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Biochemistry

Phenolic Compounds of Wines from Georgian Autochthonous Grapes, Rkatsiteli and Saperavi, Prepared by Georgian (Kakhetian) Technology

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ABSTRACT. The qualitative composition and the quantitative content of phenolic compounds of wines from the Georgian autochthonous grapes, white Rkatsiteli and red Saperavi, prepared by Kakhetian technology are studied. According to the obtained data, in white wine Rkatsiteli the content of catechines is the following: (+)-catechine (32.6 mg/l), (-)-epicatechine (58.6 mg/l), (-)-gallocatechine (43.7 mg/l), and in red wine Saperavi: (+)- catechine (115.4 mg/l), (-)-epicatechine (29.5 mg/l), (-)-gallocatechine (174.4 mg/l), respectively. It is shown that a significant difference in the contents of catechines is observed between white and red wines. Red wine Saperavi contains 3.4 times and 4 times more (+)-catechine and (-)-gallocatechine, and 2 times less (-)-epicatechine than the white wine Rkatsiteli. Out of flavonols kaempferol (13.2 mg/l), quercetin (11.2 mg/l) and rutin (2.6 mg/l) were found in red wine Saperavi, while in white wine Rkatsiteli those compounds are absent. The white wine Rkatsiteli does not contain resveratrol, while in red wine Saperavi its amount is equal to 1.47 mg/l. Out of hydroxybenzoic acids the protocatechuic acid and gallic acid were not found in white wine Rkatsiteli, while in red wine Saperavi their amount is equal to 14.5 mg/l and 21.8 mg/l. Of hydroxycinnamic acids, in red wine Saperavi caffeic acid in the amount of 7.4 mg/l, and o-coumaric acid and syringic acid as traces were found. These acids were not found in the white wine Rkatsiteli. Thus, in qualitative composition and quantitative contents of phenolic compounds the red wine Saperavi considerably surpasses the white wine Rkatsiteli. *© 2012 Bull. Georg. Natl. Acad. Sci.*

Key words: *phenolic compounds, Georgian (Kakhetian) wines, catechines, flavonols, stilbenes, hydroxybenzoic acids, hydroxycinnamic acids, HPLQ.*

A major challenge in human health over the next 50 years will be in the area of chronic diseases, many types of cancer, type 2 diabetes and obesity [1]. For protection against the cited diseases phenolic compounds, ubiquitously distributed in vascular plants, have on important value. The molecules of phenolic compounds have several biological effects, including inhibition of LDL oxidation *in vitro* and *in vivo* and protection of DNA from oxidative damage; they also have antithrombotic, antimutagenic properties

[2]. In addition, phenolic compounds are characterized by extremely high antioxidant properties [3]. Proceeding from this, identification of such foodstuffs which are rich in phenolic compounds having medical activity is extremely important. In this aspect interest attaches to white and red wines prepared by Kakhetian technology which contain phenolic compounds in the amount of 1.330-2.430 mg/l and 2.898-4.416 mg/l, respectively [4]. The purpose of this research is studying the qualitative composition and the quantitative content of phenolic compounds of Kakhetian white and red wines prepared in clay vessels (*kvevri*) by means of a method of high-pressure liquid chromatography.

Materials and methods. Analyzed samples were prepared by us in autumn 2010 from autochthonous grapes (*Vitis vinifera L)*, cultivated in Georgia white Rkatsiteli and red Saperavi. Grapes Rkatsiteli and Saperavi were collected in Kakheti region, respectively in villages Chumlaki and Velistsikhe of Gurjaani municipality. Grapes Rkatsiteli (80 kg) and Saperavi (70 kg), together with other parts of grape cluster (stem, skin, seeds), were crushed in juicer and were placed in clay vessels (kvevri) dug in the ground.In the case of Rkatsiteli, the fermentation took place in a closed cap (t = 21 °C) and at the further ripening of wine on husks of grapes within 120 days.In the case of Saperavi the fermentation occurred with an open cap (t = 23 °C) within 9 days, at regular agitation (daily) of emerged on surface of the husks of grapes, submerging the cap by hand with wooden paddle. In both cases natural yeasts were used. The fermented wines were drained into a glass vessel and stored in a cellar (t = 14 °C).

In the analyzed wines Rkatsiteli and Saperavi the sum of phenolic compounds is 2160 mg/l and 4320 mg/l, respectively [5]. A method of extraction of liquid/liquid was used for fractionation of phenolic compounds of the analyzed wines [6]. For this purpose, alcohol was removed from 1000 ml of each wine sample (dealcoholization) on the rotational evaporator at 40°C.The dealcoholized wine, pH of which was brought up to 2 (pH = 2), was extracted by ethyl acetate, after evaporation of ethyl acetate in vacuum (t = 30°C), the residue was dissolved in water, pH was brought up to 7 (pH = 7) and extracted again by ethyl acetate. The organic phase which substantially contains flavanols and flavonols, and a water phase was obtained. The organic phase was evaporated in vacuum (t = 30 °C), dissolved in methanol and marked as fraction X1 of Rkatsiteli white wine (RWWF X1) and fraction X3 of Saperavi red wine (SRWF X3). The pH of water phase was brought up to 2 (pH = 2) and extracted again by ethyl acetate. Extract of ethyl acetate was evaporated in vacuum (t = 30 °C), the residue was dissolved in methanol and marked as RWWF X2 and SRWF X4. These two fractions contain phenolic acids and flavonols. Identification of phenolic compounds from the obtained fractions was carried out by means of the high-pressure liquid chromatograph "Gilson 116", with UV detector. Chromatographic separation was carried out on "Zorbax ODS" column (25 cm x 4.6 mm); system of solvents: methanol - 4% acetic acid; 0.2 ml/min, at 280 nm, and 24 °C; injected volume 20 μl. Identification of compounds was carried out by comparison with retention time of authentic phenolic compounds. As authentic samples were used: (+)-catechine, (-)- epicatechine, (-)-gallocatechine, kaempferol and gallic acid (Sigma), quercetin and rutin (Chemapol), protocatechuic acid, o-coumaric acid and caffeic acid (Reachim), resveratrol (Bio-Tech Co.).

Results and discussion. According to the obtained data (Table), in white wine Rkatsiteli (fraction X1), the content of catechines is the following: (+)-catechine (32.6 mg/l), (-)-epicatechine (58.6 mg/l), (-)-gallocatechine (43.7 mg/l), and in red wine Saperavi (fraction X3): (+)- catechine (115.4 mg/l), (-)-epicatechine (29.5 mg/l), (-)-gallocatechine (174.4 mg/l), respectively. It is visible that under the contents of catechines, between white and red wines a significant difference is observed. Red wine Saperavi contains 3.4 times and in 4 times more (+)-catechine and (-)-gallocatechine, and 2 times less (-)-

Table. The contents of phenolic compounds in wines of grapes Rkatsiteli and Saperavi (mg/l)

Authentic phenolic compound	Retention time (min)	Fractions of white wine Rkatsiteli		Fractions of red wine Saperavi	
		X1	X2	X3	X4
(+)-Catechine	19.7	32±0.169		115.4±0.292	
(-)-Epicatechine	32.3	58.6±0.365		29.7±0.438	
(-)-Gallocatechine	9.1	43.7±0.204		174.5±0.432	
Kaempferol	57.5	not found		13.2±0.373	
Quercetin	53.9	not found		7.3±0.279	3.9±0.274
Rutin	50.6	not found		2.6±0.392	
Resveratrol	51.8	not found		1.47±0.323	
Protocatechuic acid	14.4		12.8±0.219		14.5±0.396
Gallic acid	9.2		26.7±0.288		21.8±0.287
O-coumaric acid	37.7		not found		traces
Caffeic acid	25.1		not found		7.4±0.357
Syringic acid	29.8		not found		traces

epicatechine than the white wine Rkatsiteli. Of flavonols (Table), in red wine Saperavi (fraction X3) were found kaempferol (13.2 mg/l), quercetin (11.2 mg/l) and rutin (2.6 mg/l), and in white wine Rkatsiteli (fraction X1), these compounds are absent.It is necessary to note that a certain amount (3.9 mg/l) of quercetin was revealed in fraction X4 of red wine Saperavi.

The white wine Rkatsiteli (fraction X1) does not contain resveratrol, while in red wine Saperavi (fraction X3) its amount is equal to1.47 mg/l. Of hydroxybenzoic acids (Table), in white wine Rkatsiteli (fraction X2) the protocatechuic acid and gallic acid were not found, while in red wine Saperavi (fraction X4) their amount is respectively equal to 14.5 mg/l and 21.8 mg/l. Of hydroxycinnamic acids (Table), in red wine Saperavi (fraction X4) the caffeic acid in the amount of 7.4 mg/l, o-coumaric acid and syringic acid as traces were found . These acids were not found in the white wine Rkatsiteli (fraction X2). Thus, in qualitative composition and the quantitative contents of phenolic compounds, the red wine Saperavi considerably surpasses the white wine Rkatsiteli. Exclusive interest attaches to a comparison of the quantitative contents of phenolic compounds of wines from grapes of white Rkatsiteli and red Saperavi, and wines prepared in the various countries. In particular, in the French white wines the quantitative contents of (+)-catechine and (-)-epicatechine on the average makes 9.8 mg/l and 5.3 mg/l, respectively, while in the French red wines the quantitative contents of (+)-catechine changes within the limits of 22.1-130.7 mg/l, and the quantitative contentsof (-)-epicatechine changes within the limits of 7.8-39.1 mg/l [7]. In wines prepared from red varieties of grapes (Tempranillo, Graciano, Cabernet, Merlot), cultivated in Spain, (+)-catechine content is equal to 16.01, 32.78, 41.76 and 27.09 mg/l, respectively [8], whereas the red wine Saperavi contains this compound in the amount of 115.4 mg/l (Table). The red wine Saperavi contains 11.2 mg/l of quercetin, whereas, in the above-mentioned red wines, the contents of this compound re-

spectively is equal to 1.88, 6.90, 5.00 and 4.67 mg/l. Red wine Saperavi contains 1.47 mg/l of resveratrol (Table), red wines Tempranillo, Graciano and Merlot contain this compound respectively in the amount of 0.73, 1.36 and 0.21 mg/l, and in red wine Cabernet it is detected as traces. Red wine Saperavi contains gallic acid and protocatechuic acid in amounts of 21.8 mg/l and 14.5 mg/l (Table), while in red wines of grapes Tempranillo, Graciano, Cabernet and Merlot the contents of these acids respectively varies in the limits of 12.20 - 14.5 mg/l and 0.81 - 1.52 mg/l. It is interesting that in red wine Saperavi, syringic acid was found as traces, however, in the above-cited Spanish four red wines this compound was found in the amount of 2.60 - 5.76 mg/l. According to our data, in white wine Rkatsiteli (the harvest of 2010), kaempferol, quercetin and rutin were not found (Table), however, in a commercial wine from grapes of the variety Chardonel cultivated in China (the harvest of 2005), these compounds were identified in the amount of 0.01, 0.06 and 0.17 mg/l, respectively. In commercial wine from the same variety of grapes, from the 2004 harvest, kaempferol was not found, however the contents of quercetin and rutin were equal to 0.25 mg/l and 0.40 mg/l, respectively [9]. Also it is interesting to compare the quantitative contents of flavonols of wine from grapes of Saperavi (the harvest of 2010) and of commercial wines from 5 varieties of red grapes cultivated in China [9]. Red wine Saperavi contains kaempferol 13.2 mg/l, quercetin 7.3 mg/l and rutin 2.6 mg/l (Table), however, in commercial red wine from grapes of varieties Cabernet Souvignon, Cabernet Franc, Merlot, Marselan and Petit Verdot, cultivated in China (the harvest of 2005), these compounds were identified in the amount of 0.01-0.04 mg/L, 0.2-0.94 mg/l and 0.49-0.72 mg/l, respectively. Apparently, the qualitative composition and the quantitative contents of phenolic compounds in wines are influenced by the variety of grapes and the geographical environment.

Conclusion. In this paper we have shown that the wines from the Georgian autochthonous grapes, white Rkatsiteli and red Saperavi, prepared by Georgian (Kakhetian) technology, in kvevri, contain biologically active phenolic compounds in abundance: catechines, flavonols, hydroxybenzoic acids hydroxycinnamic acids also are the best medical and preventive means.

ბიოქიმია

საქართველოს ავტოქტონური ვაზის ჯიშების რქაწითელის და საფერავის ყურძნიდან ქართული (კახური) წესით დაყენებული ღვინის ფენოლური ნაერთები

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შესწავლილია საქართველოს ავტოქტონური ვაზის ჯიშების თეთრი რქაწითელისა და წითელი საფერავის ყურძნიდან კახური წესით ქვევრში დაყენებული ღვინოების ფენოლური ნაერთების თვისებრივი შედგენილობა და რაოდენობრივი შემცველობა. რქაწითელის ყურძნიდან დაყენებულ ღვინოში იდენტიფიცირებულია: (+)-კატექინი (32.6 მგ/ლ), (-)-ეპიკატექინი (58.6 მგ/ლ), (-)-გალოკატექინი (43.7 მგ/ლ), პროტოკატექინმჟავა (12.8 მგ/ლ) და გალმჟავა (26.7 მგ/ლ); საფერავის ყურძნიდან დამზადებულ ღვინოში - (+)-კატექინი (115.4 მგ/ლ), (-)-ეპიკატექინი (29.5 მგ/ლ), (-)-გალოკატექინი (174.4 მგ/ლ), კემპფეროლი (13.2 მგ/ლ), კვერცეტინი (11.2 მგ/ლ), რუტინი (2.6 მგ/ლ), რესვერატროლი (1.47), პროტოკატექინმჟავა (14.5 მგ/ლ), გალმჟავა (21.8 მგ/ლ) და ყავამჟავა (7.4 მგ/ლ).

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