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BIOCHEMISTRY OF SPRING SAP OF DIFFERENT GRAPEVINE CULTIVARS AND ITS IMPACT ON CROP QUANTITY AND QUALITY

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Abstract

The aim of the study is to investigate the spring sap of 15 different grape varieties during the spring season – April 2024 in an experimental vineyard of the Institute of Agriculture – Kyustendil, Bulgaria. The methods used in this research have been: physico-chemical and agro-ecological studies of the spring grape sap and determination of the oxidation sustainability of the sap for each studied grape cultivar, using the Pourbaix pH-Eh water diagram. 1) main conclusion is that the grape sap consists salt on the level of saturation, and this delicate equilibrium in water-salt balance must not be disturbed; 2) the sap is very susceptible to oxidation and adding of ascorbic acid as antioxidant in the soil will be useful for the common physiology of the grapevine cultivars, 3) ascorbic acid circulates in nature and might pass through the root onto the plant, moreover, the roots of the plants may synthesizec ascorbic acid; 4) grape sap is a bio-indicator for contamination of the environment, and specially of the soil as cyanuric acid and nitrates passes through the root to the plant, but some other contaminators as Pb, As, Radiation cannot be claimed from the present study.

Key words: *biochemistry, physiology, grape, cultivars, sap, crop quality, soil fertility, Bulgaria*

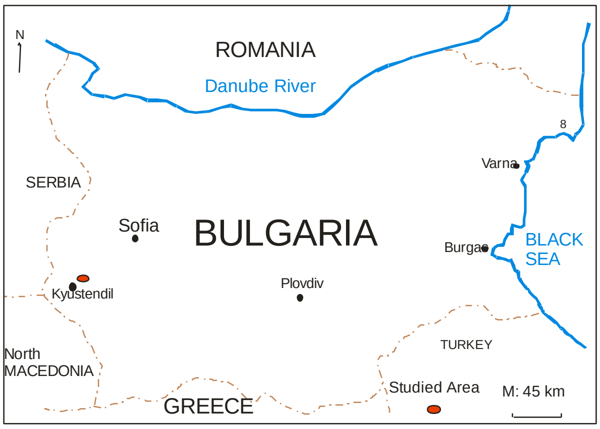
INTRODUCTION

The importance of this study in the context of the literature in the field.
Its originality, novelty, actual importance.
Make the link between the biochemistry of spring sap of various grape varieties and wine quality. The present study was provoked by an increased relative salt content in grape, cherry, and apple fruits from the Kyustendil region, The measured salt contents of grape and other fruit juices were superior to the total dissolved solids. Last winter 2022/2023 was dry, and early summer fell large amounts of precipitation during the ripening in June-July 2023. According to data of the meteorological station “Meteobot” of the Institute of Agriculture - Kyustendil, from mid-June to mid-July rainfalls were 22 days. Many of the rainfall fell in 1 hour exceeded half the monthly norm. It is assumed that this precipitation is unusual and is the result of climate changes. As a result, the grape harvest in the Kyustendil region was of poor quality, the fruits were small, cracked, with low amount. Many grape cultivars had not produced crop. The death of plants began. The reason is the activation of sodium cations, as a result of heavy rainfall, which leads to blockage of the root systems of cultivars. The mineral halite is formed – sodium chloride in the soil, which disbalance the equilibrium of the salt-water balance in the plants. Study was implemented at the experimental fields and laboratories of the Institute of Agriculture, town of Kyustendi, which is a part of Agricultural Academy, city of Sofia, Bulgaria. It is located in the geometric center of Balkan Peninsula, Europe, of about 3 km North-Eastern from the town of Kyustendil, which is settled in a valley with the same name, on the right side of the cross-border Struma River and at the foot of the Mountain "Osogovo". The studied area is located in the central part of the Balkan Peninsula exactly on the border between Bulgaria, Serbia and North Macedonia, and close to Greece. Its altitude is 512 m (Fig. 1).
The climate of the town of Kyustendil is transitional continental to Mediterranean. Rainfall in Kyustendil is not heavy. Their

average annual amount is about to 589 mm. There is a tendency to alternate dry with wet years or periods. By seasons they are distributed fairly evenly. Snowfall is usually from November to March, with snow cover up to 30 cm thick and lasting for up to 15 days. However, the water wealth of Kyustendil and its surroundings is not small. There are many rivers, springs, dams, mineral and ground waters [12].

The largest river in the region is the Struma River as Bistritsa River is the biggest tributary of Struma River on the territory of the municipality of Kyustendil. The Bistritsa River is one possible source for irrigation of the experimental fields of the Institute of Agriculture, town Kyustendil [8].

It is 51 km long and borders directly to the studied experimental fields. It springs at 2,182 m above sea level in Osogovo Mountain, northeast of Mount Ruen, and it flows on the right into the Struma River at 462 m above sea level, southwest of the village of Konyavo [4].



Map 1. Location of the area of study
Source: own drawing.

In this context, the purpose of this research work was to monitor the water-salt balance of the grapevine sap in different grape cultivars and to take measures to keep it through appropriate methods. If the balance is disturbed by dry period, the measure is artificial irrigation. If balance is disturbed by flooding – it is proposed to treat the soil with the mineral gypsum – soil plastering or the so-called gypsum fertiliser.

MATERIALS AND METHODS

The research was carried out in an experimental vineyard of the Institute of Agriculture – Kyustendil, Bulgaria in 2024.

The object of the study were grapevine cultivars with different genetic origin and direction of use: 5 red wine vine cultivars - Pamid (*Vitis Vinifera*), Cabernet Sauvignon (*Vitis Vinifera*), Kaylashki rubin (Pamid x Hybrid VI 2/15) x (Gamay noir x *Vitis Amurensis*), Trapezitsa (Dunavska Gamza x Noir hatif de Marseille) and Otelo (Clinton x Black Hamburg; 3 white wine vine cultivars – Tamianka (*Vitis Vinifera*), Slava (Dunavska Gamza x Tsvetochnyi) and Druzhba (Muskat hamburg x S.V.12375) x (Zaria Severa x Muskat hamburg); Table grapevine cultivars - Super early Bolgar (Italia x Yantar), Prista (Bolgar x Mimosa), Ryahovo (Trakia x Black Rose), Garant (Hybrid V25/20 x Druzhba), Dunav (Bicane x Ribi mehur) x Cardinal), Velika (self-pollinating Hybrid 3/23 (Bolgar x Alphonse Lavallee) and Misket plevenski (Muscat hamburg x Perle Von Csaba).

The vines of the wine cultivars were planted in 2015 and the table grapevine cultivars in 2007. They were grafted onto the rootstock Berlandieri x Riparia SO4. The wine cultivars are stem-formed, and the table cultivars are formed on the ground. Pruning is according to the Guyot system.

The aim of the study is to investigate the spring sap of 15 different grape cultivars. Digital instruments were used *in situ* and in laboratories as follow: Refractometer "Milwaukee Brix MA871"- Hungary to measure the total sugar content by Brix (%) and refractometer "Atago-Pal 1", Australia for control. Glucose content (mmol/l, %) was measured through the Austrian "Wellion WF073" glucometer. Instrument "Lovibond-SensoDirect 150"-United Kingdom and Bluetooth compatible water quality intelligent tester "Yieryi BLE-C600"-China for control are used for determination of total acidity (pH), electrical conductivity (EC, μS/cm), total dissolved solids (TDS, ppm), total salt content (Salt, ppm,%), Specific Gravity (S.G.), Eh - Redox potential (mV) of the grape sap and juices, and temperature (°C). Sap was received as each grape cultivar was cut, during spring season and collected in sterile dishes.

The juices of the fruits were produced by the cold-pressing method, using 2.5 pressure of the press, speed of the grinder 35 Hz, speed of the belt-press 48 Hz, temperature 20°C in regular air environment. Minimum 5 measurements of technological parameters were used for each kind of fruits and vegetables at the present study. Fruit juice (100%) is obtained by the method of cold pressing with a single-shaft juicer Star Light SJB-150 R, unpasteurized, without additives. Radiation (μSv/h) of the sap and juice from grape and the common radiation background were measured with a Geiger counter "Radex"RD1503, which performed 5 automatic measurements and calculated the average value. Ascorbic acid in the liquids was measured through semi-quantity colorimetric test strips with range 0-25-50-100-200-400 mg/l. Bromine is determined through test strips with range 0-0.5-1-2-6-10-20 mg/l, Fluoride 0-25-50100-200 mg/l, and Iodine 0-0.02-0.04-0.08-0.10-0.15 mg/l.

Nitrate and nitrite content in the liquids were measured by using test strips with a range of 0-10-25-50-100-250-500 mg/l. Arsenic content was measured by the usage of test strips with a range of 0.005-0.0010-0.0025-0.05-0.1-0.25-0.5 mg/l. Lead content in studied liquids is measured by through blei-test and strips with a range of 20-40-100-200-500 mg/l.

The statistical processing of the data was performed by using computer application XLStat-Pro [5].

Pourbaix pH-Eh water diagram is used for visualizing of equilibrium of oxygen plus hydrogen ions to form water. Figure 2 shows the E-pH diagram for water with no metal involved. Line (a) represents the equilibrium reaction for hydrogen ions to evolve hydrogen gas. At any potential and pH below this line the hydrogen ion in water will react with electrons to evolve hydrogen gas. Line (b) represents the equilibrium of oxygen plus hydrogen ions to form water. At any potential and pH above this line water is oxidized to evolve oxygen gas and hydrogen ions (the reverse of Equation (3)). For potential and pH conditions between lines (a) and (b), water is thermodynamically stable and there is no gas evolved [10].

RESULTS AND DISCUSSIONS

Table 1 and Table 4 show the results from the measured biochemical content of the spring sap, collected by 15 grape plant cultivars during the spring season – April 2024 at the mentioned above geographic region. Sterile laboratory dishes collected the sap for 24 hours, but the leakage was not permanent, because of this reason the data given in the tables is not debit of the leaking per hour, it is just collected amount in grams. Total sugar content by Brix (%) was measured also as the red varieties of grape fruits had higher sugar content as the sweetest sap is of Pamid, Kaylashki rubin, and Otelo. The same red varieties demonstrated the highest content of glucose as the grape variety Otelo with very different origin and very different place of growing, had 1.3% sugar which is 83,37% glucose.

Grape products as wine, vinegar and cold-pressed juice are given only for comparison.

At the histrogram Fig. 1 and Tables 2 and 3 may be seen the statistical processing of the data for acidity of the solutions. It is obvious that the acidity of the spring grape plant sap has low acidity between 5. 08 and 6.17 or average – mean 5.564 and standard deviation 0.348 for 16 observations and 0 missing results. Most of the measurements between 4.6 and 5.6.

For comparison the grape products as wine, juice and vinegar are more acid pH=3.65-3.76, but the sugar content is higher and glucose is a smaller part from the total sugar content.

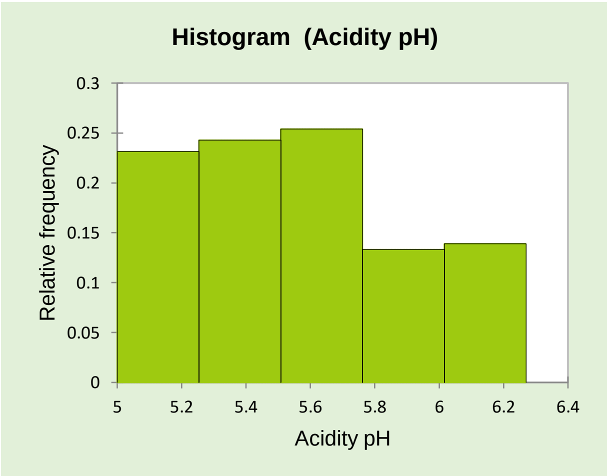


Fig. 1. Histogram of the relative frequency of the pH measurements of the spring sap of different grape cultivars
Source: own calculations with XL Stat [5].

Table 1. Physico-chemical parameters of spring sap of different grape cultivars, n.d.-no data

Cultivar	Collected amount for, g	Total Sugar Content, Brix, %	Glucose, 5 of total sugar, %	Glucose, mmol/l	Acidity pH	Redox Oxigation-reduction Eh, Mv	Electro conductivity EC, µS	Total Dissolved Solids TDS, ppm	Salt, ppm	Salt, %	Specific Gravity of Salt, S.G.
Cultivars for red wines											
Pamid	113	0.6	15.63	3.9	6.14	17	538	269.0	269.0	0.02	1.000
Kaylashki rubin	123	0.5	4.81	1.2	5.91	15	687	343.5	343.5	0.03	1.000
Trapezitsa	220	0.1	<1.1	<4.4	5.71	35	957	478.5	478.5	0.04	1.000
Cabernet Sauvignon	32	0.1	<1.1	<4.4	5.90	27	693	346.5	346.5	0.03	1.000
Otelo	54	1.3	83.37	20.8	5.58	-50	807	403.5	403.5	0.04	1.000
Cultivars for white wines											
Slava	22	0.1	<1.1	<4.4	5.50	2	717	358.5	358.5	0.03	1.000
Tamianka	322	<0.1	<1.1	<4.4	5.62	25	732	366.0	366.0	0.03	1.000
Druzhba	155	<0.1	<1.1	<4.4	6.17	30	609	304.5	304.5	0.03	1.000
Table grapevine cultivars (white)											
Super early Bolgar	70	<0.1	<1.1	<4.4	5.61	46	457	228.5	228.5	0.02	1.000
Prista	129	0.1	<1.1	<4.4	5.38	60	718	359.0	359.0	0.03	1.000
Ryahovo	142	0.1	<1.1	<4.4	5.13	83	652	326.0	326.0	0.03	1.000
Garant	256	0.2	<1.1	<4.4	5.30	64	884	442.0	442.0	0.04	1.000
Table grapevine cultivars (red)											
Dunav	44	0.1	<1.1	<4.4	5.08	83	528	264.0	264.0	0.02	1.000
Velika	208	<0.1	<1.1	<4.4	5.14	73	616	308.0	308.0	0.03	1.000
Misket plevenski	183	0.1	<1.1	<4.4	5.15	83	796	398.0	398.0	0.03	1.000
Other grape products											
Wine (Otelo)	-	9.8	11.62	2.9	3.72	98	3,340	1,670	1,690	0.16	1.000
Vinegar (Otelo)	-	8.0	11.22	2.8	3.65	92	5,590	2,790	2,810	0.28	1.001
Fruit juice (Velika)	62	14.8	n.d.	n.d.	3.75	230	2,140	1,430	1,090	0.11	1.075
Fruit juice (Seper early Bolgar)	57	17.7	n.d.	n.d.	3.76	234	1,910	1,297	986	0.09	1.068
Fruit juice (Prista)	59	15.4	n.d.	n.d.	3.72	233	1,612	1,082	812	0.08	1.055

Source: own data.

Table 2. Summary statistics

Variable	Observations	Obs. with missing data	Obs. without missing data
Acidity pH	15	0	15
Min.	Max.	Mean	Std. Dev.
5.080	6.170	5.564	0.348

Source: own calculations with XL Stat [5].

Redox potential Eh, mV is an important physics-chemical parameter. It is essential for redox oxidizing ability of the studied solution. It is visible that the oxidizing ability of the sap is very high. As smaller is the measured Eh, the danger from oxidizing is higher. The very healthy parameters usually have negative values as the sap from grape variety Otelo has Eh=-50.

Table 3. Descriptive statistics for the intervals

Lower bound	Upper bound	Frequency	Relative frequency	Density
5.000	5.254	20.5	0.231	0.910
5.254	5.508	21.54	0.243	0.956
5.508	5.762	22.52	0.254	1.000
5.762	6.016	11.81	0.133	0.524
6.016	6.270	12.31	0.139	0.547

Source: own calculations with XL Stat [5].

Fig. 2 presents pH-Eh water diagram and where the studied solutions are located, according to their pH and Eh and which processes are usual for their, what may we expect as behavior of the solutions. All of them are located at the lower part of the middle area between line a) and line b).

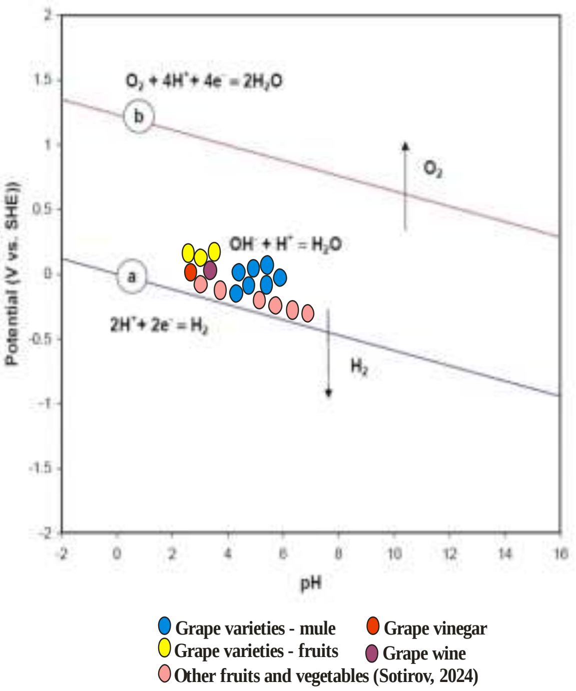


Fig. 2. Pourbaix pH-Eh water diagram for the spring sap, grape juices, wine and vinegar
Source: [1]; [7]-provided data for fruit and vegetable juices.

All types of cold-pressed juices – grape as well as apple, orange, strawberry, cherry, melon, carrot, and etc. are located directly in the entire side of the line a) in the middle part of the diagram, which is characterized with loosing of hydrogen and oxidizing of the liquid. Because of this reason, measured in situ at the terrain some of the saps had high amount of ascorbic acid (Vitamin C), which oxidized very quickly to zero. The conclusion, which is necessary after a year of observation and experiments, is that the addition of ascorbic acid as an antioxidant in the soil passes into the plant through the roots and further supplies hydrogen to the very delicate balance in the grape sap and supports the physiological processes of the plant.

Other important measured parameters of the spring grape sap is the Electroconductivity, Total Dissolved Solids and Salt content (Fig. 3). It presents TDS-Salt diagram. At this diagram the sap liquid is situated exactly on the line of salt situation. Here the ratio between TDS and Salt – TDS/Salt = 1 or TDS=Salt. It is valid for all studied samples. For comparison is shown the place of other grape products as juice, wine and vinegar. Grape juice and all other investigated in previous studies fruit and vegetable juices are above the line of saturation of the salts where Ratio TDS/Salt>1, but the products of grape as wine and vinegar have little higher salt content or ratio TDS/Salt<1. Main decision is that he grape sap consists salt on the level of saturation.

Specific gravity of the water is S.G.=0.999, the sap has S.G.=1.000 and wine, but the vinegar has specific gravity 1.001.

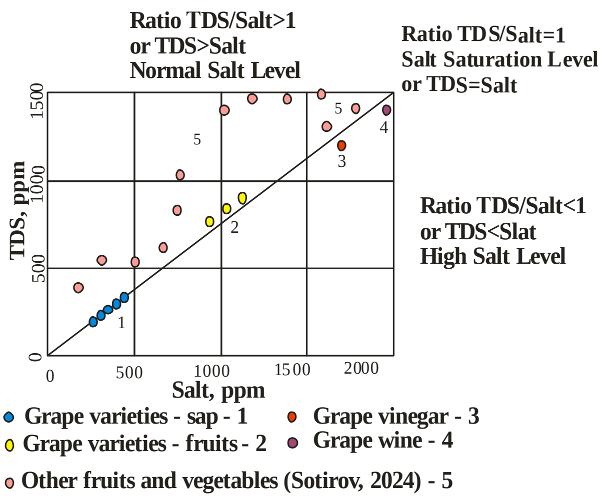


Fig. 3. TDS-Salt Diagram of the spring sap, grape juices, wine and vinegar
Source: own diagram.

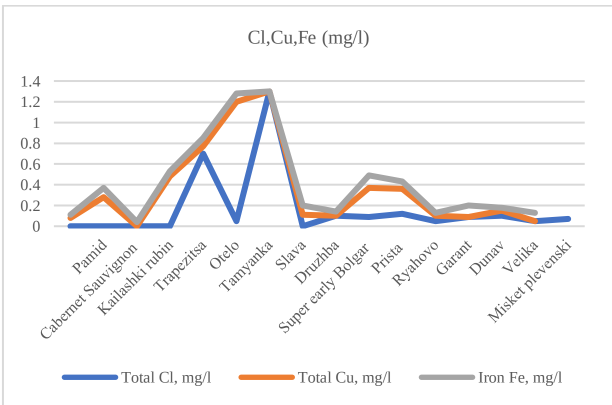


Fig. 4. Diagram of the distribution of the Cu, Fe, and Cl by cultivars
Source: own data Excel.

Several other parameters were measured also presented in Table 4 as Cl, Fe, Cu, which appears as trace elements in low amount, but they are connected and related each other, which might be seen on Fig. 4. Sometimes the Cl is connected with the Cu and sometimes with the Fe, as in these cases it has opposite relation with the Cu. Mainly white wines are connected with high content of Cu and Cl. Fe content is almost stable for all samples.

Figure 5 shows the hardness different and total alkalinity ($CaCO_3$) distributed by the samples of the studied sap. The alkalinity of water is calcium carbonate equivalent or mg of $CaCO_3$/l equivalent. Hardness of the water is the sum of the concentrations of positively-charged ions (cations) with more than one positive charge, such as Ca^{++}, Mg^{++}, Fe^{++}, and Fe^{+++}. As we may expect both parameters are very dependent each other and has almost matching graphs. Sap investigated is threated as drinking water, according to requirements described by [6] Fig. 5.

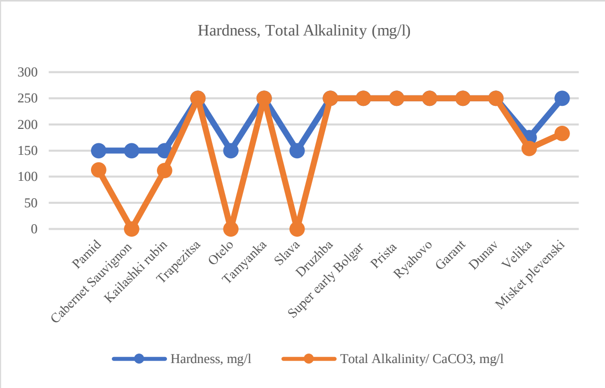


Fig. 5. Diagram of the distribution of the Hardness and Total Alkalinity by cultivars; 0-no data
Source: own data Excel.

Table 4. Physico-chemical parameters of spring sap of different grape cultivars, n.d.-no data

Cultivar	Total Cl. mg/l	Cianuric acid CYA mg/l	Hardness mg/l	Total alkalinity, $CaCO_3$, mg/l	Total Cu, mg/l	Iron, Fe, mg/l	Ascorbic acid, C vitamin	Nitrate NO^2, NO^{2-N}, mg/l	Nitrite NO^3, NO^{3-N}, mg/l
Cultivars for red wines									
Pamid	<0.05	14	150	113	0.08	0.03	200	50	<1
Cabernet Sauvignon	n.d.	21	150	n.d.	0.28	0.09	50	50	<1
Kailashki rubin	<0.05	20	150	112	<0.05	0.04	50	50	<1
Trapezitsa	0.70	15	>200	>200	0.48	0.05	0	50	<1
Otelo	0.05	14	150	n.d.	0.07	0.08	0	50	<1
Cultivars for white wines									
Tamyanka	1.30	7	>200	>200	1,15	0.08	0	50	<1
Slava	n.d.	20	150	n.d.	n.d.	n.d.	0	50	<1
Druzhba	0.10	10	>200	>200	0.11	0.09	0	50	<1
Table grapevine cultivars (white)									
Super early Bolgar	0.09	5	>200	>200	<0.05	0.04	0	50	<1
Prista	0.12	18	>200	>200	0.28	0.12	0	50	<1
Ryahovo	0.05	6	>200	>200	0.24	0.07	0	50	<1
Garant	0.09	17	>200	>200	0.05	0.03	0	50	<1
Table grapevine cultivars (red)									
Dunav	0.10	13	>200	>200	<0.05	0.11	0	50	<1
Velika	0.05	14	175	154	0.05	0.03	0	50	<1
Misket plevenski	0.07	19	>200	183	<0.05	0.08	0	50	<1
Other grape products									
Wine (Otelo)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Vinegar (Otelo)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0	n.d.	n.d.
Fruit juice (Velika)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Fruit juice (Super early Bolgar)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Fruit juice (Prista)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Source: own data.

Content in the studied samples of spring grape sap vary between 5 and 21 mg/l as mean is 14 mg/las many cultivars has higher content of CYA (Table 4).

Cyanuric acid is a generalized concept of waste products of the chemical industry, often used in everyday life such as bleach, adhesives, disinfectants, paints, cosmetics, chlorine stabilizers (protect chlorine compounds from rapid decomposition of sunlight and UV light) and many others. It is invariably present as an infiltrate (leachate) in places where there are domestic landfills and sewers. It was first found in urine. It is difficult to form independently in nature and its presence is a good indicator of anthropogenic activity. The formula may be different, but generally can be summarized as $(CNOH)_3$ (1,3,5-triazine-2,4,6-triol). One of its analogues is melamine [3]. This is the end product of decomposition and is a strong pollutant. Estimated dietary exposure to melamine from use of cyromazine as a pesticide ranged from 0.04 μg/kg body weight per day to 0.27 μg/kg body weight per day [11].

Below method sensibility were measured also several agroecological parameters (Table 5). Nitrate content is low - about 50 mg/l and Nitrite is about zero mg/l in the studied sap samples. Some other parameters were measured as radiation of the radioactivity of the sap and common radiation background for comparison. Results were normal for the region and crop products about 0.12-0.18 μSv/h. Obviously the plants adsorbs only the necessary elements [2] or there is no contamination of the soil with the elements given in Table 5 [9].

Table 5. Summary data of all studied grape cultivars about some agroecological parameters

Nitrate NO^2/NO^{2-} ^{N}, mg/l	**Nitrite NO^3, NO^{3-} ^{N}, mg/l**	**Radiation. sap, μSv/h**	**As, mg/l**	**Pb, mg/l**	**Br, mg/l**	**Fl, mg/l**	**I, mg/l**
50	1	0.12	0	0	0	0	0

Source: own data.

Economic impact of the study.

Monitoring of the grapevine sap for physico-chemical and agro-ecological parameters helps for corrections of the quality and quantity of the grape crop during time of ripening. Controlling the water-salt balance in the sap helps to be taken measures against salinization of the soils, it does not matter the reason: dry period and concentration of the salts or flooding and increasing of the sodium and respectively halite content into the soil. The study proved that the ratio Total Dissolved Solids (TDS)/Salts=1 in the grapevine sap in normal conditions is exactly 1 or TDS=Salt. When the salt content is higher, causes salinization of the fruits and worsening of the quality crop or full absent of crop. Because of high rain amount during early summer 2023, result was several times lower crop was received and with bed quality and short durability. Corrective measures are: is salinity is high during a dry period – artificial irrigation is necessary. If salinity is high after wet period and flooding of the terrain – using of gypsum mineral fertilizer is needed to be added in the soil. By this way the sodium drainages deep below the roots and does not block them. Moreover the salt content in the salts leads to higher content of the salt in the fruits, which harm not only fruits, but also all products processed by the fruits as whine, because of beginning of liberation of hydrogen (see Fig. 2) or oxidation processes are available.

CONCLUSIONS

As a result of the present study, the following conclusions have been drown:

The main results emphasized that the grape sap during spring 2024 is a stable salt-water solution with values of pH and Eh almost in the middle of the stable water area of the Pourbaix pH-Eh water diagram, out of values of liberation of hydrogen or oxygen where corrosion process starts, or it is a process that involves electrochemical as well as chemical reactions, which impact negatively to the grapevine crop. Other result is that the grapevine spring sap does not consist Pb, As, and high radioactivity. Third result is that the roots of the grapevine cultivars may produce ascorbic acid or also the plant may adsorb ascorbic acid through the roots. The sap of grape cultivars with established high amount of Ascorbic acid into them as Pamid, Cabernet Sauvignon and Kaylashki Rubin are less prone to oxidation or they have reservoir of hydrogen which reacts preferentially to oxygen from the environment and thus protects sap and fruit juices from oxidation.

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