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The $^{18}O/^{16}O$ Ratio of Retail Moravian Wines from the Czech Republic in Comparison with European Wines

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Abstract

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About 50 samples of retail Czech wines from the South of Moravia (vintage years 2008 to 2015) were measured for $\delta^{18}O$ values in wine water together with more than 60 European wines. The aim of the study was to compare Moravian wines (not measured for $\delta^{18}O$ up to date) with regional European wines and published data from the European wine databanks. The observed variability of $\delta^{18}O$ values with vintage year corresponds to the variability of German wines from the Rhine region. We did not observe any significant admixture of must from other regions. The method of ^{18}O measurement appears to be very sensitive to small differences in the climate of the region (comparison of South Moravia and the near Malé Karpaty region).

Keywords: ^{18}O isotope composition; water in wine; South Moravian wine region; Czech wines; EU wines

In 1990, the EU decided to install a system of data banks for all wine-producing countries within the EU (EU Regulation 2676/90) to protect and control the authenticity of European wines. The analysis of stable isotopes as genuine markers of wine origin becomes an important part of the authentication process. Determination of the site-specific D/H ratio in wine ethanol by NMR was the first officially adopted stable isotope method (Martin *et al*. 1983). In subsequent years, further applications of the stable isotope method were adopted ($^{13}C/^{12}C$ values of ethanol, $^{18}O/^{16}O$ in wine water). Finally, all these methods were recommended to detect fraud in wine production (ECR 1990; CEN 1996; OIV 2012). $^{2}H/^{1}H$ and $^{13}C/^{12}C$ values of ethanol are used to detect the addition of exogenous sugar before or during the fermentation process (Dordevic *et al*. 2013). Beet and cane sugar, the two main additives, have different isotope ratios in comparison with wine must. $^{18}O/^{16}O$ in wine water is used to detect the addition of water in must. Tap or spring water has a lower ^{18}O concentration than the original grape juice (Guyon *et al*. 2006).

The European wine databank (2004) collects more than 1400 samples of grapes annually according to wine regions and production (France or Italy 400 samples, Czech Republic 20 samples). The databank serves quality control purposes, and every member state has access to its own data only (or that of a country of possible import). Aside from this official databank, a number of studies were published on stable isotope data with respect to various phenomena. These data can be used for the comparison and interpretation of stable isotope patterns in selected wines. We analysed more than 50 samples of Czech production (South Moravian region vintage 2008–2015) and another 60 samples produced by European or other countries to study the variation of $^{18}O/^{16}O$ ratio in wine water. Up to now, no ^{18}O data have been published from the area,

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and a comparison with European production (both published and directly measured data) would be interesting and useful for future analyses.

MATERIAL AND METHODS

Wine samples. Only wines labelled as PDO (protected designation of origin) (AOC in France, DOC in Italy) were considered for testing. According to the European Community trademark law (No. 1493/1999), wine to be considered as a 'quality wine psr' must be produced in a specified region. Some foreign wines were available only as Protected Geographical Region (PGR, IGP in France). Table wines or wines from a tank were measured occasionally but not considered in the study.

Methods. The original method from Epstein and Mayeda (1953) using the equilibration reaction between CO_2 and water for the measurement of $\delta^{18}O$ in water was modified for must and wine measurements (ECR 1990; CEN 1996; ECR 2009). This technique is a standard method for $^{18}O/^{16}O$ equilibration that utilises the GasBench II periphery together with the isotope ratio mass spectrometer Delta V or Delta Advantage (all Thermo Fisher Scientific, USA). A helium mixture with 0.4% CO_2 is equilibrated for isotope exchange with water in wine or some other solution at room temperature for about 20 hours. After equilibration, the gas above the sample is measured against CO_2 equilibrated with the international reference materials (Vienna Standard Mean Ocean Water – V-SMOW2) and related to internal references. $\delta^{18}O$ is calculated according to Eq.1:

$$\delta^{18}O\ (‰) = \frac{(^{18}O/^{16}O)_{sample} - (^{18}O/^{16}O)_{standard}}{(^{18}O/^{16}O)_{standard}} \times 1000 \quad (1)$$

where: $(^{18}O/^{16}O)_{standard} = (^{18}O/^{16}O)_{V\text{-}SMOW2})$ – equal to 0.0020052

External reproducibility of measurement is better than 0.1%. Stable Isotope Laboratory of the Czech Geological Survey participates regularly in International Water Isotope Inter-Comparison Tests of the International Atomic Energy Agency – last time in 2016 (WICO 2016).

The usual values of $\delta^{18}O$ in wines range from –3 to +2 for Central Europe and from –1 to +7 for Southern Europe (Christoph *et al*. 2015). For comparison, tap water $\delta^{18}O$ values in Central Europe range from –10 to –9%; those in Southern Europe are about –5%.

As isotopic composition is the molecular property of monitored water, the simple mass balance is valid for, multiplied by the total volume (V) of water in the wine sample:

$$\delta^{18}O_{tot} \times V_{tot} = \delta^{18}O_{wine} \times V_{wine} + \delta^{18}O_{water} \times V_{water} \quad (2)$$

where: tot – total mixture; wine and water to wine and admixed water

Theory. The distribution of isotopes of bioelements ($^{13}C/^{12}C$, $^{2}H/^{1}H$, or $^{18}O/^{16}O$) in sugar, organic acids, water, or fermented components such as ethanol is controlled by fractionation processes. The fractionation of oxygen isotopes results in the typical $^{18}O/^{16}O$ isotope ratio for a given year of vintage or geographic origin, which can be used further for the comparison of wines. Water itself changes the $^{18}O/^{16}O$ ratio during evaporation and condensation (depletion with evaporation, enrichment during condensation, depletion of precipitation with increasing latitude, enrichment with increasing temperature, less depletion with decreasing latitude, etc.). Generally, precipitation at a given location generates groundwater and soil water of a typical $\delta^{18}O$ value, which is taken up by the roots of the vine and subsequently transported to the leaves. This part of water supply is without any significant isotope fractionation (White 1989). However, $\delta^{18}O$ is higher in grape (enriched with ^{18}O) as compared to soil water because of enrichment during evapotranspiration in leaves and grape skin as well as exchange with atmospheric vapour (Tardaguila *et al*. 1997; Rossmann *et al*. 1999). Final changes in $\delta^{18}O$ of grape water occur in the period between veraison (berries start to change colour) and harvest (Rossmann *et al*. 1999). Climatic conditions during the harvest period are most important. The dry and hot environment of the end of summer produces wines with relatively stable and positive $\delta^{18}O$ values (e.g., +1 to +8‰). Later harvest (September or even October) in the higher latitude regions (north of France or Italy, Central Europe, or Germany) takes place at lower temperatures, with higher air humidity and raining, which shifts $\delta^{18}O$ of the must to variable and frequently even negative values (+1 to –4‰). The fermentation process does not change the $\delta^{18}O$ value significantly: must and wine differ only 0.2% or less (Rossmann *et al*. 1999). Generally, any addition of compounds with different $^{18}O/^{16}O$ ratio (added sugar, bentonites, etc.) can change the $\delta^{18}O$ value of must according to Eq. 2. But amounts of such additives are too small to change

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Table 1. The $^{18}O/^{16}O$ ratio of retail wines from the Czech Republic and European countries

Country of origin	Year	Region	Producer	Variety	$\delta^{18}O$ (‰)
Czech Republic	2008	Mikulov, Sedlec	Roztoky	white Burgundy	–1.4
	2009	Mikulov, Horní Věstonice	Wine pod Martinkou	Riesling	0.4
		Mikulov	Wine Dietrichstein	Riesling	2.4
		Mikulov, Dolní Dunajovice	Wine Valtice	Aurelius	0.0
	2010	Mikulov, Perná	Vinselekt Michlovský	Chardonnay	7.8
	2011	Mikulov, Popice	Wine maker Tomáš Krist	Pálava	–2.1
		Mikulov, Pouzdřany	Wine Kolby	blue Portugaise	1.6
	2012	Mikulov, Novosedly	Wine Ludwig	Neuburger	0.4
		Mikulov, Dolní Dunajovice	Mikrosvín Mikulov	Chardonnay	0.1
		Mikulov, Valtice	Valtice wine school	Vetltliner	–2.4
	2013	Mikulov, Popice	Wine Gotberg	red Traminer	–2.7
		Mikulov, Valtice	Venerice	BlauFrankish	–4.9
		Mikulov, Popice	Wine Gotberg	Pálava	–4.3
		Mikulov, Novosedly	Wine Ludwig	white Burgundy	–2.5
		Mikulov	Wine Dietrichstein	white Burgundy	–3.8
		Mikulov, Popice	Wine Gotberg	red Traminer	–2.5
	2014	Mikulov		red Burgundy rose	–1.4
		Mikulov, Bavory	Wine Palavín	Merlot rosé	–3.7
		Mikulov	Víno Mikulov Motýl	BlauFrankish	–3.7
		Mikulov, Valtice	BlacQin	Chardonnay	–3.6
		Mikulov		Veltliner	–2.9
	2008	Slovácko, Hovorany		Chardonnay	3.7
		Slovácko, Čejč	Vinselekt Michlovský	Riesling	–0.4
	2012	Slovácko, Milotice	Wine Babíček, Vacenovský B/V	BlauFrankish	–1.6
	2013	Slovácko, Tvrdonice	Wine JanBalga Solen	red Traminer	–3.1
		Slovácko, Hýsly	U dvou lip	red Traminer	–4.1
		Slovácko, Bzenec	Chateau Bzenec	Chardonnay	–2.9
	2014	Slovácko, Prušánky	Wine Košut	white Burgundy	–3.9
		Slovácko, Bzenec	Wine P. Bunža	red Burgundy rose	–3.0
	2015	Slovácko, Blatnice		blue Burgundy	–3.5
	2008	V. Pavlovice	Ludwig, Bořetice	red Traminer	–1.3
		V. Pavlovice, Boleradice		Riesling	1.3
	2011	V. Pavlovice	Wine maker Kubik	Merlot barique	1.8
		V. Pavlovice, V. Bílovice	Habánské sklepy	Riesling	–0.4
	2012	V. Pavlovice	Wine Baloun	Zweigeltrebe	–0.4
		V. Pavlovice, Rakvice	Wine Michlovský	André	0.9
	2013	V. Pavlovice, Čejkovice	Templars cellars	Riesling	–3.5
	2014	V. Pavlovice, Čejkovice	Templars cellars	Grey Burgundy	–2.8
		V. Pavlovice, V. Bílovice	Wine Madl	Muskat ottonel	–3.1
	2015	V. Pavlovice	Vinum	red Burgundy	0.8
		V. Pavlovice	Vinum	Zweigeltrebe	–0.3
	2013	Znojmo, Dobšice	Wine Lahofer	white Burgundy	–5.3
		Znojmo, Dolní Kounice	Wine Trpělka & Oulehla	white Burgundy	–2.0
		Znojmo, Vrbovec	Pavel Zbojník	Riesling	–2.9
	2014	Znojmo, Stošíkovice	Znovín	Riesling	–3.3
		Znojmo, Dolní Kounice	Regina Coeli, Trpělka & Oulehla	Merlot rosé	–3.5
		Znojmo, Vrbovec	Ampelos Znojmo	Chardonnay	–4.6
		Znojmo, Vrbovec	Vinice Hnanice	Cabernet Sauvignon	–4.5
	2015	Znojmo, Nové Bránice	Wine Oulehla	BlauFrankish	–3.2
		Znojmo, Dyje	Wine Lahofer	Zweigeltrebe rosé	–3.2

doi: 10.17221/205/2016-CJFS

Table 1 to be continued

Country of origin	Year	Region	Producer	Variety	$\delta^{18}O$ (‰)
Slovak Republic	2011	Svatý Ján	Vinařství Chowianec, Svatý Jur	Cabernet Sauvignon	2.1
	2014	Modra		Burgundy	–2.7
		Pezinok	Wine Matyšák	BlauFrankish	1.8
	2015	Pezinok	Wine Cech	Leanka	0.4
		Modra, Králová	Lalinwine	Lalinawine, Sylvaner	1.4
		Modra, Hliny	Vinko Klimko	Saint Laurent	0.6
		Modra, Grefty	Vinko Klimko	Muller Turgau	0.7
		Modra, Noviny	Lalinwine	white Burgundy	1.1
		Modra, Plázle	Lalinwine	Muscat Otonel	1.3
		Modra, Grefty	Vinko Klimko	Muller Turgau	0.7
		Modra	Vinko Klimko	Chardonnay	0.5
Hungary	2013	BalatonForedi		Cabernet Sauvignon	–1.0
	2014	Matra	Szola	Muscat Ottonel	0.6
		BalatonForedi		Veltliner	–0.8
		Eger	Egri Korona Borház kft., Demjén	Muscat Ottonel	–1.7
Italy	2009	Chianti	San Caseinao Val di pesa Torteza Colli	Chianti	2.9
	2010	Toscana, Poggibonsi	Corte alle Mura	Chianti	3.1
	2013	Salento		Negroamaro, Salento Rosso	3.7
		Garganega, Corte Viola, Trebliano		Bianco di Custoza, Tokaj	0.8
		Dorgali	da Cantina Sociale Dorgali	Cannonau di Sardegna	5.2
	2014	Veneto		Merlot	0.7
		Codici nero D'Avola		Negro	3.3
		Salento, Messapico, SRL in Tuglie – Lecce	Da Agricola Capo Leuca	Negroamaro	2.3
	2015	Cossano Belbo	Canti	Merlot rosé	2.7
France	2010	Bordeaux La Chateau Pirouette, Medoc	Global Wines cz	Empereur	2.3
	2011	Massif central		Basalt	4.1
	2013	Narbonne	Les Vignerons de la Méditerranée	Chardonnay	3.2
		Bordeaux		Bordeaux (Merlot, Sauvignon)	1.1
		Bordeaux		Bordeaux	1.5
	2014	Bordeaux	Grand Desir	Bordeaux	3.1
		Bordeaux		Medoc	2.5
		Ventoux	Bedoin	Harmonie rose	2.3
		Gironde, Chateau Cardonna, Lahourcarde		Medoc	2.7
		Languedoc	Familie Castle	Merlot	3.8
		Vin de la vallee du Rhone		Merlot	5.2
		Fleurs des Templiers		Bordeaux	2.6
		Languedoc Roussillon	J.P.Chenet	Grenache-Cinsault	3.0
		Bordeaux	Fernand, Carignan de Bordeaux	Merlot Cabernet Sauvignon	4.3
		Marquis Delplanque, Costières de Nîmes		Merlot	2.6
		Bordeaux	SARL VR F33540, Empereur	Merlot Cabernet Sauvignon	2.2

doi: 10.17221/205/2016-CJFS

Table 1 to be continued

Country of origin	Year	Region	Producer	Variety	$\delta^{18}O$ (‰)
Spain	2007	Marques de Campoblanco		Tempranillo	7.8
	2009	Valencia	Torre Oria	Temprenillo 0.6, Cabernet 0.4	4.5
		Valencia	La Emperatriz S.L. Baños de Rioja		1.6
	2010	Catalonia, Terra Alta	Vespral	Cabernet	5.6
		Castillo San Simón, Monastrell	Bodegas	Cabernet	5.8
	2012	Jumila	Bodegas	Alaja Crianza	6.3
	2014	Cosecha, Rioja	Viña Nobile Rioja, Cosecha	Tempranillo	–1.4
		Valencie	Velada	Muscat	5.5
		Navarra		Ravel	0.5
		Campo de Borja		Tempranillo	4.2
		Navarra	Bodegas BrañaVieja S.L.C.	Ravel blanco	4.3
		La Tierra da Castilla	Felix Solis	Cabernet Sauvignon	8.6
		Navarra		Garnacha	2.3
Portugal	2014	Douro		Tempranillo	2.6
		Portas Tejo, Regional Tejo		Castelao Aragones	5.1
Austria	2014	Osterreichischer Landwein-Weinland	Weingut Neustifter, Poysdorf	Cuvée Noir/Blauburger/Merlot	–2.7
Bulgaria	2014	Rose valley		Merlot	0.4
Gruzia	2014	Mukuzani	Mukuzami Wine	Saperavi	3.5
Moldavia	2015	Orhei	Chateau Vartely	Cabernet Sauvignon	2.7

the resulting balance of $^{18}O/^{16}O$ in a measurable way and need not be considered.

RESULTS AND DISCUSSION

Regional ^{18}O variations. The sample measurements are summarised in Table 1 according to the country of origin and vintage year. The $\delta^{18}O$ values of European wines are presented in Figure 1, plotted against the latitude of vine cultivation. The figure also contains published values from the wine databanks of Germany, France, Italy, and Spain (Rossmann *et al*. 1999; ; Gómez-Alonso & García-Romero 2009). Measured $\delta^{18}O$ values can be roughly interpreted as an increase in $\delta^{18}O$ with decreasing latitude (i.e., with increasing air temperature and lower precipitation and air humidity) (Figure 1). Further variation for a given location manifests as variation of $\delta^{18}O$ with a given vintage year. For these reasons, the EU wine databanks collect samples every year to provide comparison for suspicious samples of the same vintage and origin. Updating the data banks is a laborious task, and comparable samples are not always available. Supplementary methods and models are created to estimate probable $\delta^{18}O$ values of wine from certain locations. Models are constructed on meteorological parameters such as relative humidity, mean air temperature, and GIS parameters. Models were tested for American wines from Washington, Oregon, and California (West *et al*. 2007) and German wines from Rhine, Pfalz, and Mosel regions (Hermann & Voerkelius 2008). For the tested years, models offer good quality data for the comparison of local wines using the data banks.

Temporal ^{18}O variations. Samples of Czech wines (open squares in Figure 1) show a significant range of $\delta^{18}O$ values in a relatively small area of the South Moravian region. This information is given by the time variation of the vintage (Figure 2).

While in the South of Europe vintage takes place at the dry and relatively warm end of the summer, in Central Europe (or, generally, in regions of higher

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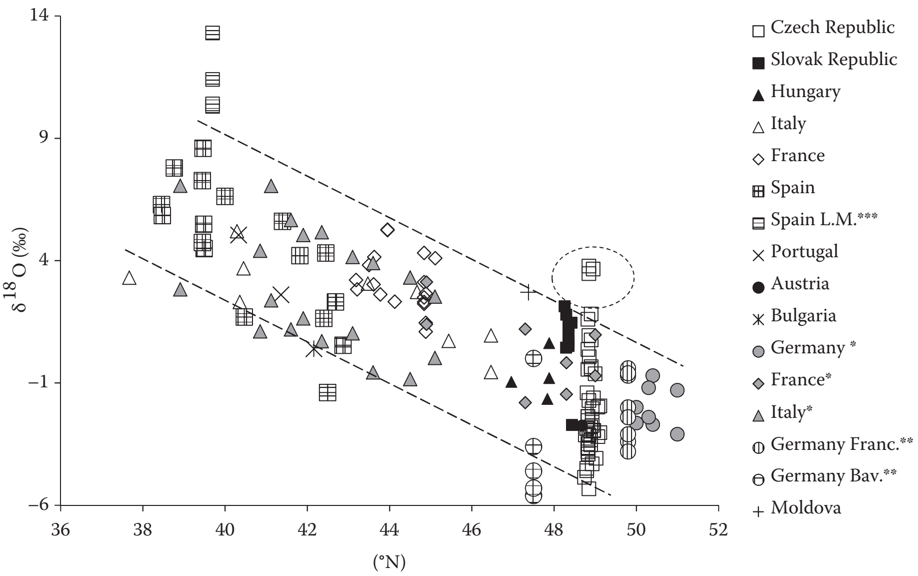

Figure 1. $\delta^{18}O$ of the Czech and European wines plotted against the latitude of the production region; samples in the dotted area have higher (more positive) $\delta^{18}O$ values, corresponding to the range of Czech wines during the period from 2008 to 2015

*Rossmann *et al.* (1999)
**Christoph *et al.* (2003)
***Gómez-Alonso & García-Romero (2009)

latitude) grape harvest takes place in September (even in October) under frequently changing climatic conditions. During the period before harvest, a few days' change of temperature, rain, or fog can dramatically shift the $\delta^{18}O$ values of the must-water and consequently of wine (Rossmann *et al.* 1999). Early harvest under dry and warm conditions produces wine with more positive $\delta^{18}O$ values, or very positive values for a very dry region (Figure 1, data from Spain L.M. (La Mancha region)) (Tardaguila *et al.* 1997) may mean that no drying effect is observed for irrigated grapes (Gómez-Alonso & García-Romero 2009). Later harvest produces less positive or even negative $\delta^{18}O$ values (). Comparable Slovak wines from the very near Little Carpathian region are systematically enriched with ^{18}O (Figure 2). These data correspond to different microclimatic conditions in both regions: the Little Carpathian region is drier and warmer than the South of Moravia.

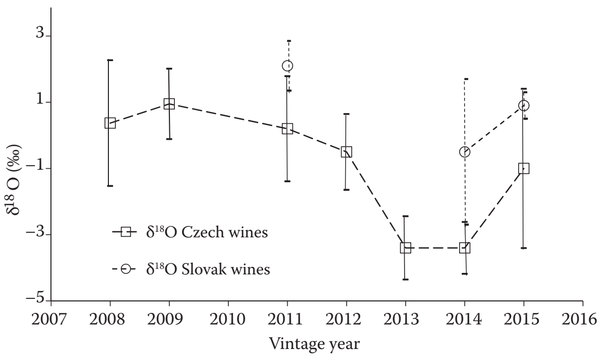

Figure 2. Mean $\delta^{18}O$ values of wine water from the measured Czech wines produced in the period 2008–2015 in the South Moravian region; error bars correspond to 95% confidence intervals as calculated from the measured values for each vintage year; Slovak wines from the near Little Carpathian region are plotted together

We examined Moravian wines for a possible relationship to air temperature, precipitation, and the ^{18}O isotopic composition of precipitation last month before harvest (all data are from the South Moravian region in September) (CHMI 2016) (Figure 3). All plots show expected effects of the $\delta^{18}O$ values of wine water: a temperature decrease (Figure 3A), extreme precipitation (Figure 3B), and a decrease in $\delta^{18}O$ of precipitation (Figure 3C).

Because examining the wine water for the addition of water or must of some other origin is a comparative method, it is important to know the range of reliable $\delta^{18}O$ values for a given region and vintage. With the known time variation (Figures 2 and 3), we can identify samples with higher $\delta^{18}O$ values (dotted area in Figure 1) as possible mixtures of local must with must from regions of lower latitude with higher temperature and evapotranspiration (for example from Moldavia or Romania). Only 15% of grapes from the other region are allowed for PDO. Wines with more negative $\delta^{18}O$ values than corresponds to Figure 2 are likely to contain additional water ($\delta^{18}O$ of local tap water varies from −9 to −9.5%). We observe this phenomenon frequently in association with 'pure' fruit juice or must. For example, apples from the 2015 harvest have $\delta^{18}O$ values from −2.2 to −2.6% (apple juice has lower variability than grape must), but the retail product has the $\delta^{18}O$ value of −5.6 or less. Using Balance Equation 2 and $\delta^{18}O$ of −9.5‰ for tap water, we get about 45% of additional water in '100% juice'.

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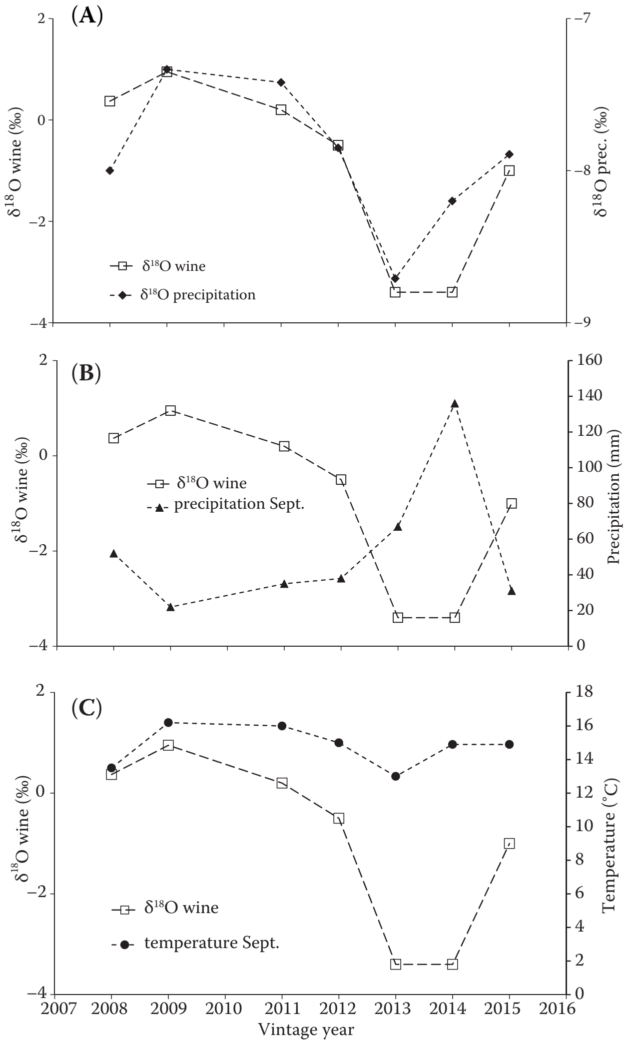

Figure 3. Time plot of mean $\delta^{18}O$ values of the measured Czech from the South Moravian region wines plotted against: (**A**) mean air temperature in September; (**B**) total precipitation in September; (**C**) $\delta^{18}O$ values of accumulated September precipitation

CONCLUSIONS

The wine samples studied from the South of Moravia have $\delta^{18}O$ values corresponding to the must of particular regions and vintage years. The observed dependence of $\delta^{18}O$ values on temperature and precipitation during harvest resembles the data published for German wines from the Rhine region.

A few samples had higher $\delta^{18}O$ values (enriched with ^{18}O) than the observed range for vintage years. This corresponds to the admixture of grape must from warmer or dry regions. In such a case, another isotopic method (D/H NMR) can be used to elucidate the admixture process.

Measurements of $\delta^{18}O$ in wine-water are sensitive to microclimatic effects. Small climate differences between the South of Moravia and the near Little Carpathian region can be differentiated. This supports the necessity of differentiating the wine production regions based on climatic and geographic diversity. Only the systematic collection of musts from the given region can help in the differentiation of admixtures of grapes from other regions.

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