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Radiocarbon dating of microliter sized Hungarian Tokaj wine samples

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ABSTRACT

Radiocarbon-based age determination of wine samples has a great tradition worldwide, but most of the applied techniques, such as liquid scintillation counting and gas proportional counting analyses, have had large sample size requirements. However, accelerator mass spectrometry (AMS) based radiocarbon dating methods require much lower amount of carbon. Up to now, only a few available studies applied the AMS method to the dating of wine. We tested a preparation and measurement protocol for wine radiocarbon dating, not only for the ethanol fraction but for the distillation residue and submilliliter level preparation method of the wine sample without separation was also applied, using capillaries of the twenty wine samples from the Hungarian Tokaj wine region. The reliability of our method was verified by a comparison of wine time series with the Northern Hemisphere Zone 1 atmospheric ^{14}C data as a calibration curve. The measured ^{14}C values of the two different fractions, the ethanol and distillation residue, and the milliliter-sized non-separated samples also were in good agreement with each other, which shows both fractions could be used for radiocarbon dating of wine samples. Small sample size (~10 μL) wine radiocarbon dating does not destroy a significant part of a bottle of wine.

1. Introduction

Nuclear bomb tests increased the atmospheric level of the natural radioactive carbon isotope, radiocarbon (^{14}C, C-14), which also was observed worldwide in the biosphere. These tests approximately doubled the atmospheric radiocarbon budget in the 1960s, but this level and the ratio of ^{14}C compared to the stable carbon isotope (^{12}C) has continuously decreased since the Nuclear Test Ban Treaty (1963). The increasing ^{14}C trend was result of the nuclear tests, the maximum of this curve occurred in 1963–64, and the decline after the Nuclear Test Ban Treaty created the "radiocarbon bomb-peak" (Hua et al., 2021, 2013). The reason for the rapid decline is not the half-life of the radiocarbon isotope, but the hydrosphere-atmosphere CO_2 exchange, mainly within the oceans. The atmospheric radiocarbon bomb peak and curve from the 1950s are also widely used for the dating of modern samples with forensic, medical, and environmental-related studies. The bomb-curve based on tree ring and atmospheric CO_2 monitoring samples has even better than the yearly resolution. In some cases, the dating based on the measurement of the $^{14}C/^{12}C$ ratio by accelerator mass spectrometers (AMS) can be performed with an accuracy of less than 1 year. For both the decay counting and accelerator mass spectrometry (isotope ratio measurement) based dating methods, the mass (isotope) fractionation is corrected by the measurement of the fractionation of stable isotopes ($^{13}C/^{12}C$), so the final ^{14}C results are normalized to the same value of $^{13}C/^{12}C$ (Kutschera, 2013, 1983). Recently, the decline of the atmospheric ^{14}C level is not as rapid as before, because the $^{14}C/^{12}C$ ratio is already close to the previous natural level, and the yearly decrease is smaller than the AMS-method measurement uncertainty so that 1 year or better than 1-year precision is no longer feasible for recent years (Bergmann et al., 2012; Heinke et al., 2022; Hua et al., 2021; Levchenko and Williams, 2016; Martin and Thibault, 1995; Rinyu et al., 2019; Zoppi et al., 2004).

Most of the plant-produced organic materials represent the actual $^{14}C/^{12}C$ ratio of atmospheric CO_2 due to carbon dioxide uptake during

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photosynthesis (Cook et al., 2001; Garbaras et al., 2018; Rakowski, 2011). For this reason, most part the organic material of wines, specifically the sugars, should also reflect the atmospheric ^{14}C ratio bomb-peak such as tree rings (Hua et al., 2021; Palstra et al., 2008). The radiocarbon-based dating of modern wine samples has a long tradition worldwide in different laboratories (Burchuladze et al., 1989; Palstra et al., 2008; Povinec et al., 2020; Sakurai et al., 2013; Schönhofer, 1992; Zoppi et al., 2004). The aim of these studies can be quite different, as the reason can be the verification of the harvest year for fraud investigation, which would need a small sample size, or the reconstruction of past atmospheric $^{14}CO_2$ level, where the sample size is secondary. Most of these published studies used liquid scintillation counting (LSC) and gas proportional counting (GPC) based dating methods (Burchuladze et al., 1989; Kaizer et al., 2018; Martin and Thibault, 1995; Schönhofer, 1992, 1989). Accelerator mass spectrometry (AMS) based methods were only applied in a few published cases for wines and other alcoholic products, like whiskey (Cook et al., 2020; Palstra et al., 2008; Sakurai et al., 2013; Zoppi et al., 2004). Furthermore, most of the studies used only a separated (chemical) fraction of the wine samples, although other components also may have been used for the dating (Kaizer et al., 2018; Palstra et al., 2008; Povinec et al., 2020). Distillation of the ethanol fraction is a time-consuming and destructive method and requires a relatively high amount of the material for the ^{14}C analysis. Ideally, the prepared wine amount for the ^{14}C age determination has to be reduced to the current expectations, to save most of the (old and expensive) bottles. A whole bottle of wine should not be destroyed for the analyses, so the quantity of the prepared material should be as low as possible. AMS-based dating methods can be excellent solutions to minimize the amount of wine used, as this method need not more than 0.5 mg of carbon for the proper $^{14}C/^{12}C$ isotopic ratio measurement (Kutschera, 2013; Linick et al., 1989; Molnár et al., 2013). This way, AMS ^{14}C-based radiocarbon dating as a tool of forensic science can effectively help the studies and fight against wine counterfeiting and supplement other analytical techniques (Bridle and García-Viguera, 1996; Bronzi et al., 2020; Herrero-Latorre et al., 2019).

Hungary has a great tradition in wine production and has several dedicated wine regions around the country with different typical, characteristic wines. The Tokaj wine region has been a UNESCO World Heritage site since 2002 and is famous for its wines which are also produced in great quantity for export (Szepesi et al., 2017).

We obtained wine samples from the Tokaj-Hétszőlő estate for this study, where the wines are made with an organic approach with their grapes. The studied „aszú" type wine collection covers a continuous period (1999–2018), the individual vintages of which are well known. Therefore, we determined the radiocarbon content of each vintage, which provides the ages of valuable Tokaj wine specialties such as aszú. The 20 wine samples were measured by the AMS-based radiocarbon dating technique at the International Radiocarbon AMS Competence and Training Center (INTERACT), Debrecen, Hungary (Molnár et al., 2013). The main aim of our study was the testing a low-amount carbon method for harvest year verification of Hungarian wines, using known age wine with approved harvest years.

2. Methods

2.1. Wine samples

Tokaji aszú is one of the most famous export wines of Hungary (Gomes et al., 2022; Hajós et al., 2000; Kiss and Sass-Kiss, 2005; Machyňáková et al., 2021). Twenty samples of wines (sweet "Tokaji aszú") were obtained from the Tokaj-Hétszőlő estate, a winery which is located in the Tokaj wine region. This region is a UNESCO World Heritage area in the Zemplén mountains, North-East Hungary. Due to regulations, the grapes could only be grown in a narrow area (Fig. 1.), which represents a non-extended geographical origin with the same soil and climate conditions. The obtained wines were produced between

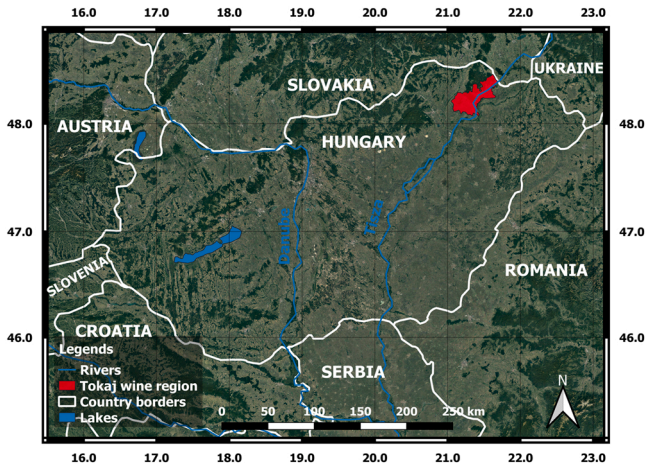

Fig. 1. Location of the Tokaj wine region in Hungary.

1999 and 2018 with yearly resolutions. The wine stock was kept in the cellar of the Hétszőlő estate, after which the prepared fractions were stored in the refrigerator at 5 °C.

In Tokaj, the bud break of the grape starts in the middle of April, the blooming starts in early June and lasts long only in the middle of June, the ripening of the grapes starts in the middle of August, and the harvest period usually lasts from the second half of October to the middle of November. In this season, grapes that have been infected by noble mold (*botrytis cinerea*) are harvested and sorted to make Aszú wine (Magyar and Soós, 2016). The selected aszú berries are put in a tub for a while to be pressed by their weight. The dripping juice is the so-called essence, which contains the most sugars and extracts. This essence is the most valuable part of the crop because it contains concentrated trace elements and minerals uptaken from the soil. After the essence has been drained, the aszú grapes are pressed into an aszú paste. The resulting mass is poured with must or wine of 136 liters, stirred from time to time, and then pressed (Eperjesi, 2010).

The term puttony is used to classify the quality of Aszú wines, which is based on the amount of aszú berry added to the mixture, which is called puttony (one puttony is about 25 kg). According to the recipe, an Aszú wine should be made with a minimum of 3 and a maximum of 6 puttony. The sugar content is between 60 and 150 g/litre, and the ethanol content is about 12%. After fermentation, the Aszú wines are aged for at least two years in wooden barrels (Kerényi, 2013).

2.2. Preparation and AMS measurement

A group of wine samples was prepared by distillation. During the distillation process, three fractions can be separated: ethanol, water, and residue. The ethanol fraction was separated by boiling the wine at 78.3 °C, while the ethanol vapor was condensed and collected in a plastic centrifuge tube. The second fraction, i.e. water, was obtained by boiling it at 100 degrees in a glass flask, which was collected in a glass bottle. For distillation, 500 ml wine samples were used, where we collected around 100 ml ethanol fraction., but most of this distilled fraction was used for tritium measurement, which is not part of this study. From this bigger fraction, subsamples were separated for radiocarbon measurement. Then, after the evaporation of water, the remaining fraction was transferred in to another closed centrifuge tube. Then, about 2 mg of ethanol and distillation residue samples were transferred into preheated borosilicate glass tubes as reaction cells with 300 mg MnO_2 reagent for sealed tube combustion (Janovics et al., 2018). From the unseparated, (non-distilled, whole) wine, only ~ 6 mg (below 10 μL) sample was used for the analyses and the samples were measured into the combustion tubes using sterile capillaries (Marienfeld capillary for melting point determination, 80 ×0.6 mm). Using these

capillary tubes, we could measure the wines precisely, without loss at the wall of the narrow glass tubes into the reaction cells.

The bottom of borosilicate glass combustion tubes was cooled by an isopropyl alcohol-dry ice mix bath (~−70 ℃), to prevent the loss of volatile parts of the wine samples, while sealed with flame on their top under vacuum. Then the sealed tubes containing the samples were heated in a furnace at 550 ℃ for 12 h. After the combustion procedure, the sample tubes were placed in a tube cracker and the gas was removed in a dedicated vacuum line for extraction and purification of the produced CO_2 gas. The amount of trapped CO_2 gas from the samples was measured in a calibrated volume to calculate and measure the yield of the combustion and preparation. IAEA-C9 (wood) and -C6 (sucrose) reference samples, using the same mass range as the samples, were prepared together with the samples and handled in the same way as the wine samples as well. The dedicated vacuum line and preparation procedure with MnO_2 regent was presented in our earlier study by Janovics et al. (2018). This vacuum line is suitable for small sample sizes (even below 50 μg C) to normal AMS measurement size samples (1–2 mg C).

The pure CO_2 gas from the samples (with >0.5 mg carbon) was transferred into borosilicate glass reagent tubes, which contained 10 mg TiH_2, 60 mg Zn, and 4.5 Fe mg reagents for sealed tube graphitization. The detailed procedure is presented in our former study by Rinyu et al. (2013).

The $^{14}C/^{12}C$ ratio of the samples was measured in the International Radiocarbon AMS Competence and Training Center (INTERACT) in Debrecen, Hungary using a MICADAS-type AMS. The "Bats" software optimized for the MICADAS system was used for the data reduction and calculating radiocarbon data (Molnár et al., 2013; Synal et al., 2007; Wacker et al., 2010). The ^{14}C results of wine samples were compared to the site-specific Northern Hemisphere Zone 1 record as a calibration curve for the post-bomb atmospheric radiocarbon level published by Hua et al. (2021), which is generally used as a reference curve in environmental, food, forensic and medical related radiocarbon studies. This reference curve is representative of the Tokaj wine region until 2019. The aim of this comparison is to validate the measured values by the reference, expected values with a comparison to each other in the given, known age of bottled wines.

For calibration of the dates of the wine samples, the OxCal (v4.4.4) online software (Ramsey, 2017) was used based on the Post-bomb atmospheric Northern Hemisphere Zone 1 curve (Hua et al., 2021; Reimer et al., 2020).

3. Results and discussion

3.1. Comparison of ^{14}C data

Three fractions (alcohol, distillation residue, and whole wine) of the twenty Tokaji aszú wine samples were measured by AMS for the $^{14}C/^{12}C$ ratio.

Sample preparation details of the combustion (sample amount used and C yields) are reported in Table 1. for the three different preparation techniques. As can be seen, even in the case of whole-wine samples, the obtained size of > 0.5 mg carbon is sufficient for a reliable AMS C-14 measurement.

In general, the results of twenty aszú wine samples from the three different fractions are in good agreement with the expected value, compared to the NH Zone I results in (Hua et al., 2021) (see data in Table 2). The comparison of measured data and its 2.8x measurement error compared to the Hua et al. (2021) NH zone 1 boreal summer (May-Aug) data and its variability are in good agreement. Comparing the measured and expected $\Delta^{14}C$ values, the R^2 correlation coefficient is 0.98 in the case of the three different fractions.

Lower values were observed in 2002, both for the ethanol and distillation residue fractions and in 2011 in the ethanol fraction compared to the NH Zone I (Fig. 2). Local differences can be observed in different regions due to local natural and anthropogenic emission that can cause shift compared to the applied reference curve. Local atmospheric CO_2 or tree ring data should be applied to similar studies due to the local and regional Suess effect (Suess, 1955) or nuclear emissions can cause the above-mentioned shifts. A former study shows that in the Carpathian basin, at the Hegyhátsál (HUN) regional background site, small but observable differences have been observed compared to the Jungfraujoch data (atmospheric $^{14}CO_2$ data measured at the Swiss Alpine monitoring station), not only in the winter heating period but during summer as well (Major et al., 2018). Unfortunately, from 2002 there is no available local atmospheric radiocarbon data, but in 2011, our wine measurement data is within the range of vegetation period and the yearly fluctuation of atmospheric CO_2 $\Delta^{14}C$ data. The HUN atmospheric site is a background site, but the fossil origin CO_2 excess at non-background sites can be higher which can cause lower ^{14}C ratios compared to the expected values. Also, fraudulent wines, i.e. selling the younger ones as older wines, can show lower ^{14}C values, as younger wines have lower radiocarbon signals due to the continuously decreasing trend of the atmospheric radiocarbon bomb spike. However, the ^{14}C results of the whole wine fraction by the unseparated

Table 1
Combusted sample amounts and C yields for the three different preparation.

Nr.	Year	Combusted sample amount (mg)			C yield (m/m %)		
		ethanol fraction	residue fraction	whole wine	ethanol fraction	residue fraction	whole wine
1.	2018	3.71	3.41	6.45	24.9	32.1	10.1
2.	2017	3.56	3.50	6.68	25.7	24.7	8.5
3.	2016	3.46	3.86	6.21	29.5	18.2	9.9
4.	2015	3.53	3.58	4.90	24.5	28.9	9.6
5.	2014	3.73	4.01	6.93	22.6	31.0	8.1
6.	2013	3.73	4.14	4.77	23.8	29.6	10.2
7.	2012	3.52	4.07	6.56	21.9	30.0	8.9
8.	2011	4.35	5.46	5.81	23.4	23.5	10.1
9.	2010	3.83	4.65	6.34	25.0	32.1	11.4
10.	2009	3.44	4.26	5.55	22.8	30.7	10.6
11.	2008	3.51	3.96	5.42	24.0	33.7	10.0
12.	2007	3.69	4.33	4.79	27.6	30.7	9.5
13.	2006	3.52	4.38	6.66	22.1	32.6	11.1
14.	2005	3.82	3.49	5.89	21.1	26.5	11.0
15.	2004	3.99	4.57	4.70	25.9	37.7	10.7
16	2003	4.78	4.21	5.80	20.2	31.3	9.9
17.	2002	4.25	4.35	5.85	25.2	29.4	11.3
18.	2001	4.02	4.57	5.08	23.4	31.2	10.8
19.	2000	3.64	4.80	5.61	26.7	33.7	10.9
20.	1999	3.53	3.94	5.19	23.4	36.3	11.5

Table 2
AMS radiocarbon results of the selected twenty wine sample.

		Ethanol (alcohol fraction)				Difference from theHua et al. (2021) boreal summer	Distillation residue fraction				Difference from theHua et al. (2021) boreal summer	Whole wine fraction, without separation, using capillary tubes (< 10 μL)				Difference from theHua et al. (2021) boreal summer	Hua et al. (2021) Zonal and hemispheric $\Delta^{14}C$ for the boreal summers (May-Aug)	
Nr.	Year	pMC	±pMC	$\Delta^{14}C$	$\pm\Delta^{14}C$	Ethanol ($\Delta^{14}C$)	pMC	±pMC	$\Delta^{14}C$	$\pm\Delta^{14}C$	Distillation residue ($\Delta^{14}C$)	pMC	±pMC	$\Delta^{14}C$	$\pm\Delta^{14}C$	Whole wine ($\Delta^{14}C$)	$\Delta^{14}C$	$\pm\Delta^{14}C$
1.	2018	101.65	0.20	8.1	2.0	4.1	101.57	0.20	7.4	2.0	3.40	101.78	0.29	9.4	2.9	5.40	4	1
2.	2017	101.52	0.20	6.9	2.0	-1.1	101.59	0.21	7.7	2.1	-0.30	102.29	0.30	14.6	3.0	6.60	8	1
3.	2016	101.80	0.20	9.8	2.0	-2.2	102.38	0.19	15.6	1.9	3.60	102.60	0.29	17.8	2.9	5.80	12	1
4.	2015	102.43	0.20	16.2	2.0	1.2	102.29	0.20	14.9	2.0	-0.10	102.98	0.23	21.6	2.3	6.60	15	2
5.	2014	103.08	0.19	22.8	1.9	2.8	103.14	0.19	23.4	1.9	3.40	102.96	0.29	21.6	2.9	1.60	20	1
6.	2013	103.11	0.20	23.2	2.0	0.2	103.33	0.19	25.4	1.9	2.40	103.25	0.25	24.6	2.5	1.60	23	1
7.	2012	103.86	0.19	30.8	1.9	-0.2	103.78	0.20	29.9	2.0	-1.10	104.26	0.29	34.8	2.9	3.80	31	1
8.	2011	103.37	0.23	26.1	2.3	-9.9	104.10	0.22	33.3	2.2	-2.70	104.75	0.30	39.7	3.0	3.70	36	2
9.	2010	104.46	0.20	36.9	2.0	-3.1	105.11	0.19	43.5	1.9	3.50	104.80	0.28	40.3	2.8	0.30	40	2
10.	2009	105.08	0.20	43.3	2.0	-1.7	104.84	0.21	40.9	2.1	-4.10	105.27	0.30	45.1	3.0	0.10	45	3
11.	2008	105.39	0.20	46.5	2.0	-1.5	105.12	0.19	43.8	1.9	-4.20	105.69	0.31	49.4	3.1	1.40	48	1
12.	2007	105.48	0.20	47.5	2.0	-3.5	105.90	0.20	51.7	2.0	0.70	105.42	0.25	46.9	2.5	-4.10	51	1
13.	2006	106.27	0.20	55.5	2.0	-1.5	105.83	0.23	51.1	2.3	-5.90	106.62	0.27	58.9	2.7	1.90	57	2
14.	2005	106.02	0.25	53.1	2.5	-5.9	106.60	0.23	58.8	2.3	-0.20	107.16	0.29	64.5	2.9	5.50	59	2
15.	2004	106.67	0.23	59.7	2.3	-5.3	106.95	0.23	62.5	2.3	-2.50	106.73	0.23	60.3	2.3	-4.70	65	2
16.	2003	107.17	0.23	64.8	2.3	-4.2	107.49	0.24	68.0	2.4	-1.00	107.45	0.30	67.6	3.0	-1.40	69	3
17.	2002	106.91	0.24	62.3	2.4	-12.7	107.01	0.23	63.3	2.3	-11.70	107.51	0.29	68.3	2.9	-6.70	75	2
18.	2001	108.34	0.23	76.6	2.3	-4.4	109.11	0.23	84.4	2.3	3.40	108.90	0.32	82.2	3.2	1.20	81	3
19.	2000	108.82	0.24	81.6	2.4	-4.4	109.11	0.23	84.4	2.3	-1.60	109.78	0.31	91.1	3.1	5.10	86	3
20.	1999	109.79	0.24	91.4	2.4	-1.6	109.99	0.23	93.4	2.3	0.40	110.65	0.32	99.9	3.2	6.90	93	3

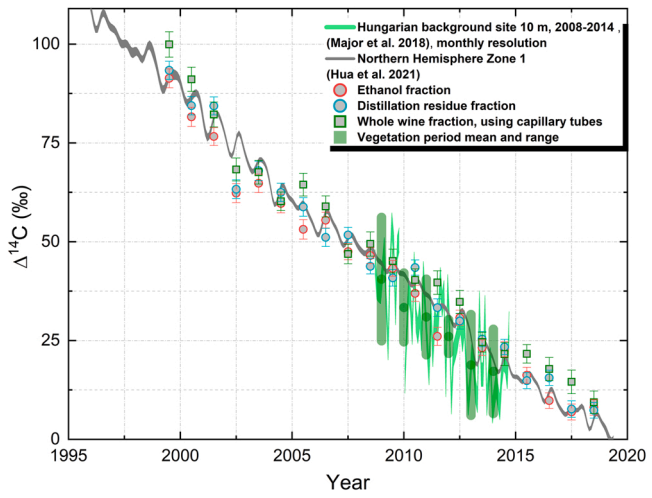

Fig. 2. Results of the AMS measurements with 1σ uncertainty of three different wine fractions (ethanol, distillation residue, and whole wine without separation) compared to the bomb-peak of the Northern Hemisphere Zone 1 (Hua et al., 2021) and a Hungarian background site monthly mean data and vegetation period mean data from 2008 to 2014 (Major et al., 2018).

capillary-based measurements of the 2002 and the distillation residue-based measurement of the 2011 year samples show good agreement with the expected value from Hua et al. (2021). If we compare the measurement data of all three fractions of the 2011 year sample to the Hungarian background site vegetation period ^{14}C data, all three fit the results. For the year 2002, there is no Hungarian background data available.

The difference between the three fractions was generally less than the 3σ measurement error (3σ= 9‰ $\Delta^{14}C$) and the whole wine fraction using the capillary-based method generally gives slightly higher results compared to the ethanol and distillation residue fractions (Fig. 3a). The capillary-based, whole wine analyses technique gave 2.8 ± 4.1‰ and 4.8 ± 4.0‰ ($\Delta^{14}C$) higher results compared to the distillation residue and ethanol fractions, while the difference between the ethanol and distillation residue fraction is only − 2.0 ± 3.5‰ ($\Delta^{14}C$). The explanation of the differences lies in the differences between the three fractions' carbon sources. While the whole wine fraction contains both the ethanol and distillation residue fractions it may contain many other fractions, materials, and molecules that we lost during the distillation. It may contain materials from the soil, probably from the wooden cask, for the detection of ^{14}C signal of well-specified materials, the compound-specific radiocarbon analysis would be suitable (Druffel et al., 2010; Haghipour et al., 2019; Ziolkowski and Druffel, 2009).

The methods compared to the Hua et al. (2021) NH Zone I corresponding yearly ^{14}C data (Fig. 3b) show that the ethanol and distillation residue fractions are slightly lower (−2.8 ± 3.9‰ and −0.7 ± 3.8‰ $\Delta^{14}C$) results, while the capillary-based, whole wine method resulted in a bit higher (2.0 ± 3.9‰ $\Delta^{14}C$).

The ^{14}C result of the ethanol and distillation residue fraction is generally lower, and the whole wine fraction using the capillary method is generally higher than the expected value of the corresponding year from Hua et al. (2021). If one takes into account the fluctuation of the atmospheric $^{14}C/^{12}C$ ratio in CO_2 and its yearly cycle in Hungary (Major et al., 2018), the mean difference between the yearly maxima and minima was ~26‰ $\Delta^{14}C$ between 2009 and 2014. That value is a little less, 20.5‰ $\Delta^{14}C$ during the vegetation period (from March to September between 2009 and 2015) when the plants were photosynthetically active (Fig. 2). This shows much higher fluctuation in a given year than the 3σ measurement error, but our results generally fit the expected value within this 3σ range.

The z-score of every individual measurement of three different wine fractions was calculated, using the 1-sigma uncertainty of the measurements. The mean z-score of the ethanol and distillation residue fractions were − 0.62 and − 0.004 respectively, while the z-score of the whole wine fraction using the capillary-based measurement was 0.62. This statistical analysis shows that all the three, different preparation techniques of different wine fractions can be applied equally, and there is no significant difference between the final results. All of the results are within the ± 2 z-score range. These results show that all of the applied fractions have no systematic errors, and there are no significant differences between the ^{14}C results of the three different fractions of each wine.

Povinec et al. (2020) measured wines from the Tokaj region but from the Slovakian area, not from the Hungarian region. In the paper published by Povinec et al. (2020) only the samples from 2015 are comparable with our measurements, as that paper shows generally older samples from 1958 to 1965 around the maxima of the radiocarbon bomb peak. Our measurements of the ethanol and distillation residue fractions show somewhat lower values (16.2 ± 2.0‰ and 14.9 ± 2.0‰ $\Delta^{14}C$) in 2015 than Povinec et al. (2020) (29.4 ± 6.4‰ $\Delta^{14}C$ for Green Veltliner wine and 34.4 ± 6.3‰ $\Delta^{14}C$ for Red Cuvée wine). On the other hand, our data on the ethanol and distillation residue fractions of wine samples from 2015 completely fit the expected value compared to the NH Zone I results (Hua et al., 2021), (Fig. 2). The ^{14}C result (2015) based on the whole wine fraction using the capillary method is a bit higher than the other fractions, but it is also lower than the sample from the same year published by Povinec et al. (2020).

It is important to note that the level of atmospheric radiocarbon has a

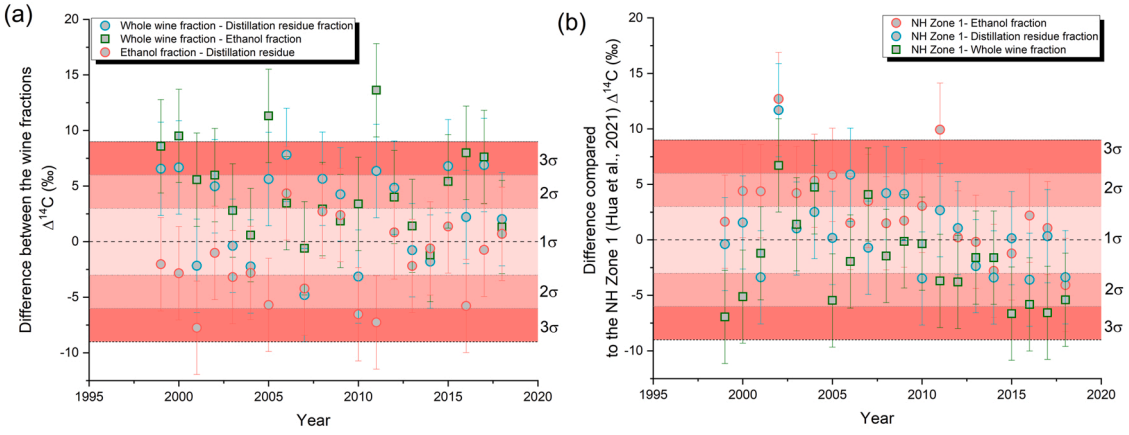

Fig. 3. The radiocarbon results compared to the corresponding value of the Northern Hemisphere Zone I. from Hua et al., (2021) (a). Difference between the three different wine fractions (ethanol, distillation residue, and whole wine sample by capillary method (b).

yearly seasonal cycle. If we use the atmospheric ^{14}C data of a previously published paper by Major et al. (2018), these shows that the cycle in Hungary causes a shift compared to the expected yearly ^{14}C data of Hua et al. (2021). With this significant yearly atmospheric ^{14}C level fluctuation (Fig. 2), all of the measured data are in good agreement with the expected value. Fig. 4.

Fahrni et al. (2015) dated wines without opening the bottles, by connecting the bottle of wine to a vacuum line and pumping the system then trapped the so-called angel's share, the trace amount of ethanol and other gases that diffuse through the cork of the bottle what was cryo-trapped. That method used an even lower amount of ethanol than our method, but the measurement uncertainty is higher in some cases. Their method is non-invasive but presumably could not use for metal-capped wines.

3.2. Comparison of calibrated ages

Although, the raw ^{14}C (pMC or $\Delta^{14}C$) results are comparable within the defined measurement uncertainties and the measurement results fit well to the calibration curve of Hua et al. (2021), the main purpose of the presented methods is the grape harvest or wine production year verification based on the radiocarbon measurements. For this reason, we have calibrated the dates of the measured wine samples. The raw results of the calibrated ages are shown in the Supplementary material S1 file and Fig. 5. The calibration intersects the calibration curve at a minimum of two points, due to the increasing and decreasing section of the curve (there is a minimum of 2 points with quite similar ^{14}C value), but we only discuss the most likely value and calibrated date. These selected probabilities are higher than 63% in every case. As Fig. 5d shows, the calibrated dates cover probability intervals, not exact years. The results of the three different fractions overlap, but in some cases, only the edge of the time intervals overlap, as in the case of the samples of 2017, where the whole wine fraction seems older, in 2016, when the ethanol fraction seems younger, in 2011, when the distillation residue overlap both with the ethanol and whole wine fraction, but the whole wine and ethanol do not overlap with each other. In other cases, the time ranges overlap well. The mean covered the time of the calibrated dates are 2.2 ± 0.5 (ethanol fraction), 2.1 ± 0.5 (distillation residue), and 2.5 ± 0.7 (whole wine) years, respectively. This shows the whole wine fraction covers a slightly wider period generally, but as it is mentioned before, these time ranges generally overlapped. The mean differences between the expected year (known age of the bottle of wine, the middle of the vegetation period of the grape) and the calibrated date are -0.3 ± 0.8 (ethanol fraction), 0.1 ± 0.9 (distillation residue) and 0.8 ± 1.0 (whole wine) year, respectively. This shows that the mean difference between the calibrated age and the expected value is slightly higher in the case of the whole wine fraction, but it is still under 1 year. This one-year average difference does not mean that the calibrated date has one year of precision. As it is above mentioned the covered time interval, in this case, the accuracy of the calibrated date is wider, that is between 1.2 and 3.6 years. The size of this interval, and the accuracy does not change over time, due to the slower decrease of the bomb peak between 1999 and 2018. Figs. 5a-5c show, almost all of the covered periods of the calibrated dates overlap with the expected year's intervals (vegetation period of the grapes at the Tokaj wine region), which demonstrates that all three fractions can be applied properly for the grape harvest year verification, for the radiocarbon dating of wine samples. As the above-mentioned calculation and Fig. 5b show, the most accurate measurements were performed from the distillation residue, but the two other fractions also performed well.

Fig. 4. Z-score of the AMS ^{14}C measurement of three different wine fractions. The horizontal dot line shows the zero z-score, and the two horizontal dash line means the $\pm$ 2 z-score.

These results show that the yearly precision of the wine production year verification is no longer available with the radiocarbon method after 1999, with the present measurement precision ($\sim \pm 2$‰ $\Delta^{14}C$) as the yearly decrease of the atmospheric $^{14}C/^{12}C$ ratio is now lower.

4. Conclusion

An accelerator mass spectrometry-based radiocarbon dating method of different fractions of wine samples has been successfully applied in the Hungarian International Radiocarbon AMS Competence and Training Center (INTERACT). Most of the measured $^{14}C/^{12}C$ ratios of the ethanol, distillation residues, and the whole wine samples (by the capillary method) were in good agreement with the expected value, the Northern Hemisphere Zone I atmospheric radiocarbon data. There were no significant differences found between the three investigated fractions and all three fractions are suitable for radiocarbon-based harvest year verification of modern wine samples. Our applied method shows that distillation residue and the whole-wine sample without separation of wines can be used for radiocarbon dating, as well as the ethanol fraction. A z-score analysis also shows there is no significant difference between the results produced by the three preparation methods. The covered time period of the calibrated dates shows a good agreement with the expected harvest year period, but between 1999 and 2018 time period, the yearly precision was not achievable, as the decreasing trend of the calibration curve is lower than the AMS ^{14}C measurement precision. The achievable accuracy of the calibrated dates during this period is around 2–3 years. To achieve more realistic results, local calibration curves, based on local atmospheric $^{14}CO_2$ or tree ring radiocarbon data should be applied. The presented capillary-based preparation and accelerator mass spectrometry measurement technique, without the distillation step, could also be more acceptable to wine collectors, due to the small sample volume (below 10 μL), but our presented method can be used in forensic science, for the verification of harvest year or period of the grape as well. Our results are one of the firsts with a preparation method that uses a much lower sample volume than 1 ml wine samples. We suggest this method can be applied to other types of wine that have lower sugar or organic compound content, as the wine's alcohol content is generally above 10%, and the capillary method preserves the ethanol fraction due to the cooling during the preparation and sealing of the reaction tubes.

CRediT authorship contribution statement

TV contributed significantly to the conceptualization of the paper, its design and writing, data curation, data interpretation, and formal analysis. MM contributed significantly to the formal analysis of the data, participated in the writing and supervision of the paper. AM contributed significantly to investigation and methodology. TJ and LP contributed significantly to the supervision of the paper. LE contributed significantly to the sample collection, methodology, writing and supervision of the

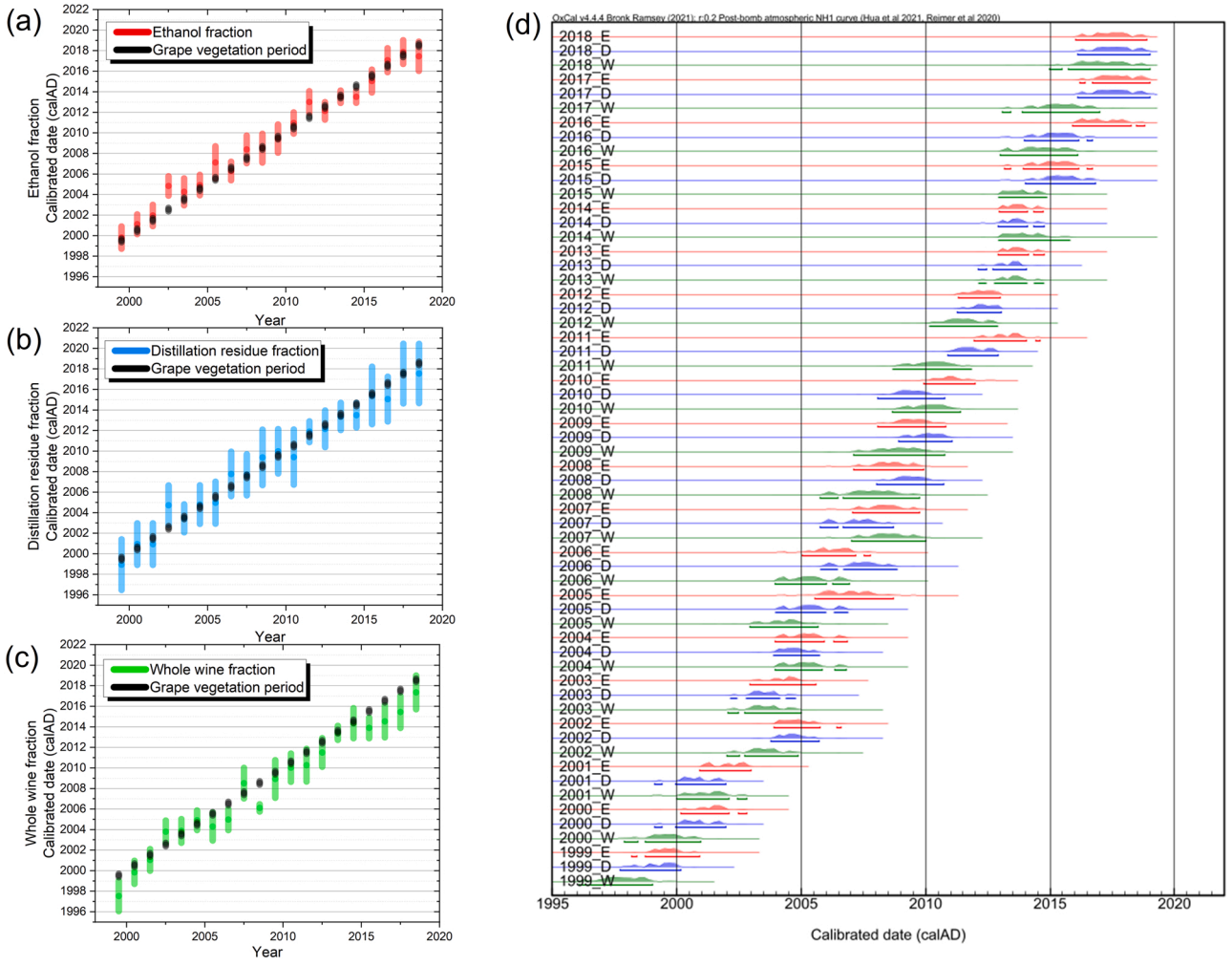

Fig. 5. Results of the calibrated dates based on the AMS ^{14}C measurements. Calibrated date of the ethanol fraction (a), distillation residue (b) and whole wine fraction (c) compared to the expected time period. The red, blue and green shaded areas shows the covered time period of the wine calibrated dates, the black shaded areas show the expected time period, the vegetation period of the grapes. The (d) shows the calibrated dates where the "E", "D" and "W" letters after the years mean the "ethanol", "distillation residue" and "whole wine" fractions.

paper.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Tamas Varga reports financial support was provided by Institute for Nuclear Research. Elemer Laszlo reports financial support was provided by Ministry for Innovation and Technology. Mihaly Molnar reports financial support was provided by International Atomic Energy Agency. Elemer Laszlo reports financial support was provided by Hungarian Academy of Sciences. Tamas Varga reports financial support was provided by European Regional Development Fund. Tamas Varga reports financial support was provided by Ministry of Innovation and Technology.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2023.105203.

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