

Scientific study of $^{13}\text{C}/^{12}\text{C}$ carbon and $^{18}\text{O}/^{16}\text{O}$ oxygen stable isotopes biological fractionation in grapes in the Black Sea, Don Basin and the Western Caspian regions

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Abstract. The report presents the results of a study of carbon and oxygen stable isotopes in carbohydrates and intracellular water of red and white grapes of 2016 wine-growing season in the Crimean peninsula areas, South-west coast of the Greater Caucasus, the Don basin and the Western Caspian region. The mass concentration of reducing sugars in the studied grape samples has been from 17.5 to 25.0 g/100 ml, titrated acids concentration (based on tartaric acid) – from 6.0 to 9.1 g/l, the buffer capacity 34.1–63.2 mg-Eq/l. Red and white wine made from respective grapes contained from 0.5 to 3.6 g/l of residual sugar; from 11.1 to 14.5% ethanol by volume; buffer capacity was 35.2–52.6 mg-Eq/l. 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 for the studied geographical areas are ranging from -26.74 to -20.74‰ (the Crimean peninsula); from -27.31 to -21.58‰ (South West Coast of the Greater Caucasus), from -27.33 to -24.73‰ (Don Basin) and from -26.64 to -23.17‰ (West Caspian). The $\delta^{13}\text{C}_{\text{VPDB}}$ values for ethanol of the red and white dry wines range from -28.52 to -24.26‰ (the Crimean peninsula); from -29.23 to -24.52‰ (South West Coast of the Greater Caucasus); from -28.97 to -26.22‰ (Don Basin); from -29.14 to -25.22‰ (Western Caspian). Compared with the surface water and groundwater (averages from $\delta^{18}\text{O}_{\text{VSMOW}} -13.90$ to -6.38‰) and with precipitation (averages from $\delta^{18}\text{O}_{\text{VSMOW}} -10.30$ to -9.04‰) the $\delta^{18}\text{O}_{\text{VSMOW}}$ values in intracellular water of grapes are the following: for the Crimean peninsula grapes, from 0.40 to 4.97‰; the South West Coast of the Greater Caucasus, from -2.11 to 6.29‰; the Don Basin, from -2.21 to 6.26‰; the Western Caspian, from -0.24 to 1.44‰. It has been noted that in conditions of water shortage caused by low rainfall or lack of rainfall and irrigation, grapes are prone to the accumulation of “heavy” ^{13}C carbon isotope due to changes in isotopic exchange with the environment through a partial reduction in the intensity of transpiration and photosynthesis.

1. General climatic conditions in the areas of the study

1.1. Black sea

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 5 viticulture areas of the Crimean Peninsula – Western Maritime-Steppe area (villages Romashkino and Plodovoye), Western Foothills-Maritime area (village Uglovoye), Eastern Mountain-Valley Maritime zone of the South Coast area (village Morskoye) and East foothill zone of the South Coast area (village Koktebel). The total area of sampling vineyards made 512.0 ha. The main areas on the South West Coast of the Greater Caucasus are the Temryuk, Krymsk and Anapa districts, and also areas

close Novorossiysk. The total area of vineyards on South West Coast, in which in a season of 2016 grapes samples have been taken, has made 215.02 ha. Scientific data on the Black Sea areas, including climate, soils and grapes varieties have been reported in the previous publication about results of the study of 2015 [1].

1.2. Don basin

The grapes were studied in two areas – Tsimlyansky (Tsimlyansky Reservoir coast) and Martynovsky districts (Fig. 2). The total area of the surveyed vineyards has made 80.60 ha.

1.3. Western caspian

The main area of viticulture is the The Republic of Dagestan. The list of areas of this region, in which



Figure 1. Black Sea areas.



Figure 2. Don Basin areas.

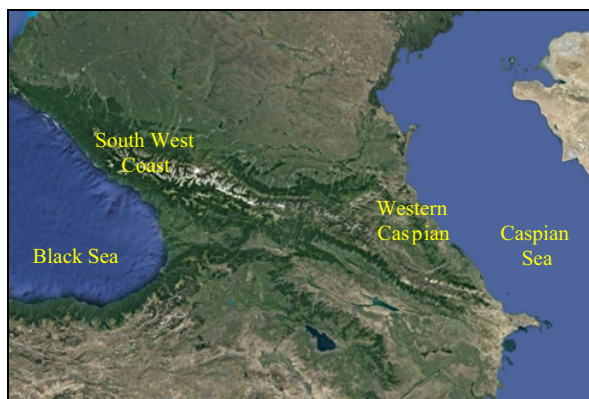


Figure 3. Western Caspian.

grapes samples have been taken, had included the Derbent, Sergokalinsky, Suleyman-Stalsky and Magaramkentsky districts. The total area of the surveyed vineyards has made 375.04 ha.

2. Grape samples

2.1. Varieties

The samples of the 9 white and red grape varieties were used for the study – Aligote, Rkatsiteli, Cabernet Sauvignon, Sauvignon Green (Crimean Peninsula); Cabernet Sauvignon, Chardonnay (South West Coast – Krasnodar region); Bianka, Saperavi Severniy, Tsimlyansky Cherniy (Don Basin); Rkatsiteli, Riesling, Cabernet Sauvignon (Western Caspian – The Republic of Dagestan). The

sampling of the authentic fresh grapes was carried out in 15 viticulture areas in shown four regions. The total area of sampling vineyards made 1182.66 ha. The sampling was conducted according to the requirements described in [2].

2.2. Main physical-chemical characteristics

The research program included 43 samples of grapes of 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.

36 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.

The mass concentration of reducing sugars in the studied grape samples has been from 17.5 to 25.0 g/100 ml, titrated acids concentration (based on tartaric acid) - from 6.0 to 9.1 g/l, the buffer capacity 34.1–63.2 mg-Eq/l. Red and white wine made from respective grapes contained from 0.5 to 3.6 g/l of residual sugar; from 11.1 to 14.5% ethanol by volume; buffer capacity was 35.2–52.6 mg-Eq/l. The additional scientific data about buffer capacity measurements in musts and wines are published in [3,4].

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 [5,6].

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) and ethanol by the FC-IRMS/SIRA method. The composition of oxygen isotopes ¹⁸O/¹⁶O was studied by the EQ-IRMS/ SIRA method directly in musts and dry wines without prior isolation of water from the samples.

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

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 2016).

Grape variety	Area ¹	$\delta^{13}\text{C}_{\text{VPDB}}, \text{‰}$	
		carbohydrates (sugars)	ethanol
Aligote	Uglovoye	-23.83 ± 0.21	-25.69 ± 0.05
Cabernet Sauvignon		-23.71 ± 0.16	-25.43 ± 0.05
Rkatsiteli	Morskoye	-26.74 ± 0.02	-28.52 ± 0.01
Cabernet Sauvignon		-23.14 ± 0.03	-24.82 ± 0.04
Rkatsiteli	Plodovoye	-23.88 ± 0.07	-26.52 ± 0.05
Cabernet Sauvignon		-25.08 ± 0.17	-27.29 ± 0.05
Aligote	Koktebel	-24.78 ± 0.06	-26.81 ± 0.03
Cabernet Sauvignon		-20.74 ± 0.03	-24.26 ± 0.04
Cabernet Sauvignon	Romashkino	-21.91 ± 0.08	-24.58 ± 0.03
Sauvignon Green		-22.74 ± 0.04	n.d. ²

Notes to the Table 1:

¹ For area's description see 1.1.

² Not determined.

Table 2. Composition of stable carbon isotopes (¹³C/¹²C) in carbohydrates (sugars) of grape varieties and ethanol of dry wines of the selected areas of the South West Coast of the Greater Caucasus (crop of 2016).

Grape variety	Area ¹	$\delta^{13}\text{C}_{\text{VPDB}}, \text{‰}$	
		carbohydrates (sugars)	ethanol
Chardonnay (vineyards 1)	Temryuk district	-25.24 ± 0.37	-28.57 ± 0.04
Chardonnay (vineyards 2)		-25.29 ± 0.35	-27.11 ± 0.08
Chardonnay (vineyards 3)		-27.31 ± 0.40	-29.23 ± 0.06
Cabernet Sauvignon (vineyards 1)		-25.99 ± 0.15	-28.16 ± 0.02
Cabernet Sauvignon (vineyards 2)		-24.60 ± 0.10	-27.32 ± 0.08
Cabernet Sauvignon (vineyards 3)		-21.54 ± 0.23	-28.51 ± 0.10
Chardonnay	Krymsky district	-26.15 ± 0.21	-29.02 ± 0.02
Cabernet Sauvignon		-27.27 ± 0.19	-29.06 ± 0.01
Chardonnay	Anapsky district	-25.83 ± 0.20	-28.88
Cabernet Sauvignon		-26.17 ± 0.25	-28.62 ± 0.16
Chardonnay (vineyards 1/1)	Novoros-siysk	-23.18 ± 0.02	n.d. ²
Chardonnay (vineyards 1/2)		-23.59 ± 0.11	-25.01 ± 0.02
Chardonnay (vineyards 2/1)		-22.25 ± 0.16	n.d. ²
Chardonnay (vineyards 2/2)		-21.58 ± 0.13	-24.61 ± 0.01
Chardonnay (vineyards 3/1)		-22.33 ± 0.10	n.d. ²
Chardonnay (vineyards 3/2)		-23.45 ± 0.07	-24.52 ± 0.10
Chardonnay (vineyards 4/1)		-26.35 ± 0.22	n.d. ²
Chardonnay (vineyards 4/2)		-23.67 ± 0.16	-28.17 ± 0.05
Cabernet Sauvignon (vineyards 1/1)		-23.84 ± 0.02	-26.69 ± 0.05
Cabernet Sauvignon (vineyards 2/1)		-22.77 ± 0.23	-24.96 ± 0.23
Cabernet Sauvignon (vineyards 3/1)		-23.10 ± 0.17	-25.00 ± 0.08
Cabernet Sauvignon (vineyards 4/1)		-24.75 ± 0.18	-27.91 ± 0.08

Notes to the Table 1:

¹ For area's description see 1.1.

² Not determined.

3.1.2. Water samples

To study the composition of stable isotopes of oxygen (¹⁸O/¹⁶O) 19 water samples were taken in all studied areas - two samples from the Crimean Peninsula (water from atmospheric precipitations & underground source), two samples from the Don Basin (water from atmospheric precipitations & underground source), twelve samples from the South West Coast (water from atmospheric precipitations, tap water used for irrigation of vineyards, water from the Anapka river, city tap water and water from underground sources) and three samples from the Western Caspian (water from atmospheric precipitations, city tap water and water from underground sources).

The water samples were stored before the isotopic measurements at a temperature from 2 to 4 °C in sealed sterile glass containers without access of air.

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

The study of fractionation effect of the stable isotopes of carbon (¹³C/¹²C) in grape and wine components (carbohydrates (sugars), ethanol) using the FC-IRMS/SIRA method 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);

Table 3. Composition of stable carbon isotopes ($^{13}\text{C}/^{12}\text{C}$) in carbohydrates (sugars) of grape varieties and ethanol of dry wines of the selected areas of the Don Basin (crop of 2016).

Grape variety	Area ¹	$\delta^{13}\text{C}_{\text{VPDB}}, \text{‰}$	
		carbohydrates (sugars)	ethanol
Bianka	Tsimlyan-sky district	-26.48 ± 0.10	-27.74 ± 0.05
Saperavi Severniy		-25.38 ± 0.01	-26.73 ± 0.09
Tsimlyansky Cherniy		-27.33 ± 0.14	-28.97 ± 0.11
Bianka	Martynov- sky district	-24.73 ± 0.20	-26.22 ± 0.09

Notes to the Table 3:

¹ For area's description see 1.2.

Table 4. Composition of stable carbon isotopes ($^{13}\text{C}/^{12}\text{C}$) in carbohydrates (sugars) of grape varieties and ethanol of dry wines of the selected areas of the Western Caspian (crop of 2016).

Grape variety	Area ¹	$\delta^{13}\text{C}_{\text{VPDB}}, \text{‰}$	
		carbohydrates (sugars)	ethanol
Rkatsiteli (vineyards 1)	Derbent district	-26.75 ± 0.04	-27.79 ± 0.11
Rkatsiteli (vineyards 2)		-25.92 ± 0.20	-28.07 ± 0.01
Cabernet Sauvignon (vineyards 1)		-26.20 ± 0.01	-28.15 ± 0.06
Cabernet Sauvignon (vineyards 2)		-26.64 ± 0.11	-29.14 ± 0.04
Cabernet Sauvignon	Sergokalin-sky district	-23.17 ± 0.04	-25.22 ± 0.01
Rkatsiteli	Magaram- kent district	-26.20 ± 0.16	-27.77 ± 0.03
Riesling	Suleiman-Stalsky district	-23.66 ± 0.08	-25.78 ± 0.11

Notes to the Table 4:

¹ For area's description see 1.3.

Table 5. Composition of stable oxygen isotopes ($^{18}\text{O}/^{16}\text{O}$) in intracellular water of grape varieties from selected areas of the Crimean Peninsula (crop of 2016).

Grape variety	Area ¹	$\delta^{18}\text{O}_{\text{VSMOW}}, \text{‰}$
Aligote	Uglovoye	3.57 ± 0.06
Cabernet Sauvignon		2.34 ± 0.30
Rkatsiteli	Morskoye	0.40 ± 0.10
Cabernet Sauvignon		1.23 ± 0.16
Rkatsiteli	Plodovoye	1.92 ± 0.17
Cabernet Sauvignon		2.77 ± 0.15
Aligote	Koktebel	2.32 ± 0.36
Cabernet Sauvignon		4.11 ± 0.13
Cabernet Sauvignon	Romashkino	4.45 ± 0.03
Sauvignon Green		4.97 ± 0.29

Notes to the Table 5:

¹ For area's description see 1.1.

- (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, $\delta^{13}\text{C} = -27.771 \text{‰}$) 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.

Sample preparation – extraction and purification of carbohydrates (sugars) from musts obtained from fresh grapes – was performed according to the method described in [7]. 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. Ethanol was extracted from dry wines by using of the laboratory glass distillation unit according to the conditions for the ethanol extraction as described in [5,8]. Extracted ethanol samples 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 [1,9–11]. 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. Ethanol samples were injected directly into oxidation reactor of the elemental analyzer (A) by using of a microsyringe. The injection volume of each ethanol sample was from 0.08 to 0.1 μl .

The conditions of carrying out measurements of isotope composition of carbon in the preparations of carbohydrates (sugars) and ethanol by using the stated above laboratory facilities corresponded to the optimum modes which are developed during the preliminary runs. Quantitative combustion of carbohydrates (sugars) and ethanol 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 $^\circ\text{C}$ in the presence

Table 6. Composition of stable oxygen isotopes (¹⁸O/¹⁶O) in intracellular water of grape varieties from selected areas of the South West Coast of the Greater Caucasus (crop of 2016).

Grape variety	Area ¹	δ ¹⁸ O _{VSMOW} , ‰	
Chardonnay (vineyards 1)	Temryuk district	2.28 ± 0.12	
Chardonnay (vineyards 2)		2.82 ± 0.30	
Chardonnay (vineyards 3)		2.74 ± 0.07	
Cabernet Sauvignon (vineyards 1)		-1.87 ± 0.32	
Cabernet Sauvignon (vineyards 2)		-0.83 ± 0.14	
Cabernet Sauvignon (vineyards 3)		-2.11 ± 0.18	
Chardonnay	Krymsky district	1.00 ± 0.23	
Cabernet Sauvignon		-1.20 ± 0.06	
Chardonnay	Anapsky district	1.55 ± 0.03	
Cabernet Sauvignon		-1.98 ± 0.20	
Chardonnay (vineyards 1/1)	Novorossiysk	3.99 ± 0.13	
Chardonnay (vineyards 1/2)		2.96 ± 0.25	
Chardonnay (vineyards 2/1)		6.29 ± 0.16	
Chardonnay (vineyards 2/2)		2.71 ± 0.12	
Chardonnay (vineyards 3/1)		2.49 ± 0.42	
Chardonnay (vineyards 3/2)		2.07 ± 0.09	
Chardonnay (vineyards 4/1)		2.61 ± 0.07	
Chardonnay (vineyards 4/2)		1.70 ± 0.09	
Cabernet Sauvignon (vineyards 1/1)		-0.35 ± 0.12	
Cabernet Sauvignon (vineyards 2/1)		Novorossiysk	4.91 ± 0.17
Cabernet Sauvignon (vineyards 3/1)			5.09 ± 0.10
Cabernet Sauvignon (vineyards 4/1)			0.32 ± 0.06

Notes to the Table 6:

¹ For area's description see 1.1.

Table 7. Composition of stable oxygen isotopes (¹⁸O/¹⁶O) in intracellular water of grape varieties from selected areas of the Don Basin (crop of 2016).

Grape variety	Area ¹	δ ¹⁸ O _{VSMOW} , ‰
Bianka	Tsimlyansky district	2.74 ± 0.01
Saperavi Severniy		1.87 ± 0.25
Tsimlyansky Cherniy		-2.21 ± 0.25
Bianka	Martynovsky district	6.26 ± 0.27

Notes to the Table 7:

¹ For area's description see 1.2.

of molecular high purity oxygen 99.999% (Linde Gas RUS, Russian Federation). The separation of elementary gases resulting from the combustion of carbohydrates (sugars) or ethanol 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 ¹³C/¹²C, 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 ¹²C¹⁷O¹⁷O and ¹³C¹⁷O¹⁶O 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 ¹³C and ¹⁷O in the nature (respectively 1.11 and 0.0375%).

Table 8. Composition of stable oxygen isotopes (¹⁸O/¹⁶O) in intracellular water of grape varieties from selected areas of the Western Caspian (crop of 2016).

Grape variety	Area ¹	δ ¹⁸ O _{VSMOW} , ‰
Rkatsiteli (vineyards 1)	Derbent district	1.12 ± 0.15
Rkatsiteli (vineyards 2)		0.79 ± 0.26
Cabernet Sauvignon (vineyards 1)		-0.02 ± 0.08
Cabernet Sauvignon (vineyards 2)		0.96 ± 0.29
Cabernet Sauvignon	Sergokalinsky district	-0.24 ± 0.02
Rkatsiteli	Magaramkent district	0.96 ± 0.29
Riesling	Suleiman-Stalsky district	1.44 ± 0.42

Notes to the Table 8:

¹ For area's description see 1.3.

3.1.4. EQ-IRMS/SIRA method for δ¹⁸O_{VSMOW} analysis

The study of fractionation effect of the stable isotopes of oxygen (¹⁸O/¹⁶O) in the intracellular water in grapes (musts) and water 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);

Table 9. Composition of stable oxygen isotopes (¹⁸O/¹⁶O) in water samples from selected areas of the Crimean Peninsula, South West Coast of the Greater Caucasus, Don Basin and Western Caspian (study of 2016).

Sample description	Area ¹	δ ¹⁸ O _{VSMOW} , ‰
Tap water	Western Caspian (Derbent)	-9.42 ± 0.09
	South West Coast of the Greater Caucasus (Temryuk - Taman)	-9.31 ± 0.08
	South West Coast of the Greater Caucasus (Temryuk - Vinogradnyy)	-9.19 ± 0.08
	South West Coast of the Greater Caucasus (Krymsk)	-13.90 ± 0.01
Water of atmospheric precipitations	Western Caspian (Derbent)	-9.09 ± 0.08
	Don Basin (Tsymlyansky district)	-9.04 ± 0.12
	South West Coast of the Greater Caucasus (Temryuk - vineyard 1)	-10.10 ± 0.01
	South West Coast of the Black Sea (Temryuk - vineyard 2)	-9.62 ± 0.40
	South West Coast of the Greater Caucasus (Temryuk - vineyard 3)	-9.20 ± 0.08
	South West Coast of the Greater Caucasus (Krymsk)	-10.30 ± 0.01
Water from underground sources	Western Caspian (Foothills of the Caucasus)	-8.20 ± 0.09
	Don Basin (Martymovsky district)	-11.34 ± 0.09
	South West Coast of the Greater Caucasus (Temryuk – Taman, vineyard)	-12.78 ± 0.19
	South West Coast of the Greater Caucasus (Temryuk – Taman, winery)	-9.39 ± 0.04
	South West Coast of the Greater Caucasus (Anapsky district)	-7.88 ± 0.15
Water from surface sources	South West Coast of the Greater Caucasus (Anapsky district - river)	-9.55 ± 0.08
	South West Coast of the Black Sea (Anapsky district lake)	-6.38 ± 0.03

Notes to the Table 9:

¹ For area's descriptions see 1.1-1.3.

Table 10. Ranges for δ¹³C_{VPDB} and δ¹⁸O_{VSMOW} in carbohydrates (sugars) and intracellular water of white and red grape varieties of the selected areas of the Crimean Peninsula, South West Coast of the Greater Caucasus, Don Basin and Western Caspian (crop of 2016).

Area	δ ¹³ C _{VPDB} , ‰ carbohydrates (sugars)	δ ¹⁸ O _{VSMOW} , ‰ intracellular water
Crimean Peninsula	-26.74...-20.74	0.40...4.97
South West Coast of the Greater Caucasus (Krasnodar region)	-27.31...-21.58	-2.11...6.29
Don Basin (Rostov region)	-27.33...-24.73	-2.21...6.26
Western Caspian (The Republic of Dagestan)	-27.67...-23.66	-0.24...1.80

Table 11. Range for δ¹³C_{VPDB} in ethanol of dry white and red wines made from grape varieties of the selected areas of the Crimean Peninsula, South West Coast of the Greater Caucasus, Don Basin and Western Caspian (crop of 2016).

Area	δ ¹³ C _{VPDB} , ‰ ethanol of dry wines
Crimean Peninsula	-28.52...-24.26
South West Coast of the Greater Caucasus (Krasnodar region)	-29.06...-24.61
Don Basin (Rostov region)	-28.97...-26.22
Western Caspian (The Republic of Dagestan)	-29.14...-25.22

- (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, δ¹³C = -27.771‰) 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 ¹⁸O/¹⁶O were used for adjustment of measurement's results: VSMOW2 (Vienna-Standard Mean Ocean Water, δ¹⁸O = 0.00‰);

GISP (Greenland Ice Sheet Precipitation, δ¹⁸O_{VSMOW} = -24.76‰); SLAP2 (Standard Light Antarctic Precipitation, δ¹⁸O_{VSMOW} = -55.50‰).

High purity gases were used in the study – flushing gas – helium 99.9999%, containing 0.4–0.5% of high 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 intracellular water was carried out by isotopic equilibration technique (EQ-IRMS/SIRA method). For isotopic equilibration reaction, 0.500 ml of grape must or 0.200 ml of water 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 reactions show that the equilibrium state occurs after at

least 18 hours. The optimum temperature for the reaction was $24.0 \pm 0.1^\circ\text{C}$.

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°C . Temperature of samples in the sample module (1) was maintained at a constant level of $24.0 \pm 0.1^\circ\text{C}$ until the end of the measurements.

Quantitative measurements of oxygen isotopes $^{18}\text{O}/^{16}\text{O}$ were performed according to conditions described above and in [12]. During the measurement cycle the WRG calibrated against a reference substance is introduced 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 $\delta^{18}\text{O}_{\text{VSMOW}}$ 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 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 1–9. 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 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 0.40‰ (for carbon isotopes) and 0.42‰ (for oxygen isotopes). For the calculation of the EMU the coverage factor of 2 was used that corresponds to a confidence level of 95%.

5. Conclusions

Summarizing the data clusters obtained within the research program on the white and red grape's samples taken in the Crimean Peninsula, South West Coast of the Greater Caucasus (Krasnodar region), Don Basin and Western Caspian allows to set the ranges of the natural fractionation of stable carbon and oxygen isotopes in components of this plant within the season of 2016 (Tables 10–11).

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 study of 2015 in the Crimean Peninsula [1] 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. It was shown that the carbohydrates (sugars) of grapes which are grown up in the conditions of water deficiency and high level of active temperatures are enriched by “heavy” ^{13}C carbon isotopes [13–23]. For example, C. van Leeuwen et al. suggested in [15], according to the results of their studies, the following dependence of the range for $\delta^{13}\text{C}$ of sugars on the water status during the grape cultivation:

- “no water deficit” ... $< -26.0\text{‰}$,
- “weak water deficit” ... from -24.5 to -26.0‰ ,
- “moderate to weak water deficit” ... from -23.0 to -24.5‰ ,
- “moderate to severe water deficit” ... from -21.5 to -23.0‰ ,
- “severe water deficit” ... $> -21.5\text{‰}$.

In our study the width of the range of $\delta^{13}\text{C}_{\text{VPDB}}$ values for natural carbohydrates (sugars) in 9 white and red grape varieties amounts to 6.09‰.

Carbohydrates (sugars) of grape varieties from four geographical regions regarding the carbon isotope composition are almost equivalent in accordance with the experimental data from this study, taking into account the quantitative levels of measurement uncertainty. In this part the results of the study correlate well with published data from other scientific researches [18–20].

The research results for water samples show the $\delta^{18}\text{O}_{\text{VSMOW}}$ values, which are characteristic for the natural fractionation of oxygen isotopes in the atmospheric and geological underground waters (Table 9).

The results of the study can be used in practice for quality assessment, including identification of commercial products offered on national and international foodstuffs markets (e.g. wines from the regions with a Geographical Indication (GI) or Designation of Origin (DO)).

However, in applying the results of this study, including above shown intervals of $\delta^{13}\text{C}_{\text{VPDB}}$ and $\delta^{18}\text{O}_{\text{VSMOW}}$ for grapes from the Crimean Peninsula, South West Coast of Greater Caucasus, Don Basin and Western Caspian, for example, when planning a scientific or an applied work, it is necessary to take into account interrelations of the obtained experimental data with agroclimatic conditions of cultivation and harvest of 2016, and also to consider the size of a data matrix which is defined by a quantity of samples, climate peculiarities sampling areas and grape varieties.

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