

Mass-spectrometric study on $^{13}\text{C}/^{12}\text{C}$ carbon and $^{18}\text{O}/^{16}\text{O}$ oxygen stable isotopes distributions in grapes and wines from the Black Sea regions

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Abstract. The present study continues the 2015–2016 research project on biological characteristics of stable isotopes fractionation in grapes taking into account the agro-climatic growth conditions of this representative of the C3-pathway of photosynthesis group of plants in different geographical Black Sea regions. The first parts of the project were presented at the 39th and 40th Congresses of OIV in Bento Gonçalves (Brazil) and Sofia (Bulgaria). The scientific data on compositions of $^{13}\text{C}/^{12}\text{C}$ carbon and $^{18}\text{O}/^{16}\text{O}$ oxygen stable isotopes in carbohydrates, organic acids, and intracellular water were obtained for grapes of 2015–2016 growing seasons in the four areas of the Crimean peninsula as well as in several areas of the Don Basin and the Western Caspian region. This report presents the results of the 2017 season study of $^{13}\text{C}/^{12}\text{C}$ carbon and $^{18}\text{O}/^{16}\text{O}$ oxygen stable isotopes in carbohydrates and intracellular water of 12 red and white grape varieties (Aligote, Rkatsiteli, Sauvignon Zeleny, Chardonnay, Cabernet Sauvignon, Sauvignon Blanc, Merlot, Riesling, Pinot Noir, Cabernet Franc, Sira, Krasnostop) as well as in ethanol of wines made from corresponding grapes from the Crimean Peninsula and South-West Coast of the Greater Caucasus. To measure the ratio of carbon isotopes $^{13}\text{C}/^{12}\text{C}$ in grape (must) carbohydrates and wine ethanol the Flash-Combustion technique (FC-IRMS/SIRA) has been used, while the method of isotopic equilibration (EQ-IRMS/SIRA) has been used for the measurement of $^{18}\text{O}/^{16}\text{O}$ oxygen isotopes ratio in the intracellular water of grapes (must) and in the water fraction of wine. The GC-Combustion technique (GC-IRMS/SIRA) has been used for the first time to measure the carbon isotopes $^{13}\text{C}/^{12}\text{C}$ distribution in ethanol of studied wines. It has been found that the $\delta^{13}\text{C}_{\text{VPDB}}$ values for carbohydrates of red and white grape varieties as a result of biological fractionation of carbon isotopes in the agro-climatic conditions of plant growth (2017 season) for the studied geographical areas formed the following quantitative ranges: from -26.72 to -23.35‰ (the Crimean Peninsula) and from -25.92 to -23.87‰ (South-West Coast of the Greater Caucasus). The $\delta^{13}\text{C}_{\text{VPDB}}$ values for wine ethanol are in the following ranges: from -28.15 to -24.47‰ (the Crimean Peninsula) and from -27.29 to -25.78‰ (South-West Coast of the Greater Caucasus). The $\delta^{18}\text{O}_{\text{VSMOW}}$ values in intracellular water of grapes of the 2017 season range from -1.24 to 2.17‰ (the Crimean Peninsula) and from 1.08 to 4.09‰ (South-West Coast of the Greater Caucasus). The results of this study show, in comparison with the results of studies of the 2015 and 2016 seasons, a decrease in the $\delta^{13}\text{C}_{\text{VPDB}}$ values for carbohydrates of grapes and ethanol of wine, which is explained by the changed climatic conditions of grapes growing in the vegetation period of 2017.

1. General climatic conditions

The grapes samples from two main regions of the Black Sea have been used in the study – Crimean peninsula and South West Coast of the Greater Caucasus (Fig. 1). The sampling of the authentic fresh grapes was carried out in 3 viticulture areas of the Crimean Peninsula – Western Maritime-Steppe area (Evpatoria), Western Foothills-Maritime area (Uglovoye) and South Coast of the Crimean peninsula (Gurzuf). The main area of study on the South West Coast of the Greater Caucasus was the Anapa district (Semigorye area). Scientific data on the Black Sea areas, including climate, soils and grapes varieties have been

reported in the previous publications about results of the studies of 2015–2016 [1, 2].

2. Grape samples

The samples of the 12 white and red grape varieties were used for the study – Aligote, Rkatsiteli, Cabernet Sauvignon, Sauvignon Green, Merlot, Chardonnay and Syrah (Crimean Peninsula); Cabernet Sauvignon, Chardonnay, Sauvignon Blanc, Merlot, Riesling, Pinot Noir, Krasnostop and Cabernet Franc (South West Coast – Anapa district). The sampling of the authentic fresh grapes was carried out in viticulture areas in shown five regions. The sampling



Figure 1. Black Sea areas.

was conducted according to the requirements described in [3].

The research program included 33 samples of grapes and wines from the above stated varieties. The fresh musts were obtained under the laboratory conditions. Gentle conditions for the extraction of musts exclude any changes in the natural composition of carbon and oxygen isotopes in the target components of grapes – carbohydrates (sugars) and intracellular water. In order to ensure the microbiological stability the musts samples have been pasteurized and filled in glass containers under aseptic conditions. Before the isotopic measurements the must samples were stored at a temperature from 2 to 4 °C in sealed sterile glass containers without access of air.

16 samples of dry white and red wines (residual sugar – from 0.5 to 3.6 g/l) have been made from the fresh musts under laboratory conditions. The fermentation of fresh musts was carried out using a pure 47 K yeast culture, which was added in an amount of 2% of the must volume. The SO₂ in an amount of 75–100 mg/l was added to musts before fermentation. Prior to the isotopic measurements the dry wines were stored at a temperature from 2 to 4 °C in sealed sterile glass containers without access of air.

3. Stable isotopes compositions of studied grape samples

Scientific study of compositions of stable isotopes of carbon (¹³C/¹²C) and oxygen (¹⁸O/¹⁶O) was carried out using two types of IRMS/SIRA¹ technique – redox transformation (Flash Combustion – FC-IRMS/SIRA) and isotopic exchange/equilibration (Equilibration – EQ-IRMS/SIRA). Methodological principles of the FC-IRMS/SIRA and EQ-IRMS-SIRA methods have been described in previous publication [1] and in OIV methods [4].

3.1. Materials and methods

3.1.1. Musts and wine samples

The musts and dry wines were used for measurements of the composition of the carbon isotopes ¹³C/¹²C in carbohydrates (sugars) by the FC-IRMS/SIRA method and ethanol by the GC-IRMS/SIRA method. The composition of oxygen isotopes ¹⁸O/¹⁶O was studied by the EQ-IRMS/

¹ IRMS/SIRA = Isotope Ratio Mass Spectrometry/Stable Isotope Ratio Analysis.

SIRA method directly in musts and dry wines without prior isolation of water from the samples.

3.1.2. FC-IRMS/SIRA method for $\delta^{13}C_{VPDB}$ analysis of carbohydrates (sugars)

The study of fractionation effect of the stable isotopes of carbon (¹³C/¹²C) in grape carbohydrates (sugars) was carried out by using of the following laboratory facilities for stable isotopes measurements:

(A) an elemental analyzer FlashEA1112[®] with oxidation & reduction reactors and autosampler (Thermo Fisher Scientific, Germany);

(B) an isotopic interface ConFlo III[®] (Thermo Fisher Scientific, Germany);

(C) an IRMS/SIRA isotopic mass spectrometer Delta V Advantage[®] (Thermo Fisher Scientific, Germany);

(D) a gas system Sigm-Plus[®] for the supply of the analytical devices with highly purified gases (Sigm-Plus Ltd., Russian Federation);

(E) a PC workstation Optiplex 745[®] (Dell, USA) for data registration and processing by the high level software Isodat NT 2.5[®] (Thermo Fisher Scientific, Germany).

The reference substance IAEA-600 (Caffeine, $\delta^{13}C = -27.771\text{‰}$) was used for the calibration of the working reference gas (WRG) – carbon dioxide 99.9999% (Linde Gas RUS, Russian Federation). The high purity helium 99.9999% (Linde Gas RUS, Russian Federation) was used in the study as carrier gas.

Sample preparation – extraction and purification of carbohydrates (sugars) from musts and wines was performed according to the method described in [6]. The isolated and purified preparations of carbohydrates (sugars) were freeze-dried at –50 °C and 0.035 mbar pressure in the laboratory lyophilic facility FreeZone¹²[®] (Labconco Corporation, USA). The preparations were stored at a temperature no higher than –20 °C in a sealed glass container.

The above mentioned laboratory facilities have been used for isotope measurements. All measurements were carried out taking into account guidelines published in [8]. Joint hinges of preparations of carbohydrates (sugars) containing from 40 to 100 ng of carbon were placed in tin capsules before carrying out the measurements. The capsules were thoroughly covered with the help of microtweezers, providing its full integrity and eliminating the contact of organic matter with the atmospheric air. The encapsulated hinges (in five replicates for each sample) were placed into the autosampler mounted on the oxidation reactor of the elemental analyzer (A). Along with the hinges of carbohydrates (sugars) the encapsulated reference substance was loaded into the autosampler for control of reliability of measurement's results.

The conditions of carrying out measurements of isotope composition of carbon in the preparations of carbohydrates (sugars) by using the stated above laboratory facilities corresponded to the optimum modes which are developed during the preliminary runs. Quantitative combustion of carbohydrates (sugars) was performed in a helium flow (flow rate 100 ml/min) in the oxidation and redox reactors of the elemental analyzer (A) at the temperature of 900 and 650 °C in the presence of molecular high purity oxygen 99.999% (Linde Gas RUS, Russian Federation). The separation of elementary gases resulting from the

combustion of carbohydrates (sugars) in the redox reactor was carried out in automatic mode by the chromatographic purification in the elemental analyzer (A). Water contained in the gas flow was removed by the trap column filled with the magnesium perchlorate.

For the quantitative measurement of carbon isotopes $^{13}\text{C}/^{12}\text{C}$, the isolated carbon dioxide is directed through isotopic interface (B) to the IRMS/SIRA mass spectrometer Delta V Advantage[®] (C). Accelerating voltage of the mass spectrometer was 3.07 kV, pressure in the ion source -1.8×10^{-6} kPa, ionization method – electron impact (electron energy 124 eV).

The conducting of a simultaneous correction of measured values of signal strength is an important aspect for control of accuracy of results to exclude the possible influence of isobars $^{12}\text{C}^{17}\text{O}^{17}\text{O}$ and $^{13}\text{C}^{17}\text{O}^{16}\text{O}$ which share is determined at the hardware level by the measurement of a signal of carbon dioxide of mass 45 taking into account the extent of the distribution of the isotopes ^{13}C and ^{17}O in the nature (respectively 1.11 and 0.0375%).

3.1.3. GC-IRMS/SIRA method for $\delta^{13}\text{C}_{\text{VPDB}}$ analysis of ethanol

The GC-IRMS/SIRA method is based on the application of an analytical complex, which includes the following special laboratory facilities and equipment:

(A) a gas chromatograph Trace GC Ultra[®] (Thermo Scientific, USA) with a capillary column with the stationary phase GsBP-5 (5% diphenylpolysiloxane in dimethylpolysiloxane) of length 30 m, internal diameter 0.25 mm, layer thickness of stationary phase 0.25 μm (General Separations Technologies, USA), an automatic feeder of the liquid samples AS TriPlus[®] (Thermo Scientific, USA) and a built-in oxidation microreactor, which is a ceramic tube with a diameter 1.55 mm (internal diameter 0.50 mm) and a length 320 mm, which is placed inside the three catalysts wire of pure copper, nickel and platinum;

(B) a control unit GC Combustion III[®] (Thermo Scientific, USA) with a built-in reducing microreactor, which is a ceramic tube with a diameter 1.55 mm (internal diameter 0.50 mm) and a length 320 mm, which is placed inside the three wire catalysts of pure copper (diameter 0.125 mm), and a split system for controlling gas flows.

Other equipment corresponds to items C, D, E described above in the Sect. 3.1.2.

The reference standard sample BCR-656 ($\delta^{13}\text{C}_{\text{VPDB}} = -26.91 \pm 0.07\text{‰}$, European Commission, Community Bureau of Reference BCR, unique identification number of the sample is 00425) was used for the calibration of the working reference gas – carbon dioxide.

Before the determination the initial samples were normalized by diluting with distilled water to about 1% of ethanol and after filtration in a volume of 1.5 ml were introduced into standard glass containers for chromatography. The containers were inserted in the holder of the automatic liquid sample feeder placed on the gas chromatograph (A). For selection and subsequent injection of the sample into the column of the chromatograph in an automated feeder of samples were used microsyringe volume of 5.0 μl (Thermo Scientific,

USA). The volume of the sample for chromatographic selection of ethanol was 0.1 μl .

The chromatographic selection of ethanol was carried out in accordance with the following conditions: injector temperature – 250 °C; carrier gas – helium (99.9999%); flow rate of carrier gas through the column – 1 ml/min; volume of sample solution is introduced into the injector – 0.1 μl ; the input mode of the sample in the column – with splitting of the flow (the coefficient of splitting 100); temperature program of the column: a) initial phase – 35 °C, exposure time 2 min, b) the temperature rise to 45 °C with a speed of 10 °C/min, c) the temperature rise to 220 °C with a speed of 100 °C/min, d) final phase – 220 °C with the extract 2 min.

The duration of chromatographic extraction according to these conditions is 7 minutes. The fraction of pure ethanol was introduced to the oxidation reactor of the system at the fifth minute after injection of the sample into the chromatograph column. The duration of oxidation-reduction conversion, measurement and recording of its results are less than 6 minutes, and the duration of the restoration of the gas chromatograph settings to the initial values is 4 minutes. So the total duration of determination for one sample in the developed GC-IRMS/SIRA method doesn't exceed 17 minutes.

Additional information about the development of the GC-IRMS/SIRA method – see the publication [19].

3.1.4. EQ-IRMS/SIRA method for $\delta^{18}\text{O}_{\text{VSMOW}}$ analysis of grape & wine water

The study of fractionation effect of the stable isotopes of oxygen ($^{18}\text{O}/^{16}\text{O}$) in the water in grapes (musts) and wine samples using the EQ-IRMS/SIRA method was carried out by using of the laboratory facilities for stable isotopes measurements:

(A) a module used for sample preparation for the analysis (Thermo Fisher Scientific, Germany);

(B) a control unit for chromatographic purification and samples injection Gasbench II[®] (Thermo Fisher Scientific, Germany);

(C) an IRMS/SIRA isotopic mass spectrometer Delta V Advantage[®] (Thermo Fisher Scientific, Germany);

(D) a gas system for the supply of the analytical devices with highly purified gases Sigm-Plus[®] (Sigm-Plus Ltd., Russian Federation);

(E) a PC workstation Optiplex 960[®] (Dell, USA) for data registration and processing by the high level software Isodat 3.0[®] (Thermo Fisher Scientific, Germany).

The reference substance IAEA-600 (Caffeine, $\delta^{13}\text{C} = -27.771\text{‰}$) was used for the calibration of the working reference gas (WRG) – carbon dioxide 99.9999% (Linde Gas RUS, Russian Federation). The high purity helium 99.9999% (Linde Gas RUS, Russian Federation) was used in the study as carrier gas. The additional reference substances (all from the IAEA, Austria) with known composition of oxygen isotopes $^{18}\text{O}/^{16}\text{O}$ were used for adjustment of measurement's results: VSMOW2 (Vienna-Standard Mean Ocean Water, $\delta^{18}\text{O} = 0.00\text{‰}$); GISP (Greenland Ice Sheet Precipitation, $\delta^{18}\text{O}_{\text{VSMOW}} = -24.76\text{‰}$); SLAP2 (Standard Light Antarctic Precipitation, $\delta^{18}\text{O}_{\text{VSMOW}} = -55.50\text{‰}$).

High purity gases were used in the study – flushing gas – helium 99.9999%, containing 0.4–0.5% of high

Table 1. Composition of stable carbon isotopes (¹³C/¹²C) in carbohydrates (sugars) of grape varieties and ethanol of dry wines of the selected areas of the Crimean Peninsula (crop of 2017).

Grape variety	Area ¹	$\delta^{13}\text{C}_{\text{VPDB}},\text{‰}$	
		carbohydrates (sugars)	ethanol
Aligote (wine)	Uglovoye	-26.30 ± 0.15	-26.00 ± 0.11
Cabernet Sauvignon (wine)		-27.26 ± 0.01	-27.17 ± 0.04
Merlot (wine)		-27.13 ± 0.14	-27.21 ± 0.25
Chardonnay (wine)		-27.73 ± 0.08	-28.20 ± 0.06
Rkatsiteli (wine)		-26.34 ± 0.10	-27.83 ± 0.19
Aligote (must)		-25.24 ± 0.13	n.d.
Cabernet Sauvignon (must)		-25.40 ± 0.20	n.d.
Merlot (must)		-25.18 ± 0.08	n.d.
Chardonnay (must)		-26.72 ± 0.09	n.d.
Rkatsiteli (must)		-26.68 ± 0.19	n.d.
Merlot (wine)	Evpatoria	-25.92 ± 0.05	-25.67 ± 0.01
Sauvignon Green (wine)		-23.77 ± 0.18	-24.47 ± 0.13
Merlot (must)		-23.54 ± 0.03	n.d.
Sauvignon Green (must)		-23.33 ± 0.10	n.d.
Syrah (wine)	Gurzuf	-27.41 ± 0.04	-28.08 ± 0.24
Aligote (wine)		-25.29 ± 0.06	-26.12 ± 0.20
Syrah (must)		-26.42 ± 0.01	n.d.
Aligote (must)		-24.56 ± 0.01	n.d.

Notes to the table 1:
¹ For area's description see 1.
² n.d. = Not determined.

purity carbon dioxide (99.9999%), carrier gas – helium 99.9999% (all from Linde Gas RUS, Russian Federation).

Sample preparation for the direct measurement of oxygen isotopes in grape & wine water was carried out by isotopic equilibration technique (EQ-IRMS/SIRA method). For isotopic equilibration reaction, 0.500 ml of grape or wine was added into a 12 ml round-bottom borosilicate glass vial. The tubes were hermetically sealed by disposable screw cap with Teflon-silicone membrane. 0.200 ml of reference substances VSMOW2, GISP and SLAP2 were added separately in similar tubes.

To carry out measurement, we formed series (groups) of tubes. Each group included the required number of sample tubes and test tubes with reference substances. A batch of tubes was placed in the cells of the sample preparation module (A). Results of preliminary experiments on the duration of the isotopic equilibration

Table 2. Composition of stable carbon isotopes (¹³C/¹²C) in carbohydrates (sugars) of grape varieties and ethanol of dry wines of the Anapa district (Semigorye area) of the South West Coast of the Greater Caucasus (crop of 2017).

Grape variety	Area ¹	$\delta^{13}\text{C}_{\text{VPDB}},\text{‰}$	
		carbohydrates (sugars)	ethanol
Riesling (wine)	Semigorye	-26.62 ± 0.07	-25.99 ± 0.06
Cabernet Sauvignon (wine)		-26.28 ± 0.01	-26.77 ± 0.06
Merlot (wine)		-27.36 ± 0.22	-27.29 ± 0.01
Chardonnay (wine)		-25.79 ± 0.01	-25.78 ± 0.19
Sauvignon Blanc (wine)		-26.62 ± 0.13	-26.28 ± 0.20
Pinot Noir (wine)		-26.88 ± 0.10	-25.94 ± 0.19
Krasnostop (wine)		-26.56 ± 0.01	-26.28 ± 0.09
Riesling (must)		-24.71 ± 0.26	n.d.
Cabernet Sauvignon (must)		-24.91 ± 0.07	n.d.
Merlot (must)		-25.92 ± 0.03	n.d.
Chardonnay (must)		-24.28 ± 0.02	n.d.
Sauvignon Blanc (must)		-25.26 ± 0.03	n.d.
Pinot Noir (must)		-24.54 ± 0.05	n.d.
Krasnostop (must)		-23.89 ± 0.07	n.d.
Cabernet Franc (must)		-23.87 ± 0.03	n.d.

Notes to the table 1:
¹ For area's description see 1.
² n.d. = Not determined.

reactions show that the equilibrium state occurs after at least 18 hours. The optimum temperature for the reaction was 24.0 ± 0.1 °C.

At the end of the isotope exchange reaction the gas mixture from each tube was transferred to the control unit Gasbench II[®](B). Gas mixture was dried over by molecular sieve membrane Nafion[®] and transferred to the gas chromatograph installed in the control unit Gasbench II[®](B). Chromatographic separation is carried out in helium flow (2 ml/min), column used – PLOT Fused Silica, stationary phase CP-PoraPLOT Q, length 25 m, diameter 0.32 mm (Varian, USA), column temperature 70°. Temperature of samples in the sample module (1) was maintained at a constant level of 24.0 ± 0.1 °C until the end of the measurements.

Quantitative measurements of oxygen isotopes ¹⁸O/¹⁶O were performed according to conditions described above and in [9]. During the measurement cycle the WRG calibrated against a reference substance is introduced

Table 3. Composition of stable oxygen isotopes (¹⁸O/¹⁶O) in intracellular water of grape varieties and wine water (selected areas of the Crimean Peninsula, crop of 2017).

Grape variety	Area ¹	$\delta^{18}\text{O}_{\text{VSMOW},\text{‰}}$	
		wine water	intracellular water
Aligote (wine)	Uglovoye	2.28 ± 0.01	n.d.
Cabernet Sauvignon (wine)		1.61 ± 0.01	n.d.
Merlot (wine)		0.82 ± 0.01	n.d.
Chardonnay (wine)		1.12 ± 0.01	n.d.
Rkatsiteli (wine)		1.28 ± 0.01	n.d.
Aligote (must)		n.d.	0.94 ± 0.01
Cabernet Sauvignon (must)		n.d.	-0.29 ± 0.01
Merlot (must)		n.d.	-1.24 ± 0.01
Chardonnay (must)		n.d.	-0.21 ± 0.01
Rkatsiteli (must)		n.d.	-0.27 ± 0.01
Merlot (wine)	Evpatoria	2.59 ± 0.01	n.d.
Sauvignon Green (wine)		3.80 ± 0.01	n.d.
Merlot (must)		n.d.	1.31 ± 0.01
Sauvignon Green (must)		n.d.	2.17 ± 0.02
Syrah (wine)		0.98 ± 0.01	n.d.
Aligote (wine)	Gurzuf	2.43 ± 0.01	n.d.
Syrah (must)		n.d.	-0.12 ± 0.01
Aligote (must)		n.d.	0.64 ± 0.01

Notes to the table 3:
¹ For area's description see 1.
² n.d. = Not determined.

into the mass spectrometer – five portions WRG at the beginning of the cycle and three portions WRG at the end. Measurement of each sample was performed in triplicate in series of 10 impulses. For subsequent calculation of the $\delta^{18}\text{C}_{\text{VSMOW}}$ value (‰), the intensities of 7, 8 and 9th impulses of the carbon dioxide with masses 46 and 44 should be used. Registration and processing of measurements results, as well as control for all devices of the analytical system is done via a PC workstation (E).

The results of measurements calculated on the basis of the recorded signals for the masses 44 and 46 are subjected to further adjustment. This adjustment takes into account the linear relationship between the values $\delta^{18}\text{O}_{\text{VSMOW}}$ of used reference substances VSMOW2, GISP, SLAP2 and the values measured for these references

Table 4. Composition of stable oxygen isotopes (¹⁸O/¹⁶O) in intracellular water of grape varieties and wine water (Anapa district – Semigorye area of the South West Coast of the Greater Caucasus, crop of 2017).

Grape variety	Area ¹	$\delta^{18}\text{O}_{\text{VSMOW},\text{‰}}$	
		wine water	intracellular water
Riesling (wine)	Semigorye	2.84 ± 0.01	n.d.
Cabernet Sauvignon (wine)		3.53 ± 0.03	n.d.
Merlot (wine)		3.51 ± 0.01	n.d.
Chardonnay (wine)		1.99 ± 0.01	n.d.
Sauvignon Blanc (wine)		1.73 ± 0.01	n.d.
Pinot Noir (wine)		3.04 ± 0.01	n.d.
Krasnostop (wine)		3.14 ± 0.03	n.d.
Riesling (must)		n.d.	3.86 ± 0.06
Cabernet Sauvignon (must)		n.d.	1.16 ± 0.13
Merlot (must)		n.d.	4.09 ± 0.14
Chardonnay (must)		n.d.	2.50 ± 0.14
Sauvignon Blanc (must)		n.d.	1.48 ± 0.17
Pinot Noir (must)		n.d.	2.40 ± 0.13
Krasnostop (must)		n.d.	3.04 ± 0.18
Cabernet Franc (must)		n.d.	1.08 ± 0.11

Notes to the table 4:
¹ For area's description see 1.
² n.d. = Not determined.

in each batch of samples. The correlation coefficient of the linear dependence must be not lower than 0.99999. Otherwise, the measurement for a particular series of samples, including measurements of reference substance included in this batch, have to be repeated until the above conditions are met.

4. Results and discussion

4.1. Compositions of stable isotopes in grapes, dry wines and waters

The results of measurements of isotope compositions of stable carbon & oxygen isotopes presented as $\delta^{13}\text{C}_{\text{VPDB}}$ and $\delta^{18}\text{O}_{\text{VSMOW}}$ values, are shown below in Tables 1–4. The tables contain the average values obtained in a batch of five parallel determinations of carbon isotopes measurements for carbohydrates (sugars) and ethanol of each studied grape variety and dry wines samples, as well

Table 5. Ranges for $\delta^{13}\text{C}_{\text{VPDB}}$ and $\delta^{18}\text{O}_{\text{VSMOW}}$ in carbohydrates (sugars) and intracellular water of white and red grape varieties of the selected areas of the Crimean Peninsula, and South West Coast of the Greater Caucasus (crop of 2017).

Area	$\delta^{13}\text{C}_{\text{VPDB}}, \text{‰}$ carbohydrates (sugars)	$\delta^{18}\text{O}_{\text{VSMOW}}, \text{‰}$ intracellular water
Crimean Peninsula	-26.72 ... - 23.33	-1.24 ... 2.17
South West Coast of the Greater Caucasus (Anapa district – Semigorye area)	-25.92 ... - 23.87	1.08 ... 4.09

Table 6. Range for $\delta^{13}\text{C}_{\text{VPDB}}$ in ethanol of dry white and red wines made from grape varieties of the selected areas of the Crimean Peninsula and South West Coast of the Greater Caucasus (crop of 2017).

Area	$\delta^{13}\text{C}_{\text{VPDB}}, \text{‰}$ ethanol of dry wines
Crimean Peninsula	-28.15 ... - 24.47
South West Coast of the Greater Caucasus (Anapa district – Semigorye area)	-27.29 ... - 25.78

as in a batch of three parallel determinations of oxygen isotopes measurements in the intracellular water of each grape variety and water samples from surface, atmospheric and underground sources.

The expanded measurement uncertainty (EMU) of each batch of determinations did not exceed 0.30‰ (for carbon isotopes) and 0.20‰ (for oxygen isotopes). For the calculation of the EMU the coverage factor of 2 was used that corresponds to a confidence level of 95%.

4.2. Conclusions

Summarizing the data clusters obtained within the research program on the white and red grape's samples taken in the Crimean Peninsula and South West Coast of the Greater Caucasus (Krasnodar region) allows to set the ranges of the natural fractionation of stable carbon and oxygen isotopes in components of this plant within the season of 2017 (Tables 5–6).

The results of our study characterizing the fractionation of carbon isotopes in native carbohydrates (sugars) and also oxygen isotopes in intracellular water as well as in wines correlate very well with the results of our studies of 2015–2016 in the Crimean Peninsula [1,2] and the published data of other researches, which were conducted in other geographical regions with similar agroclimatic conditions, frequent water deficiency and an irregular irrigation (or total absence of that) during the vegetative period [10–18].

We thank Union of Winegrowers & Winemakers of Russia (SVVR), winegrowers and wineries from the Crimean Peninsula and South West Coast of the Greater Caucasus for supporting of the scientific study, especially for assistance with sampling of authentic grapes.

References

- [1] A. Kolesnov, N. Agafonova, BIO Web of Conferences **7**, 02004 (2016)
- [2] A. Kolesnov, M. Zenina, S. Tsimbalaev, D. Davlyatshin, M. Ganin, N. Anikina, N. Agafonova, E. Egorov, T. Guguchkina, A. Prakh, M. Antonenko, BIO Web of Conferences **9**, 02020 (2017)
- [3] Г. Валуйко, *Технология виноградных вин* (Таврида, Симферополь, 2001) / G. Valuyko, *Technology of grape wines* (Tavrida, Simferopol, 2001)
- [4] *Method OIV-MA-AS312-06* (2001)
- [5] *Method OIV-MA-AS2-12* (2009)
- [6] *BS DD ENV 12140:1997-02-15* (1997)
- [7] *Method OIV-MA-AS311-05* (2011)
- [8] *Finnigan Elemental Analyzer. Operating Manual* (Thermo Electron Corporation, Carbon Measurement, 5-1...5-20, 2010)
- [9] *Gasbench II. Operating manual* (Thermo Fisher Scientific, Revision A-1118343: 5-33-5-39, 5–42, 2009)
- [10] C. van Leeuwen, O. Tregoa, X. Chone, B. Bois, D. Pernet, J.-P. Gaudillere, J. Int. Sci. Vigne Vin. **43**, 121 (2009)
- [11] S. Gomez-Alonso, E. Garcia-Romero, Aust. J. Grape Wine Res. **16**, 283 (2010)
- [12] A. Herrero-Langreo, B. Tisseyre, J.-P. Goutouly, Th. Scholasch, C. van Leeuwen, Am. J. Enol. Vitic. **64**, 307 (2013)
- [13] *Code of practice of the European Fruit Juice Association* (2014)
- [14] F. Guyon, C. van Leeuwen, L. Gaillard, M. Grand, S. Akoka, G.S. Remaud, N. Sabathie, M.-H. Salagoity, Anal. Bioanal. Chem. **407**, 9053 (2015)
- [15] N. Christoph, BIO Web of Conferences **5**, 02020 (2015)
- [16] S. Leonardelli, S.V. Dutra, G.J. Carnieli, F. Spinelli, D. de Siqueira Ferreira, R. Vanderlinde, BIO Web of Conferences **7**, 02001 (2016)
- [17] C. van Leeuwen, Ph. Darriet, J. Wine Econ. **11**, 150 (2016)
- [18] F. Camin, M. Boner, L. Bontempo, C. Fauhl-Hassek, S.D. Kelly, J. Riedl, A. Rossmann, Trends Food Sci. Tech. **61**, 176 (2017)
- [19] А. Колеснов, С. Цимбалаев, Е. Шольц-Куликов, В. Геок, Аналитика **40**, 264 (2018)