H, C, and O Stable Isotope Ratios of Passito Wine

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

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Abstract:

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

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

Introduction

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

The *Passito* wines produced in Italy have in common a key winemaking procedure, involving postharvest drying of grapes, which can last several weeks. The drying can be performed in a dedicated ventilated or unventilated fruit drying room, (called *fruttaio*), during autumn-winter, this method being more widespread in northern Italy, whereas in southern Italy the main technology is withering on the plant *(en plein–air*).[4] In both cases, during this period the grapes lose water, thus reducing total weight, whereas the concentration of sugars and other substances increases.[5] Progressive grape dehydration can encounter extremely variable trends according to the weather conditions (e.g. average daily humidity or temperature) in the case of *plein air* withering or withering in the *fruttaio*, but it can be kept under control in ventilated rooms. The dehydration process and infection with *Botritis cinerea* Pers. can influence the secondary metabolites of grapes, in particular in relation to colour, volatile compounds and phenolic composition.[6-9]

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

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

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

different traditional Italian *Passito* wines from grapes taken at different stages of withering and withered in different conditions were taken into account focusing on the $\delta^{18}O$ of water.

The final aim was to define the isotopic characteristics of Italian *Passito* wines and to check whether isotopic methods can be used for authenticity verification for this type of wine.

The novelty of this study lies in the fact that for the first time, the effect of grape withering processes (up to 5 months) on the variability of the $(D/H)_I$, $(D/H)_{II}$, $\delta^{13}C$ and $\delta^{18}O$ of wine ethanol and the $\delta^{18}O$ of wine and must water was monitored during the production of Passito wines. Considering different types of withering and different years, the monitoring carried out becomes very important, because variations in the isotopic values with withering can lead to wrong interpretation of the data and therefore to false conclusions about the authenticity of the samples.

Materials and Methods

Grape samples

The study focused on *Erbaluce di Caluso Passito* DOC a wine produced with Erbaluce white grapes grown in the province of Turin (Piedmont, north-west Italy) during 2011 and 2012.[21] The grape samples were withered in five different cellars with fruit-drying rooms (*fruttaio)* located in Caluso (N45.301045, E7.894921), identified as ER-A, ER-B, ER-C, in San Giorgio Canavese (N45.333054, E7.798944) ER-D and in Piverone ER-E (N45.445302, E8.006088) and the producers guaranteed respect of the procedural guidelines (G.U n. 248). ER-A withered the grapes by hanging them, ER-B put the product on wicker trellises, ER-C and ER-D withered the grapes in small boxes (holding about 5 kg) and ER-E used big boxes (holding about 20 kg), both in single layers. Two tons of Erbaluce grapes were withered for all cellars and years. During withering, relative humidity and temperature were monitored in each cellar using a Hobo data logger (Onset, MA, USA). In 2011, the withering process was faster due to specific climatic conditions. In particular, the RH% (Relative Humidity Percentage) in the first period of withering was low (60-65%) and the sugar increment achieved was about 10°Brix. In 2012, the RH% in the first period of dehydration was 70-82%, with a low sugar concentration (+3°Brix) in some cellars (EC-A, EC-D, EC-D). In this case, a longer processing time was necessary in order to obtain the minim sugar content for the production of *Erbaluce di Caluso Passito* wine in accordance with its Production Disciplinary. In 2012 one sample of fresh grapes from ER-C was also withered in controlled climatic conditions (25°C; 55 RH%) in a controlled chamber (ER-F).

Moreover, 78 grape samples used to produce 17 different traditional Italian *Passito* wines (Table 1) were sampled in 2011, 2012 and 2013 by inspectors of the Italian Ministry of Agriculture. For each product, the grapes were collected immediately after harvesting and at the end of withering. In some

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

Vinification

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

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

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

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

Analysis

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

$^{18}O/^{16}O$ ratio analysis of wine and must water was performed using a SIRA II, (VG Fisons, Middlewich, United Kingdom) connected to a water/CO_2 equilibration system Isoprep 1, (VG Fisons, Middlewich, United Kingdom) The procedure is described in the OIV-MA-AS2-12 method (2009).

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

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

$$\delta i\, E = \frac{(i\,RSA - i\,RREF)}{i\,RREF}$$

where i is the mass number of the heavier isotope of element E (for example, ^{13}C);

RSA is the respective isotope number ratio of a sample (such as for C: number of ^{13}C atoms/number of ^{12}C atoms or as $^{13}C/^{12}C$ approximation).[22-23]

RREF is that of internationally recognised reference materials (see above).

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

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

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

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

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

Statistical analysis

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

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

Results and Discussion

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

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

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

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

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

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

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

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

The modelling was performed by considering the average temperature and humidity values in the period before each $\delta^{18}O$ analysis. To better characterise the process, three scenarios were examined, calculating the average temperature and humidity one, two and three weeks before each $\delta^{18}O$ analysis. Each scenario was then modelled separately. In all cases a general linear model of the form $\delta^{18}O = A*Temperature + B*Humidity + C*(Temperature*Humidity) + D + \varepsilon$ was considered. This form is able to capture the separate effect of changes in temperature and humidity, the implicit contribution of time, and also the possible presence of an interaction effect between the two predictors. This separate modelling approach was preferred to a more complex linear model taking into account all the possible factors and their interactions for two reasons: a) the very small number of experimental points (only five time-resolved measurements of the isotopic ratios), b) high correlations between the averages of temperature and humidity at the one, two and three week intervals. In all three cases, the best model was found by stepwise model selection. For all three scenarios, stepwise selection indicated that the interaction term is not necessary to model the $\delta^{18}O$ shift during withering. In all cases, the optimal model had the form $\delta^{18}O = A*Temperature + B*Humidity + C + \varepsilon$, the level of significance for the two predictors in the three models being summarised in Table 3.

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

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

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

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

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

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

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

286 ***SURVEY OF ITALIAN PASSITO WINES***

287 In this section we considered $\delta^{18}O$ 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 $\delta^{18}O$
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 $\delta^{18}O$ 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 $\delta^{18}O$ 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*, $\delta^{18}O$ 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 $\delta^{18}O$ was observed. In the southern regions of Apulia, Sicily and
302 Sardinia, in most cases $\delta^{18}O$ 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) $\delta^{18}O$ decreased up to -4.6‰, probably because of precipitation, which caused an
306 exchange with water atmospheric vapour.
307 The significant variation in the $\delta^{18}O$ 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 $\delta^{18}O$ 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 $\delta^{18}O$ 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 $\delta^{18}O$ data of *Passito* wines for fraud detection.
316
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FIGURE CAPTIONS

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

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

Figure 3: Difference between $\delta^{18}O$ of must water at the end of withering ($\delta^{18}O_{passito}$) and that at harvest ($\delta^{18}O_{harvest}$). 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
in a fruit drying room (fruttaio)				
Erbaluce di Caluso Passito	North, Piedmont	on trellises, controlled temperature	Erbaluce	2011, 2012
Oltrepò Pavese Moscato Passito	North, Lombardy	on trellises, controlled temperature	Moscato	2012
Loazzolo Passito	North, Piedmont	on trellises, natural	Moscato	2011, 2012, 2013
*Ramandolo Passito **	North, Friuli Venezia Giulia	on trellises, forced ventilation	Verduzzo	2013
Colli Orientali del Friuli	North, Friuli Venezia Giulia	in boxes, controlled temperature	Verduzzo	2011
Picolit	North, Friuli Venezia Giulia	in boxes, controlled temperature	Picolit	2011, 2012
Strevi Passito	North, Piedmont	in boxes, controlled temperature	Moscato	2013
Recioto di Gambellara	North, Veneto	hung, controlled temperature	Garganega	2011, 2012
Montefalco Sagrantino Passito	Centre, Umbria	on trellises, forced ventilation	Sagrantino	2011, 2012, 2013
Verdicchio dei Castelli di Jesi Passito	Centre, Marche	on trellises, controlled temperature	Verdicchio	2012, 2013
Vin Santo del Chianti	Centre, Tuscany	on trellises, controlled temperature	Trebbiano	2011, 2012, 2013
Vin Santo del Chianti Classico	Centre, Tuscany	in boxes, controlled temperature	Trebbiano	2012, 2013
En plein air				
Albana di Romagna Passito	North, Emilia Romagna	over-ripening on the plants	Albana	2012
*Ramandolo Passito **	North, Friuli Venezia Giulia	over-ripening on the plants	Verduzzo	2011
*Ramandolo Passito**	North, Friuli Venezia Giulia	on trellises, covered	Verduzzo	2012
Moscato di Noto passito	South, Sicily	over-ripening on the plants	Moscato	2012, 2013
Passito di Manduria	South, Apulia	over-ripening on the plants	Primitivo	2012, 2013
Moscato di Trani	South, Apulia	over-ripening on the plants	Moscato	2012, 2013
Alghero passito	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	$\delta^{18}O_{VSMOW}$ / ‰, must water	*Brix	pH	Acidity g/L tartaric acid	$\delta^{18}O_{VSMOW}$ / ‰, wine water	$\delta^{18}O_{VSMOW}$ / ‰, ethanol	(D/H)I ppm ethanol	(D/H)II ppm ethanol	R ethanol	$\delta^{13}C_{VPDB}$ /‰, 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 $\delta^{18}O$ on the basis of temperature and humidity..

	Estimate	Std. Error	P value	Signif. Level[1]
Week 1 (Adjusted R^2 = 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 R^2 = 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 R^2 = 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

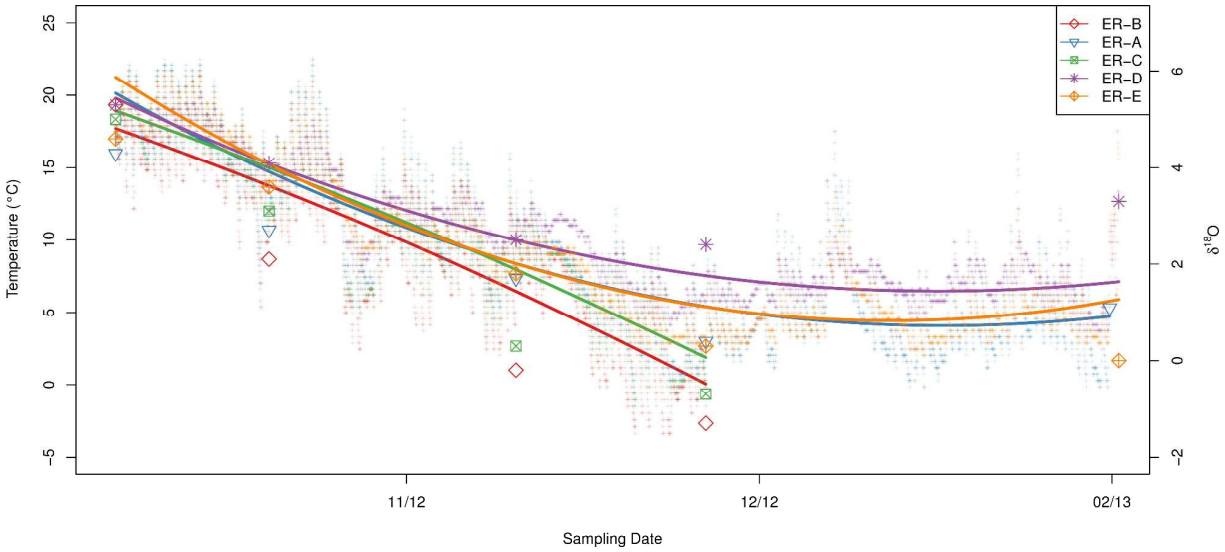


Figure 2

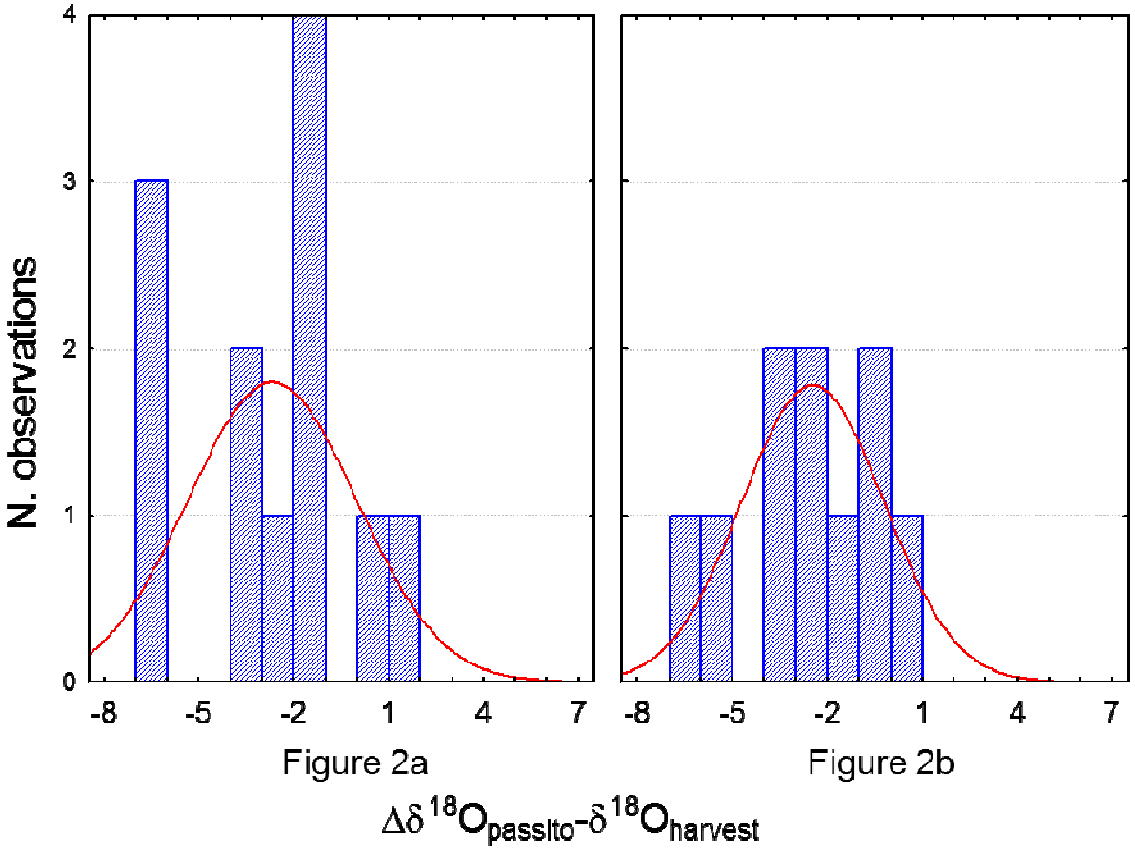
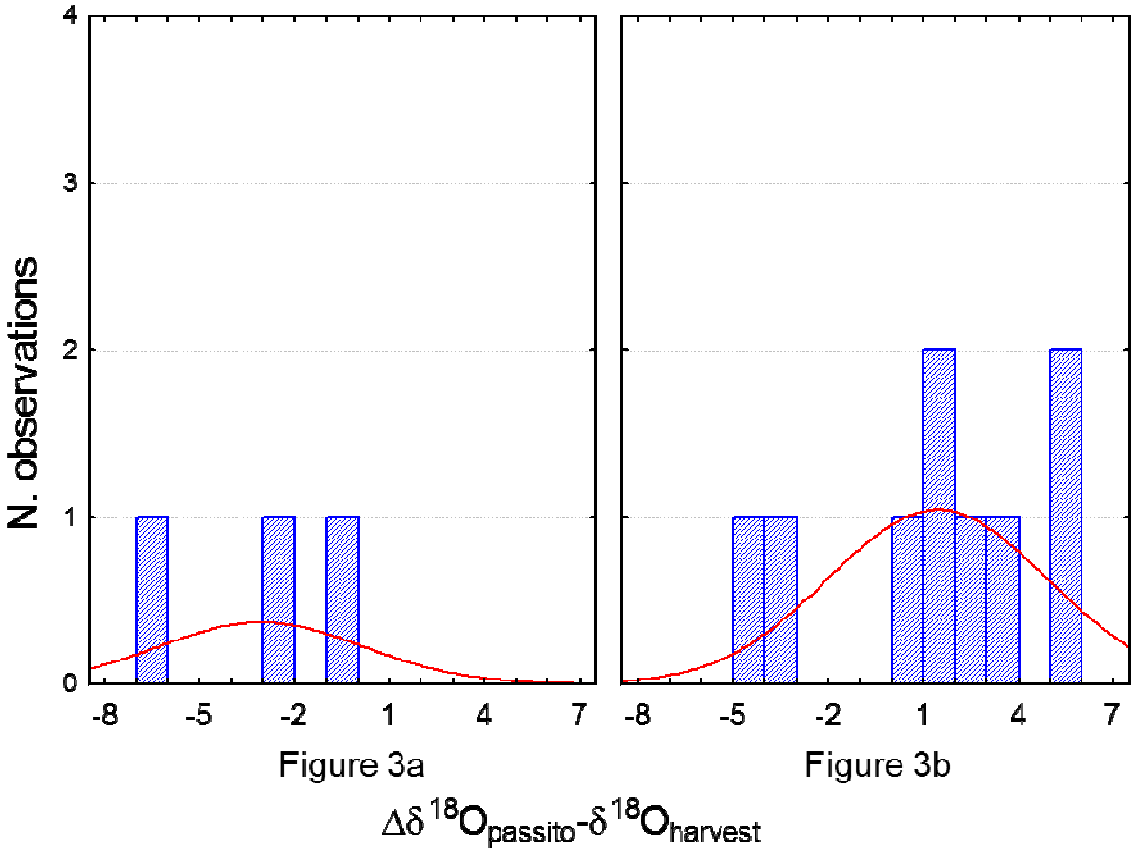


Figure 3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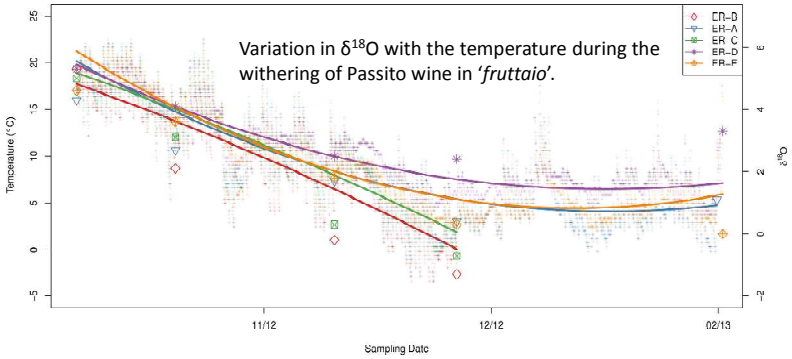
4
7
Figure 3a
Figure 3b
Δδ 18O passito-δ 18O harvest



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