Chapter 5

Polyphenols of Red Grape Wines and Alcohol-Free Food Concentrates in Rehabilitation Technologies

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Additional information is available at the end of the chapter

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Abstract

The chapter presents the results of development and testing of products, rich in polyphenols (wine, drink, extract, food grape concentrate), which has a high antioxidant capacity, and which was confirmed by research both in vitro and in vivo. It was shown that oral intake of innovative products block the development of metabolic syndrome and ischemic myocardial damage in experimental animals. Clinical studies have proved, that patients with coronary heart disease and hypertension can significantly improve their health conditions, if they add innovative products saturated with grape polyphenols into their diet, during treatment at sanatorium or resort. The chapter estimates the vectors of development in viticulture and winemaking industry for further production of healthy food for the population.

Keywords: grape polyphenols, wine, wine beverage, extract, grape food concentrates, innovative products, antioxidant capacity, metabolic syndrome, ischemia

1. Introduction

Polyphenols of cultivated grape (*Vitis vinifera*) are famous for their biologically active features, which give them broader potential in prophylactic nutrition [1–7]. In this respect, they were included in "Russian Standards" as functional ingredients determining both antioxidant- and cardiovascular-supported effects [8, 9].

Grape polyphenols are subdivided into flavonoid and nonflavonoid phenols [2–4]. Flavonoid polyphenols include anthocyanins, catechins, proanthocyanidins, and quercetins. At the same time, anthocyanins contain mainly malvidin, delphinidin, and peonidin glycosides. (+)-D-catechins, (−)-epicatechin, and (−)-epicatechin-3-O-gallate were found. In quercetin groups, quercetin itself and quercetin glycosides were determined. Proanthocyanidins, or polymerized catechins-oligomers (2–6 units) and tannins (7 and more units), compose up to 95% of polyphenols in red grape wines and polyphenolic concentrates. Non-flavonoid polyphenols include phenolic acids - trans-caftaric acid, trans-coutaric acid, gallic acid, syringic acid, also stilbenes: trans-resveratrol and viniferin as well.

Red grape wines and food concentrates rich in polyphenols contain significant amounts of high-molecular phenolic compounds, consisting of the named proanthocyanidins, their condensates with anthocyanins and protein-tannate labile agglomerates with antioxidant potential. Among wide spectrum of the anhtocyanins biological activity, ability to increase elasticity of blood vessels and improve eye vision is remarkable [10, 11]. Catechins and their polymerized forms—proanthocyanidins are the most powerful antioxidants, with activity exceeding that of C and E vitamins, and are able to inhibit prostaglandins' synthesis, being catalyzed by cyclooxygenase-2. It leads to the inhibition of inflammatory processes [12]. (−)-Epicatechin and (−)-epigallocatechin gallate are able to induce tumor cells' apoptosis [13]. Oligomeric proanthocyanidins, coming into blood, delay low-density lipoprotein oxidation in blood plasma, preventing cardiovascular pathology [14]. Besides this, they decrease cholesterol concentration, preventing development of atherosclerosis. [15]. Tannins, without penetration into blood, nevertheless, normalize intestinal microflora due to their tanning features [16].

Quercetin and quercetin glycosides as well as trans-resveratrol improve both elasticity and permeability of blood capillaries and coronary flow as well [17]. Trans-resveratrol activates cells' regulatory processes leading to apoptosis of damaged or abnormal cells and, hence, demonstrates antineoplastic activity [18].

Total polyphenols of grapes show high antioxidant activity by 2 orders of magnitude greater than the index of antioxidant activity in human's blood serum [2].

Positive experimental and clinical results are known due to the usage of total polyphenols of grapes in nonalcoholic polyphenolic food concentrate "Enoant," reducing the intoxication in a case of cytostatic therapy by cisplatin [19, 20].

American dietologists in order to reduce cardiovascular risk recommend red grape wines with total polyphenol concentration of 2.5 g/L in the dosage of 150–300 mL/day [21]. Moderate and regular consumption of red wines is favorable for the prevention of cardiovascular disaster [22–25]. At the same time, it was shown that ethanol consumption, exceeding 31 mL/day, affects circulatory system negatively. [26–28]. In this connection, it becomes essential to determine both effective and safe dosages of red grape wines, which besides polyphenols contain from 9 to 15% of ethanol. The solution of the mentioned problem is relevant both for scientific and practical enotherapy, in order to prove its effective side. Besides, standard winemaking technologies do not set norms for grape polyphenol content. This complicates the ration of daily wine consumption according to recommended norms of biologically active polyphenols about 505 mg/day [29].

2. Aims of the present study

The aims of this study are as follows:

1. Monitoring of quantitative and qualitative composition of polyphenols and antioxidant capacity of the traditional dry still red wines and grape food concentrates.
2. Monitoring of quantitative and qualitative composition of polyphenols and antioxidant capacity of the sparkling wines.
3. Evaluation of biological activity of both dry still red wines and sparkling wines using experimental models in vivo.
4. Clinical testing of experimental products rich in red grape polyphenols.
5. Clinical probation of products rich in red grape polyphenols at sanatorium rehabilitation of cardiovascular diseases.

The study was performed using the Crimean and Kuban winemaking raw materials.

2.1. Monitoring of quantitative and qualitative composition of polyphenols and antioxidant capacity in the traditional dry still red wines and grape food concentrates

Industrial samples of Cabernet Sauvignon, Saperavi, Merlot wines and grape food concentrates "Enoant," "Enoant Premium," "Fenokor" were applied for the study. Producers used the samples of 2004 vintage year [30].

Quantitative and qualitative compositions of polyphenols in juices, wines, polyphenol extracts were estimated by the method of high-performance liquid chromatography with diode array detector (LC-DAD) using a chromatographic system (Agilent 1100 series; Agilent Technologies Inc.), an autosampler, and a control module. For the separation of substances, reversed-phase C18 column (Zorbax SB, 3.5 μm, 2.1, and 150 mm; Agilent Technologies Inc.) was used. A gradient solvent system was used consisting of 0.6% aqueous solution of trifluoroacetic acid (solvent A) and water/methanol/trifluoroacetic acid, 30:70:0.4, v/v. (solvent B). The elution profile had the following proportions (v/v) of the solvent B: 0 min, 8%; 0–8 min, 8–38%; 8–24 min, 38–100%; 24–29 min, 100%; 29–33 min, 8%. The solvent flow rate was 0.25 mL/min. The column temperature was fixed at 45°C and the injection volume was chosen to be 2 μL. Quantitation was performed using an external calibration curve monitoring peak areas at 280 nm for gallic acid, (+)-D-catechin, (−)-epicatechin, and proanthocyanidins; 313 nm for the derivatives of hydroxycinnamic acids; 371 nm for quercetin; and 525 nm for anthocyanins, respectively. Compounds found in the study were identified by comparison to standards as well as by comparison with standards previously characterized on the HPLC system by both relative elution times and spectral matching. [12, 14, 31]. The content of anthocyanins was determined in terms of oenin chloride, the contents of trans-caftaric acid in terms of caffeic acid, and the content of polymeric and oligomeric proanthocyanidins produced in terms of (+)-D-catechin. To ensure uniformity of results, the content of substances was calculated in mg/kg dry pomace.

Standards for each phenolic class were prepared from commercial sources. Gallic acid, caffeic acid, (+)-D-catechin, oenin chloride, quercetin dihydrate, isoquercitrin was obtained from Fluka (Fluka Chemie AG, Switzerland) and trans-resveratrol, (-)-epicatechin, syringic acid was obtained from Sigma-Aldrich a (Sigma-Aldrich, Switzerland).

The mass concentration of total polyphenols in wine and concentrates was determined by a colorimetric method by Folin-Ciocalteu reagent [32]. Grape food concentrates were diluted 100 times. One milliliter of the tested solution was placed in a volumetric flask with a capacity of 100 mL, adding 1 mL of Folin-Ciocalteu reagent and 10 mL of sodium carbonate solution; it was adjusted to the mark with distilled water at a temperature of 20 ± 0.5°C and stirred.

In 30–40 min, the optical density of the solutions was measured in 10-mm cuvette, at a wavelength of 670 nm against control solution on the "CFC-2" photoelectric colorimeter (ZOMZ, Russia). The value of the mass concentration of phenolic substances in mg/L for gallic acid was determined according to the calibration curve. The result was taken as the arithmetic mean of two parallel definitions, with permissible difference between them not exceeding (measuring range 0.1–90 g/L) 5%.

For the evaluation of antioxidant activity of wine and concentrates, amperometric method was used for measuring mass concentrations of antioxidants according to the standard antioxidant trolox on the device "Tsvet-Yauza 01-AA" (NPO "Khimavtomatika," Russia) GOST R 54037 [33]. All determinations were carried out in three replicates. The research results were processed by standard methods of mathematical statistics [34].

The obtained experimental data on qualitative and quantitative composition of polyphenols, indicator of antioxidant capacity for still dry red wines, and food concentrates of polyphenols are presented in **Tables 1** and **2**.

As shown in **Table 1**, qualitative composition of polyphenols in red wines, produced in the Crimea and the Kuban areas from Cabernet Sauvignon, Merlot, Saperavi grape varieties, is represented by anthocyanins, quercetin-3-O-glucoside, quercetin, (+)-D-catechin, (−)-epicatechin, trans-caftaric acid, trans-coutaric acid, gallic acid, syringic acid, oligomeric and polymeric proanthocyanidins. The proanthocyanidins compose 94–95% of the total amount of polyphenols in the Crimean wines and 75–86% in the wines of the Kuban. The polyphenols in red wines are identical in their composition to polyphenols of Cabernet Sauvignon, found in grape pomace, forming the total polyphenols of wine [35].

However, it should be noted that trans-resveratrol detected in the concentration 9–92 mg/kg of dry weight pomace, is almost not present in red wines. The concentration of total polyphenols in the wines of the Crimea and the Kuban region varies in the range of 2.8–4.2 g/L (measured by high-performance liquid chromatography) and 3.8–4.5 g/L (measured by Folin-Ciocalteu), which exceeds the average content of phenolic compounds in red wines (2.5 g/L) recommended to reduce the risk of cardiovascular pathology [21]. Indicator of antioxidant capacity of red grape wine corresponds to 2.36–2.75 g/L standard antioxidant trolox, which indirectly proves high biological activity of red wines.

Index	Product manufacturers					
	Brand					
	"Massandra"			"Kubanvino"		
	Cabernet	**Merlot**	**Saperavi**	**Cabernet**	**Merlot**	**Saperavi**
Anthocyans, mg/L						
Total anthocyans	20.3	23.8	23.4	133.3	167.5	556.2
Flavones, mg/L						
Quercetin-3-O-glycoside	8.5	15.9	11.5	15.7	36.9	9.8
Quercetin	2.8	1.6	1.2	0.3	4.1	0.7
Flavan-3-ols, mg/L						
(+)-D-catechin	34.7	44.8	26.8	60.8	83.5	58.6
(−)-Epicatechin	34.5	47.4	29.7	52.9	78.8	71.2
Oxycinnamic acids, mg/L						
Trans-caftaric acid	45.6	58.0	44.3	29.9	52.7	69.6
Trans-coutaric acid	7.5	10.0	7.4	3.5	5.4	11.8
Oxybenzo acids, mg/L						
Gallic acid	39.3	42.6	33.8	78.1	67.8	63.0
Syringic acid	7.0	5.3	9.0	8.4	4.0	4.3
Proanthocyanidins, mg/L						
Oligomeric	187	222	200	221	222	212
Polymeric	3045	3723	3525	2068	2072	2380
Integrated indices, g/L						
Total phenolic substances (by HPLC)	3.43	4.2	3.91	2.67	2.79	3.44
Total phenolic substances (by Folin-Ciocalteu)	4.35	4.56	4.25	3.89	3.85	4.13
Antioxidant capacity (trolox)	2.36	2.75	2.38	2.37	2.49	2.69

Table 1. Qualitative and quantitative composition of polyphenols in industrial samples of red grape wine.

The potential technological supply of polyphenols in the grape pomace, being extracted by water-alcohol extraction at 50% moisture, is 60 g per 1 kg of pomace [35]. It means that it retrieves about 40% of polyphenols in wet grape pomace based on the concentration of polyphenols in wine (4.5 g/L) in winemaking traditional "red" method.

Thus, a large part of the total polyphenols in the production of red wines remains in the pomace, which leads to significant losses of grape polyphenols, valued at the market in the range of 2.0–2.5 USD per 1 g.

Index	Enoant	Enoant Premium	Fenokor
Total anthocyans, mg/L	18.9 ± 0.4	28.5 ± 0.6	—
Flavones, mg/L			
Quercetin-3-O-glycoside	3.1 ± 0.1	3.5 ± 0.1	15.4 ± 0.03
Quercetin	49.6 ± 1.1	81.2 ± 1.1	10.2 ± 0.2
Flavan-3-ols, mg/L			
(+)-D-catechin	177.6 ± 4.0	208.5 ± 5.1	1752.6 ± 35.1
(−)-Epicatechin	118.4 ± 2.7	127.3 ± 3.1	1374.2 ± 27.5
Oxycinnamic acids, mg/L			
Trans-caftaric acid	11.7 ± 0.3	16.9 ± 0.4	—
Trans-coutaric acid	1.8 ± 0.0	2.4 ± 0.1	—
Oxybenzoic acids, mg/L			
Gallic acid	341.1 ± 7.7	465.2 ± 11. 3	1119.2 ± 22.4
Syringic acid	22.6 ± 0.5	26.2 ± 0.6	—
Proanthocyanidins, mg/L			
Oligomeric proanthocyanidins	603 ± 14	1614 ± 39	4598 ± 92
Polymeric proanthocyanidins	28,155 ± 634	38,436 ± 932	172,662 ± 3455
Integrated indices			
Total phenolic substances (by HPLC), g/L	29.50 ± 0.70	41.01 ± 1.00	181.53 ± 3.6
Total phenolic substances (by Folin-Ciocalteu), g/L	18.51 ± 0.49	21.81 ± 0.59	82.69 ± 2.29
Antioxidant capacity (trolox), g/L	24.72 ± 0.73	36.48 ± 0.92	196.22 ± 4.92

Table 2. Qualitative and quantitative composition of polyphenols in industrial samples of grape food concentrates.

The possibility of deeper extraction of total polyphenols from pomace and other secondary raw materials of industrial grape processing is being implemented in innovative products containing polyphenols as the target components of biological activity. The number of such products, produced in different countries, including Russia, involves alcohol-free food concentrate of grape polyphenols. Their composition is shown in **Table 2** [30].

Comparative analysis of the experimental data presented in **Tables 1** and **2** shows that the integral indicators of nonalcoholic grape food concentrates by an order of magnitude greater than similar data of red grape wines. At the same time, “Fenokor,” derived from grape seed, lacks monomeric anthocyanins, oxycinnamic acids, and syringic acid. However, the qualitative composition of polyphenols in “Enoant” and “Enoant Premium” produced from the pomace of Cabernet Sauvignon variety demonstrates no difference from the composition of polyphenols in red wines.

As in red grape wines, in the food concentrate of grape polyphenols, only traces of trans-resveratrol are found, but in "Fenokor," the proportion of proanthocyanidins to the total amount of polyphenols increases up to 97.6%.

Apart from certain differences in the qualitative composition of polyphenols in red wines from different varieties of the Crimea and Kuban and concentrates, obtained from pomace and grape seed, approximation between the mass concentration of total polyphenols of grapes and indicator of antioxidant capacity of wines and concentrates is observed with high correlation coefficient R = 0.9952 [30]:

$$Y = 0.536 + 0.139X + 0.0804\,X^2 - 0.0006\,X^3 \quad (1)$$

where Y is the value of antioxidant capacity, g/L in terms of trolox and X is the mass concentration of total polyphenols in grapes, g/L reagent Folin-Ciocalteu.

Eq. (1) is valid in the limits of variation of 1,0-82,7 g/L and phenolic substances 0,76 - of 196,2 g/L in terms of antioxidant capacity.

Eq. (1) allows assessing the antioxidant capacity of products by the concentration data of grape polyphenols in wine and concentrates.

The given monitoring data prove that both polyphenols of quiet table red wines of Crimea and Kuban region and alcohol-free polyphenol concentrates, "Enoant" and "Fenokor," are qualitatively identical. Their biological activity potential is represented by the dependence of antioxidant capacity indicator from the concentration of total polyphenols in products. This dependence is supposed to be the general both for the wines and for concentrates.

2.2. Monitoring of the quantitative and qualitative composition of polyphenols and indicator of sparkling wines antioxidant capacity

In reference sources, there is absence of information about the qualitative and qualitative composition of polyphenols, and indicator of antioxidant capacity of sparkling wines in Russia. Apparently, as in the case with still grape wines, it is connected with lack of appropriate requirements in the regulations [36].

In this connection, the aim of our study was to determine the qualitative and quantitative composition of polyphenols and index of antioxidant capacity of sparkling grape wines, and evaluate the potential consumption of those as a product for healthy diet [37].

The object of our research was white, pink, red sparkling wines, delivered into the market network by their Russian manufacturers. All 26 samples of sparkling wine correspond to the regulated requirements of the normative document [36]. To determine the qualitative and quantitative composition of polyphenols and the index of antioxidant capacity in samples of sparkling wines, methods were applied, as described in Section 2.1.

Table 3 shows experimental data on the total amount of polyphenols and the level of antioxidant capacity in samples of sparkling wines.

No.	The name of the sample	Vintage year	Date of bottling	Mass concentration of phenolic substances in Folin-Ciocalteu in terms of gallic acid, g/L	Antioxidant capacity in terms of trolox, g/L
1	Wine sparkling brut white "Pinot Zolotaya Balka"	2016	June 06, 2016	0.29	0.505
2	Wine sparkling brut white "Chardonnay Zolotaya Balka"	2015	August 31, 2015	0.322	0.514
3	Wine sparkling semidry white "Zolotaya Balka"	2016	March 14, 2016	0.27	0.500
4	Wine sparkling semisweet red "Zolotaya Balka"	2015	August 07, 2015	1.744	0.933
5	Wine sparkling semisweet white "Zolotaya Balka"	2016	July 01, 2016	0.286	0.504
6	Wine sparkling brut white "Zolotaya Balka"	2016	June 01, 2016	0.284	0.505
7	Wine sparkling semisweet wine "Muscat Sparkling"	2015	August 06, 2015	0.416	0.536
8	Wine sparkling semisweet pink "Golden Beam"	2015	August 07, 2015	0.412	0.530
9	Wine sparkling semisweet "Muscat Sparkling"	2015	August 04, 2015	0.442	0.539
10	Wine sparkling semisweet white "Crimean" Trademark "Sevastopol sparkling"	2015	December 24, 2015	0.286	0.506
11	Wine sparkling semidry white "Crimean" Trademark "Sevastopol sparkling"	2015	December 25, 2015	0.304	0.511
12	Wine sparkling semidry white "Crimean" Trademark "Chersonese Tavrichesky"	2015	December 24, 2015	0.393	0.524
13	Wine sparkling brut white "Crimean" Trademark "Chersonese Tavrichesky"	2015	December 25, 2015	0.384	0.521
14	Wine sparkling semisweet red "Crimean" Trademark "Sevastopol sparkling"	2015	June 17, 2015	1.659	0.872
15	Wine sparkling aged brut red "Novyi Svet. Crimean sparkling"	2012	January 26, 2016	1.280	0.843
16	Wine sparkling aged semisweet red "Novyi Svet. Crimean sparkling"	2012	February 19, 2016	1.412	0.860
17	Russian champagne aged brut white "Novyi Svet"	2012	February 13, 2016	0.271	0.567

No.	The name of the sample	Vintage year	Date of bottling	Mass concentration of phenolic substances in Folin-Ciocalteu in terms of gallic acid, g/L	Antioxidant capacity in terms of trolox, g/L
18	Russian champagne aged white semisweet "Novyi Svet"	2013	February 12, 2016	0.289	0.560
19	Russian champagne aged semisweet pink "Novyi Svet"	2013	February 12, 2016	0.415	0.636
20	Russian champagne aged semidry white "Novyi Svet"	2012	January 27, 2016	0.284	0.588
21	Russian champagne aged semidry pink "Novyi Svet"	2012	February 18, 2016	0.330	0.607
22	Wine sparkling aged semisweet red "Premium Cabernet" Abrau-Durso	2009	November 29, 2014	2.346	1.420
23	Wine sparkling aged semisweet red "Premium Cabernet" Abrau-Durso	2009	January 10, 2015	0.313	0.580
24	Wine sparkling aged brut pink "Premium Pink"	2013	March 01, 2015	0.250	0.568
25	Russian champagne brut white "Abrau-Durso"	2013	October 06, 2014	0.207	0.566
26	Russian champagne semidry white "Abrau-Durso"	2013	December 24, 2014	0.231	0.593

Table 3. Quantitative and qualitative composition of polyphenols and indicator of sparkling wines antioxidant capacity.

Data analysis for the quantitative content of total polyphenols in grapes (**Table 3**) shows that in white sparkling wines, the massive concentration of polyphenols varies in the range from 0.21 to 0.42 g/L; in pink 0.31–0.44 g/L; and in red 0.84–2.35 g/L. The indicator of antioxidant capacity varies in the range from 0.51 to 1.42 g/L for trolox, increasing with higher content of polyphenols in sparkling wine. This dependence is well approximated by Eq. (1) obtained earlier for still red dry wines and concentrates of grape polyphenols (Section 2.1).

Thus, the range of application of Eq. (1) is expanded at the lower end to 0.21 g/L total polyphenols in Folin-Ciocalteu and to 0.51 g/L in trolox index of antioxidant capacity. Of all tested brands, white sparkling wines are characterized by the low content of grape polyphenols and, consequently, low values of the antioxidant capacity indicator.

As shown in our study of the qualitative composition of polyphenols, whole spectrum of anthocyanins is absent in white sparkling wines, due to the specific technology of its production, which greatly diminishes biologically active substances in sparkling wines.

Table 4 shows experimental data obtained in the qualitative and quantitative composition of polyphenols of grapes in pink and red sparkling wines.

Index	Wines sparkling pink					Wines sparkling red				
	Samples No.					Samples No.				
	8	**9**	**19**	**21**	**23**	**4**	**14**	**15**	**16**	**22**
Anthocyans, mg/L										
Total Anthocyans, mg/L	1.4	2.2	0.6	0	0	49.1	4.9	10.1	8.2	2.7
Flavones, mg/L										
Quercetin-3-O-glycoside	0.7	2.4	0.3	2.2	2.1	6.9	3.0	1.5	1.7	3.4
Quercetin	3.1 ± 0.1	3.5 ± 0.1	15.4 ± 0.03	0.1	0.2	0.3	0.5	0.2	0.4	1.2
Flavan-3-ols, mg/L										
(+)-D-catechin	5.1	4.6	10.1	6.3	18.1	29.2	15.4	20.0	16.1	18.1
(−)-Epicatechin	7.7	10.5	6.8	6.8	2.5	34.5	18.9	16.2	15.7	8.4
Oxycinnamic acids, mg/L										
Trans-caftaric acid	28.1	68.8	18.6	30.1	12.1	75.4	41.9	36.1	35.7	6.0
Trans-coutaric acid	1.8	1.5	2.1	3.2	1.6	2.9	2.2	5.0	4.7	1.4
Oxybenzoic acids, mg/L										
Gallic acid	17.7	11.1	13.3	10.1	6.4	34.9	40.5	33.6	34.9	66.8
Syringic acid	1.8	1.1	10.1	0.3	—	5.7	4.5	3.0	3.2	2.4
Proanthocyanidins, mg/L										
Oligomeric	144.0	116.7	46.0	63.0	76.0	185.7	151.0	109.0	104.0	105.0
Polymeric	470.7	240.0	240.0	236.0	309.0	2555.2	2353.6	1243.0	1412.0	3021.0
Integrated indies, g/L										
Total phenolic substances (by HPLC)	0.68	0.68	0.31	0.33	0.40	2.68	2.64	1.41	1.57	3.2
Total phenolic substances (by Folin-Ciocalteu)	0.41	0.41	0.41	0.33	0.31	1.77	1.66	1.28	1.41	2.30
Antioxidant capacity (trolox)	0.53	0.54	0.64	0.61	0.58	0.93	0.87	0.84	0.86	1.42

Table 4. Qualitative and quantitative composition, antioxidant capacity of pink and red sparkling wines.

Composition of phenolic complex in both red sparkling wines and still dry red wines include anthocyanins, flavones, flavan-3-ols, hydroxy-cinnamic and hydroxybenzoic acid, oligomeric and polymeric proanthocyanidins. Proanthocyanidins are 91.5–96.8% of total amount of total polyphenols in red sparkling wines; this content is several times lower than that in still dry red wines. However, index of antioxidant capacity of red sparkling wines indirectly indicates the potential biological activity. The index is at a high level and is 0.84–1.42 g/L by trolox, which allows to assess biological effects of this product in vivo in experimental models.

Hence, there is an obvious correlation between antioxidant capacity indicator and the concentration of total polyphenols in products. That fact in a case of red wines proves the high potential of their biological activity.

2.3. Evaluation of the biological activity of still dry red wines and sparkling wines on experimental models in vivo

The results of numerous epidemiological studies have shown that the consumption of grape polyphenols at a dose of 300–400 mg daily (with an estimated average of 2.5 mg/L of polyphenols in European red wines) reduces cardiovascular mortality in France by 36–56% if compared to that in the US, taking into account the same level of saturated fat consumption and cholesterol level in blood for both countries [38].

The biological activity of the total polyphenols of red grape wine in relation to cardiovascular pathology manifests itself in the cardioprotective effect which is achieved due to the following factors: the reduction of low-density lipoprotein in the blood, the inhibition of atherosclerotic transformation of the blood vessel walls, stability augmentation of cell membranes, prevention of cytolysis, and inhibition of platelet aggregation [3].

Protective properties of grape polyphenols allow us to avoid the risk of the development of arterial hypertension and atherosclerosis provoked by the metabolic syndrome which has become extremely widespread over the past few decades [39].

Biological activity of polyphenols in red still dry wines was evaluated using wine material of Cabernet Sauvignon variety with mass concentration of total polyphenols 2.5 g/L, by Folin-Ciocalteu on the model of metabolic syndrome induced by fructose feeding [39]. Experimental studies were carried out on 50 white male rats, with weight 180–200 g, and the age of 10–12 weeks. Animals of experimental groups with metabolic syndrome received standard food and 10% fructose solution as drinking water for 8 weeks. Animals of the control group received standard food and water. Fructose feeding for 8 weeks contributed to the development of metabolic syndrome: visceral adiposity, hyperglycemia, hypercholesterolemia, dyslipoproteinemia (metabolic syndrome group). Since the fifth week, the animals of the three experimental groups with correction of the metabolic syndrome received wine in the dose of 0.7 mL (300 mL of wine per 70 kg of body weight). Wine material was diluted with water, reaching a concentration of polyphenols as 0.5; 1.0; and 2.5 g/L. Each group consisted of 10 animals.

To assess biological activity, the concentration of products reacting with thiobarbituric acid (TBA-AP), peroxidase-like activity (PLA), catalase-like activity (CLA), an intracellular antioxidant superoxide dismutase activity (SOD) were studied in blood serum of the animals.

Statistical data processing was carried out using methods of variation statistics; the data were considered to be reliable at P < 0.05. Biochemical parameters obtained on model of the metabolic syndrome are shown in **Table 5**.

As shown in **Table 5**, the full normalization of biochemical parameters of blood in animals with metabolic syndrome, to the level of normal animals, was achieved by using wine materials containing 2.5 g/L of polyphenols, i.e., with a daily intake of 750 mg of polyphenols per 70 kg of body weight. It appears that this dose of the consumption of total polyphenols of grapes can be taken as an estimate in the determination of the cardioprotective effectiveness of polyphenols in the correction of the metabolic syndrome negative impact by dry red table wines.

The research into the biological effects of sparkling grape wine polyphenols was performed on the sample of the red sparkling wine (**Table 3**, sample No.22) with the mass concentration of phenolic substances of 2.35 g/L (Folin-Ciocalteu grade) and the trolox equivalent of antioxidant capacity of 1.42 g/L. The research was performed in compliance with the model of circulatory hypoxia [40, 41] on white male rats of Wistar line (25 animals) as its object, each aged 10–12 weeks and weighing 180–200 g. For the experiment, which lasted for 2 weeks, they were divided into four experimental groups.

The control group of animals (K) received standard food and drinking water. The animals of the second experimental group (K/P) received standard food and drinking water and were to undergo bloodletting within the first week of the experiment. Animals of the third experimental group (K/P + W) also underwent bloodletting in the first week of the experiment and received sparkling red wine diluted with water as a daily drink. Animals of the fourth experimental group (W) did not undergo bloodletting and received sparkling red wine diluted with water as a daily drink. Red sparkling wine was given to the animals of the third and fourth groups at a dose of 0.5 mL per 100 g of body weight, which correspond to 880 mg of total polyphenols per 70 kg of body weight. In the control and experimental groups of animals, blood samples for studies were collected by decapitation under ether anesthesia. Statistical data processing was carried out using methods of variation statistics, with the data considered to be reliable at P < 0.05.

Index	**Metabolic syndrome**	**Metabolic syndrome + correction**			**Control**
		Polyphenols concentration, g/L			
		0.5	**1.0**	**2.5**	
SOD, U/mL	116.12 ± 6.28	126.94 ± 5.54	130.55 ± 4.13	136.20 ± 5.28	136.43 ± 5.178
TBA-AP nM MDA/mL	178.11 ± 4.45	151.24 ± 15.2	143.08 ± 13.47	130.85 ± 4.69	118.43 ± 2.79
PLA mcM/gHb•s	2.75 ± 0.09	2.80 ± 0.12	3.28 ± 0.37	3.56 ± 0.13	3.11 ± 0.11
CLA mM/gHb•s	0.17 ± 0.01	0.18 ± 0.02	0.20 ± 0.02	0.23 ± 0.01	0.22 ± 0.01

Table 5. Biochemical indices of animals' blood in the correction of metabolic syndrome by red wines.

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The antioxidant capacity of the blood was estimated on the basis of the content of intracellular antioxidant enzyme superoxide dismutase (SOD), peroxidase-like activity (PLA), and catalase-like activity (CLA). The intensity of free radical lipid oxidation in the blood serum was assessed by the concentration of active products that react with thiobarbituric acid (TBA-AP).

The main results of the simulated hypoxia correction by the polyphenols of red sparkling wine are presented in **Table 6**.

As shown in **Table 6**, modeling of hypoxia by bloodletting had led to a marked increase in indicators of oxidative-antioxidant homeostasis of the blood of animals—TBA-AP, PLA, SOD indicators in the group with hypoxia (K/P) have increased if compared to the control group of healthy animals (K) that demonstrates a high level of free radical oxidation stress and exhausting of antioxidant systems. The group (K/P + W), treated with the polyphenols of red sparkling wine, demonstrates the decrease of TBA-AP by more than 8 times if compared to the same indicator group K/P with bloodletting. This index was decreased by 10 times in the group (W) which received only red sparkling wine without bloodletting.

Thus, the application of red sparkling wine polyphenols for the correction of free radical damage during hypoxia proved to be effective. The application of total polyphenols of red sparkling and quiet wines in dosages of 750–880 mg/kg on models of both metabolic syndrome and hypoxia confirms the high efficiency of the named substances. It allows stating the polyphenol concentration of 2.5 g/dm^3 adequate for the requested biological effects of red wines.

2.4. Elaboration of experimental products rich in red grape polyphenols

Reducing the risk of cardiovascular diseases and other abnormal body conditions becomes possible with a more extensive use of enotherapy potential, derived from the biological activity of the total polyphenols of red grapes, in health care practice. Despite numerous epidemiological, experimental and clinical studies, there is still no numerical index governing the content of total polyphenols in red wine varieties at a level that ensures the biological efficacy of wine as a product of functional purpose.

Given these circumstances, technical conditions and technological instructions for dry red table wine "Health," wine beverage "Health," and the extract of grape polyphenols were

Index	**Experimental groups**			**Control group**
	K/P	**K/P + W**	**W**	**K**
SOD, U/mL	233.68 ± 5.54	413.25 ± 9.79	345.55 ± 8.19	136.43 ± 5.178
TBA-AP nM MDA/mL	250.33 ± 5.92	28.56 ± 0.89	23.55 ± 0.78	118.43 ± 2.79
PLA mcM/gHb•s	5.98 ± 0.06	5.83 ± 0.06	6.84 ± 0.07	3.11 ± 0.03
CLA mM/gHb•s	1.94 ± 0.02	6.10 ± 0.02	3.68 ± 0.01	0.22 ± 0.01

Table 6. Biochemical indicators of animal blood in the correction of hypoxia by red sparkling wine polyphenols.

developed; an experimental batch of products was produced. The testing of experimental samples of the products for their biological activity in vitro and in vivo was conducted [42, 43].

Physicochemical characteristics governing the performance of these products include the increased amounts of phenolic compounds at a level of not less than 2.5 g/L for wine and beverage wine, and not less than 20.0 g/L for the extract of grape polyphenols.

Achieving a normalized indicator for the phenolic substances in wine is provided for by the traditional methods of mash fermentation; whereas in the wine beverage, blending wine material with an alcohol extract of grape polyphenols is allowed for that purpose. In the extract of grape polyphenols, the concentration of polyphenols is achieved by alcohol extraction, fermented pomace, and subsequent dealcoholization of the extract resulting in the concentration of alcohol not exceeding 15% vol.

Studies of experimental samples of wine, wine beverage, and the extract conducted in the season of grape processing of the vintage of 2015 showed that the content of polyphenols was 2.53–3.02 g/L in wine, 2.5–3.02 g/L in the wine beverage, and 21.5 g/L in the extract. Physicochemical characteristics of dry red table wine "Health," wine beverage "Health," and the extract of grape polyphenols are shown in **Tables 7** and **8**.

Normative index	**Sample***	**Actual index**		
		Grape variety		
		Cabernet Sauvignon	**Merlot**	**Saperavi**
Ethyl alcohol, % vol. from 10.5 to 15	1	10.8 ± 0.1	11.7 ± 0.1	12.0 ± 0.1
	2	10.9 ± 0.1	11.8 ± 0.1	11.9 ± 0.1
Sugar, g/L, no more than 4	1	2.5 0 ± 0.2	1.5 ± 0.2	2.7 ± 0.2
	2	2.5 ± 0.2	2.9 ± 0.2	2.8 ± 0.2
Titratable acids, g/L, at least 3.5	1	5.8 ± 0.1	5.3 ± 0.1	4.4 ± 0.1
	2	4.4 ± 0.1	4.5 ± 0.1	4.3 ± 0.1
Volatile acids, g/L, not more than 1.0	1	0.60 ± 01	0.53 ± 0.1	0.68 ± 0.1
	2	0.68 ± 0.1	0.68 ± 0.1	0.65 ± 0.1
Given extract, g/L, not less than 18.0 g/L	1	22.9 ± 1	20.5 ± 1	19.8 0 ± 1
	2	20.9 ± 1	20.0 ± 1	19.9 ± 1
Citric acid, g/L, not more than 1.0	1	0.10	0.19	0.10
	2	0.10	0.10	0.10
Total sulfur dioxide, mg/L, not more than 200	1	65.0	69.0	75.0
	2	75.0	75.0	74.0
Phenolic compound, g/L, not less than 2.5	1	2.53 ± 0.1	2.56 ± 0.1	3.02 ± 0.1
	2	3.02 ± 0.1	2.56 ± 0.1	3.00 ± 0.1

*1—Dry red table wine "Health" and 2—wine beverage "Health."

Table 7. Physicochemical characteristics of experimental samples of dry red table wine "health" and wine beverage "health".

Normative index	Actual index
Ethyl alcohol, % vol. from 10.5 to 15	10.6 ± 0.1
Titratable acids, g/L, at least 3.5	7.4 ± 0.2
Phenolic compound, g/L, not less than 2.5	21.5 ± 0.8
Mass fraction of dry substances, %, not less than 3.0	10.0

Table 8. Physicochemical characteristics of the experimental sample of extract of grape polyphenols.

The qualitative composition of polyphenols in experimental samples did not differ from the typical polyphenol composition of industrial samples of wines of the Crimea and Kuban (**Table 1**) and industrial samples of grape food concentrates (**Table 2**).

The index of antioxidant capacity in the experimental samples of wines and wine beverages corresponded to 1.51–1.72 g/L trolox equivalent and that of extract of grape polyphenols equaled 33.74 g/L trolox equivalent, which confirmed the high potential of the biological activity of experimental samples.

Evaluation of biological activity of experimental samples of red wine “Health” and the extract of grape polyphenols was performed in vivo on models of ischemic myocardial damage in rats. The conditions under which the animals were kept and the research experiments were carried out are in accordance with the requirements of international regulations “Guide for the Care and Use of Laboratory Animals” [43].

Investigations were carried out on 40 sexually mature male rats of Wistar line, each weighing 180–200 g. The animals were divided into 4 groups, with 10 animals in each group, 1 group was a control one and 3 others were experimental. Animals of experimental groups were given an aqueous solution of cobalt chloride ($CoCl_2$) for 7 days orally for the modeling of hypoxia and ischemic damage of the myocardium. For the correction of myocardial ischemia, experimental animals of two groups were administered:

- 0.25 mL/kg of the extract of grape polyphenols together with 0.5 mL/kg of water for 7 days (third experimental group);
- 2.5 mL/kg of red wine “Health” within 7 days (fourth group).

Seven days after decapitation of the animals, the components of free radical oxidation and antioxidants—TPK-AA, PLA, KLA, SOD—were detected in the serum.

Electron microscopical study of ischemic myocardial damage in heart sections of animals was carried out with the help of the electron microscope Selmi-125 at an accelerating voltage of 125 kV.

Biochemical indicators of blood on the experimental model of ischemia with cobalt chloride under the correction by polyphenols of the experimental produce are presented in **Table 9**. Statistical processing of the data was carried out by standard methods of variation statistics with the confidence probability of P = 0.95.

№	Index group	TBA-AP nM MDA/mL	CLA mM/gHb•s	PLA mcM/gHb•s	SOD, U/mL
1	Control	120.67 ± 4.52	0.21 ± 0.01	3.17 ± 0.13	140.25 ± 6.20
2	$CoCl_2$	231.25 ± 6.17	0.15 ± 0.01	2.16 ± 0.12	97.08 ± 5.10
3	$CoCl_2$ + grape polyphenols extract	152.23 ± 4.76	0.20 ± 0.02	2.57 ± 0.09	127.42 ± 4.38
4	$CoCl_2$ + wine beverage "Health"	167.80 ± 5.06	0.17 ± 0.01	2.54 ± 0.14	111.17 ± 5.18

Table 9. Biochemical indicators of blood of animals for the correction polyphenols products experimental model of ischemia with cobalt chloride ($CoCl_2$).

As shown in **Table 9**, cobalt chloride leads to a decrease in the activity of intracellular antioxidant enzyme superoxide dismutase (SOD), catalase (CLA), peroxidase (PLA), and an increase in free radical oxidation of lipids in the concentration of active products that react with thiobarbituric acid (TBA-AP). These negative effects are corrected by daily oral administration of the total red grape polyphenols with red wine and extracts of grape polyphenols in the amount of 448 and 376 mg per 70 kg of body weight. Noticeable correction has already been detected within the first week of grape polyphenol consumption.

Electron microscopical examination of slices of the hearts of animals revealed such marked pathological effects of cobalt chloride ($CoCl_2$) on the myocardium in sexually mature male rats as: intracellular edema, focal disintegration, fragmentation of muscle fibers, destruction of endothelial cells, and contractile cardiomyocytes, lysis of myofibrils, the development of anemia. The result of the influence of cobalt chloride on the heart of animals was the development of ischemic cardiomyopathy.

Morphological changes of the myocardium in the groups of animals with the correction by grape polyphenols reflect the trend toward the minimal volume of inflicted damage and the preservation of myocardial structure, manifested in the normalization of the cell structures and fibers of muscle tissue, which demonstrates the cytoprotective properties of total grape polyphenols that are promising for the prevention of cardiomyopathy.

The application of both experimental red wines and red wine drink "Health" with polyphenols concentration at least 2.5 g/dm^3 as well as alcohol-containing concentrate with polyphenols concentration at least 20 g/dm^3 had demonstrated the high efficiency in the model of myocardial ischemic damage. That opens up broad prospects of the clinical use of the named products.

2.5. The clinical testing of products rich in grape polyphenols at the health resort rehabilitation of cardiovascular diseases

The successful testing of the biological activity of total polyphenols in experimental samples of red wine and extracts of grape polyphenols in vivo on models of ischemic damage of myocardium allowed to apply them for clinical trials of cardioprotective activity at the health resort (sanatorium) "AI-PETRI" in Yalta from May to October 2016. Two groups of patients were formed: a group of patients with coronary heart disease (96 people) and a group of patients with hypertension (163 people). The research protocol was approved by the local Ethics Committee [42–44]. All patients received identical treatment as prescribed by the doctors of the institution. Additionally, they were given either red wine "Health" or the extract of grape polyphenols calculated as 10 mg of total polyphenols of grapes per 1 kg of body weight or an average of 280 mL of wine and 36 mL of extract per day.

Patients were examined both at the beginning and at the end of the course of treatment of 14 days. The functional state of the cardiovascular system, blood biochemical parameters, functional tests of the hemodynamic function of the heart, the state of lipid peroxidation and antioxidants in the blood, and the overall state of patients were studied.

The positive dynamics of the health condition was registered in all patient groups, but in the groups receiving the wine or the extract of grape polyphenols, a greater number of parameters were improved when compared with control groups.

The positive effect manifested itself to a greater extent in the rehabilitation of patients with a coronary heart disease. As shown in the histograms of **Figure 1**, the significant decrease in total cholesterol, atherogenic quotient and the amount of fibrinogen in the blood were registered, which corresponds to the reduction of both thrombosis and cholesterol plaque formation risk.

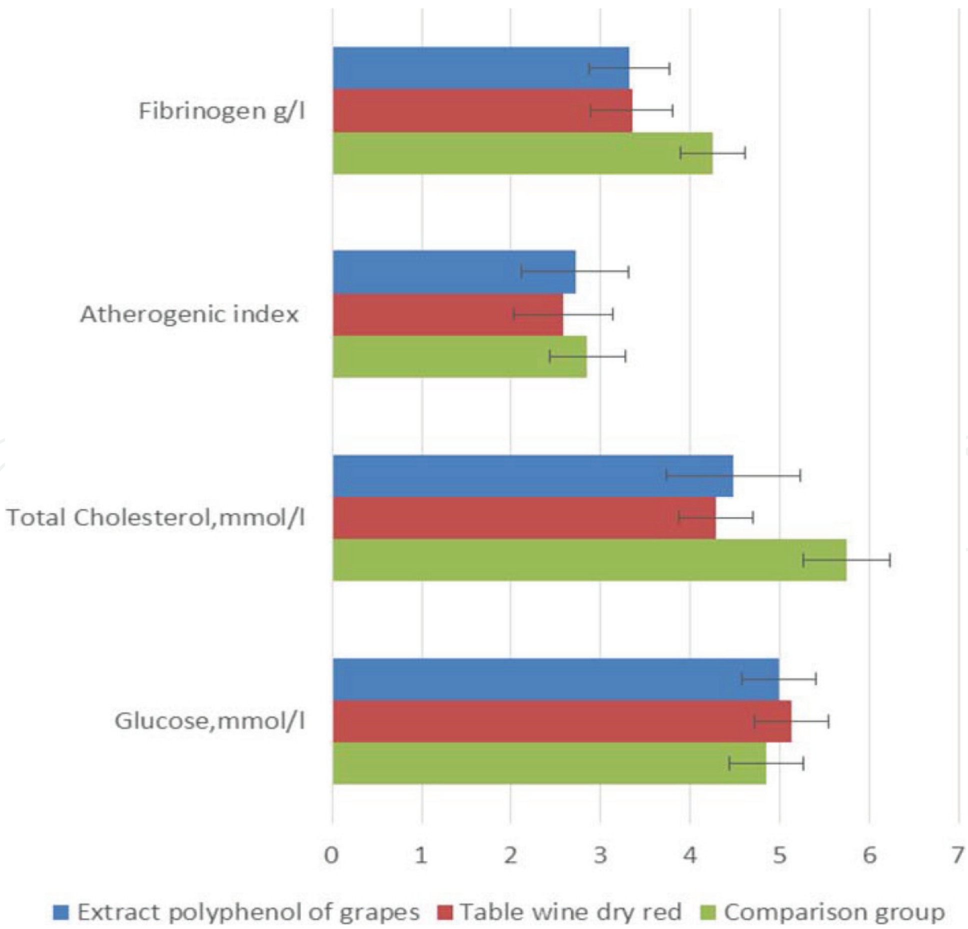

Figure 1. Changes in biochemical parameters of blood on the background of total polyphenols red wine extract in patients with coronary heart disease.

As clinically proved, the patients with the ischemic heart disease showed the twofold decrease in the need for nitroglycerin intake; the quarter of the patients had restrictions from any forms of physical activity removed; in more than 85% patients, there was both a marked decline in fatigue and the rise of tolerance to physical activity registered if compared to other patients who only received basic institutional treatment. The obtained results give us grounds to be optimistic about the possibility of using saturated total polyphenols of red grape wine and extracts of grape polyphenols for the rehabilitation of patients with a coronary heart disease and hypertension when receiving treatment at health resorts or sanatoria.

The successful rehabilitation of patients with ischemic heart disease and arterial hypertension was accomplished due to total polyphenols in red wines and polyphenols extracts despite the presence of alcohol in concentration of 10.6–12.0% ABV.

3. Conclusions

The polyphenol complex of red grape wines and grape concentrates, produced in Russia traditionally and by using innovative techniques, contains the identical groups of phenolic compounds and flavonoids, has an integral antioxidant capacity which increases with the rise of total polyphenol concentration in the products (Eq. (1)). It should be noted that 75.0–97.6% of total grape polyphenols are represented by oligomeric and polymeric proanthocyanidins.

The use of total polyphenols of grape red wines and grape concentrates calculated respectively as 376 mg, 448 mg, 750 mg, 870 mg per 70 kg of body weight in the experimental models of ischemia, hypoxia, and metabolic syndrome confirmed the high biological activity of the total polyphenols.

The possibility of successful rehabilitation of patients suffering from arterial hypertension and a coronary heart disease by taking total polyphenols of wine and the concentrate in the amount of 700 mg per 70 kg of body weight in the course of a 14-day health resort/sanatorium treatment was shown.

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