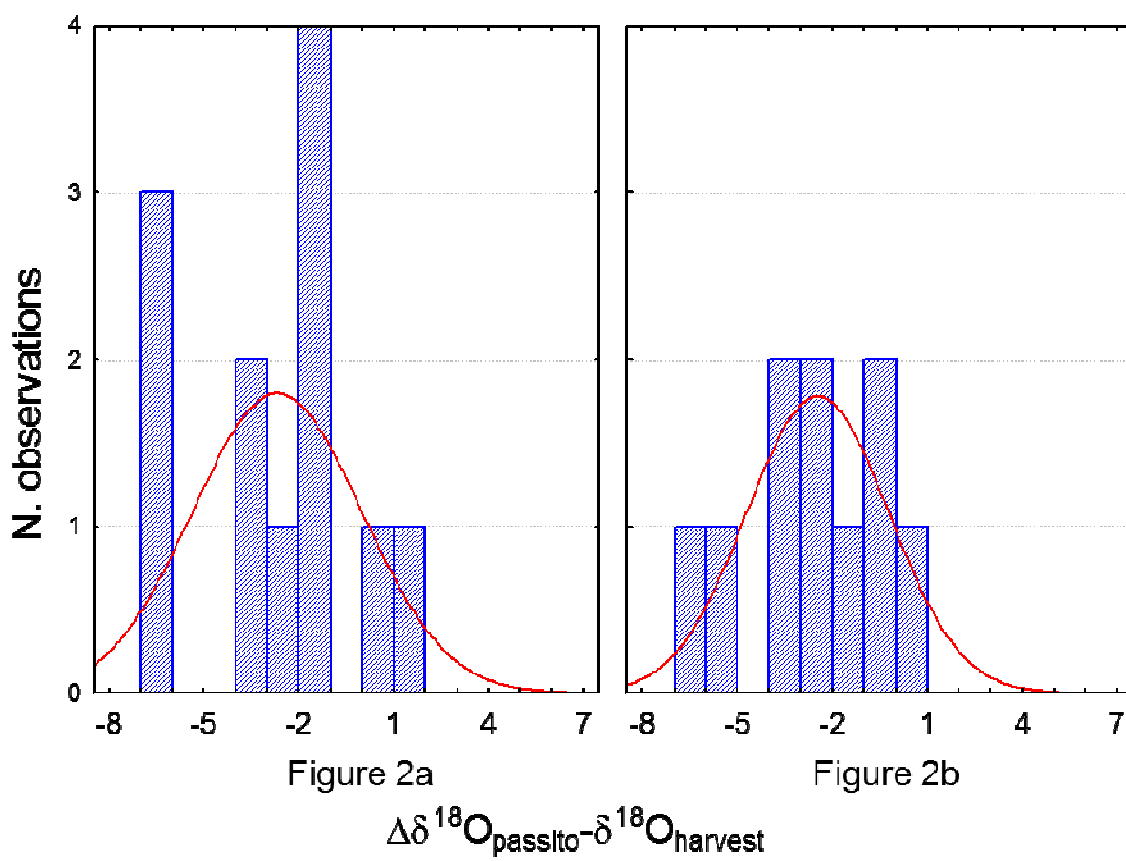
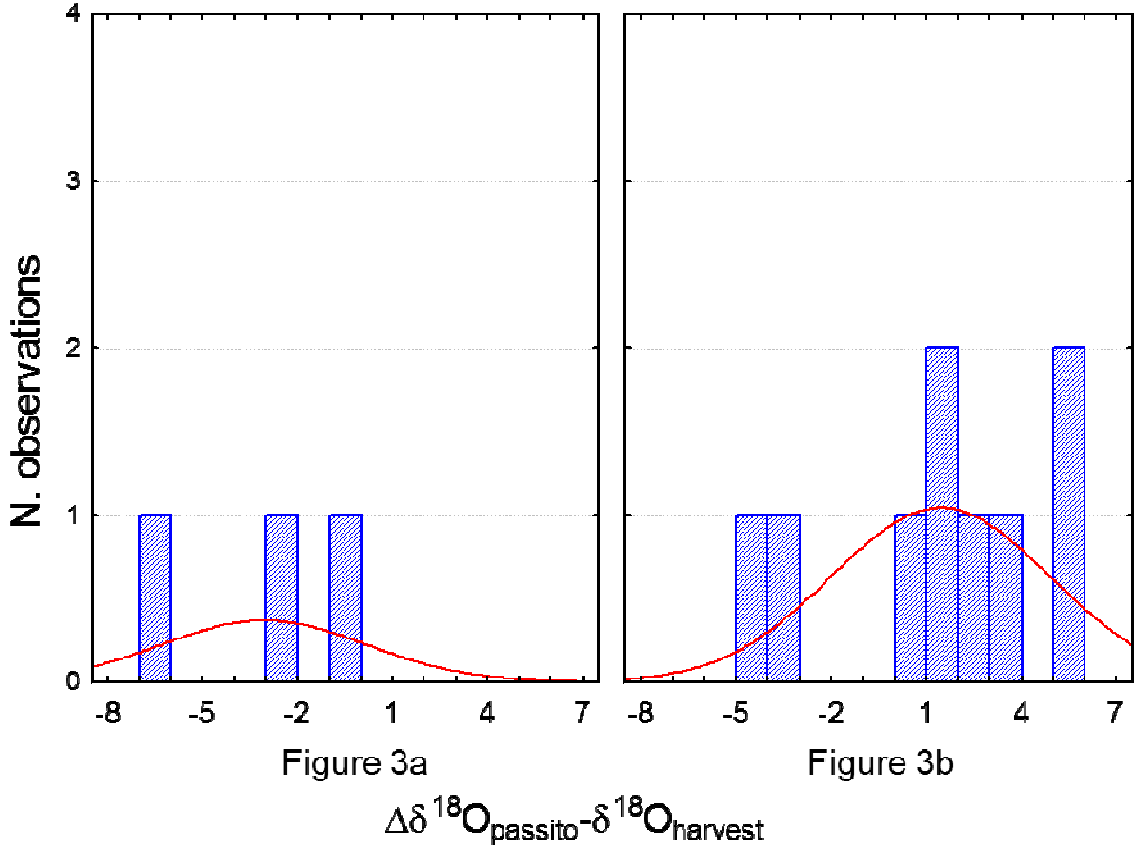
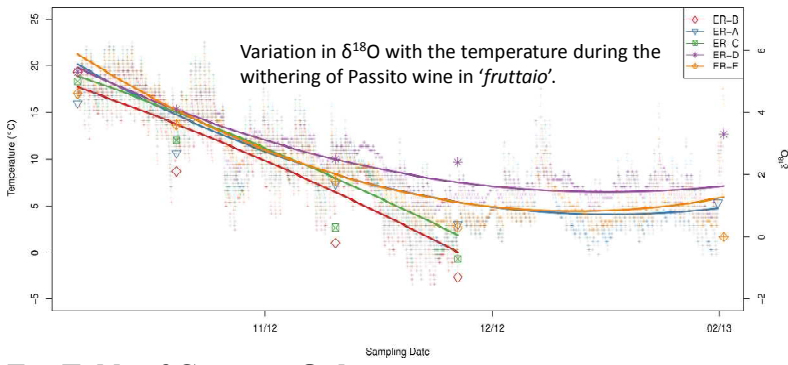


Figure 3





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