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Original Research Article

In vitro antioxidant activity and phenolic composition of Georgian, Central and West European wines



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ABSTRACT

Georgia is a traditional country of viticulture and winemaking but only a few studies have been focused on wines originating from this area. In this study, we compared antioxidant effect, total sulfite content and concentration of 14 phenolic compounds of some native Georgian red and white wines with wines commonly produced in Central and Western Europe. Georgian red wines exhibited higher antioxidant capacity in DPPH, ORAC and total phenolic content assay. Further, Georgian red wines were richer in quercetin, kaempferol and syringic acid content, while the concentration of *trans*-resveratrol was lower than in Central and West European red wines. While differences among red wines from different origins and cultivars were observed, winemaking technology was the most important factor in the case of white wines. Kakhetian method increased antioxidant effect and levels of some phenolic compounds in comparison with white wines prepared by common European method. Our findings suggest that Georgian wines deserve further attention because of their high content of phenolics and high antioxidant capacity.

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1. Introduction

Antioxidant activity of natural products has received much interest over the past few years, both in public and scientific community. Generally, it is believed that consumption of plant phenolics decreases the risk of occurrence of diseases related to

oxidative stress (Georgiev et al., 2014). This biological effect is explained by the ability of these compounds to act as antioxidant agents; i.e. act as regulators of unwanted damaging oxidative processes (Liang et al., 2014; Gris et al., 2013). Therefore, the identification of foodstuff rich in phenolic compounds is still important.

Grapevine and its products are considered to be one of the richest natural sources of phenolic compounds such as phenolic acids, stilbenes, flavonoids, and anthocyanidins (Teixeira et al., 2014). Accordingly, the moderate wine consumption is nowadays

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recognized as risk-reducing factor in several human diseases related to oxidative-stress such as cancer, type 2 diabetes, inflammation and myocardial infarction (Fagherazzi et al., 2014; Kutil et al., 2014; Rossi et al., 2014; Russo et al., 2014). Composition of health-promoting chemicals (e.g. phenolics) presented in wine is influenced by many factors such as winemaking procedure, conditions of maturation and storage, grape cultivar, soil, nutrition, climatic conditions and weather (Singleton, 1982; de Pascali et al., 2014; King et al., 2014; Ivanova-Petropulos et al., 2015). Large proportion of nearly 1400 cultivars used for commercial production of wine (Robinson et al., 2012), stays still undiscovered by means of phytochemical composition, biological activities and herewith connected health-promoting properties.

Georgia, which is considered to be cradle of the world's winemaking (This et al., 2006), is one of the countries where many local grapevine varieties are cultivated. It is believed that viticulture and winemaking first began in the South Caucasus region around 6000 years ago, and this fact is supported by several archeological findings (Imazio et al., 2013). At present, the Georgian vine gene pool contains up to 525 white and red varieties (Maghradze et al., 2012). However, to the best of our knowledge, only scarce information about phytochemical profile and antioxidant activity of Georgian wines is available. Chkhikvishvili et al. (2008) compared antioxidant activity and the content of trans- and cis-resveratrol in Georgian (from cultivars Saperavi, Kakhuri Tsarchinebuli), European, and South and North American red wines. A comparison among several red and white wines produced by Kakhetian and European method in total content of phenolics, catechins, proanthocyanidins, anthocyanins and antioxidant activity was published by Shalashvili et al. (2007). Recently Shalashvili et al. (2012) investigated the content of some flavonoids, phenolic acids and resveratrol in Georgian wines prepared from local cultivars Saperavi (red) and Rkatsiteli (white). The content of catechins, hydroxycinnamic acids, volatile compounds and their glycosides in wines prepared by Kakhetian (juice fermented with skins, seeds, and bunch stems in clay vessel [kvevri] dug into the ground for three to five months) and European wine making processes (juice fermented without stems for 7-30 days; white wines fermented without the presence of pomace) has been compared by Mikiashvili et al. (2010a,b). The content of total catechins, proanthocyanidins, flavanols, and some phenolic acids in wines produced from white cultivars Kakhuri Mtsvivani, Rkatsiteli, Kakhuri Mtsvane, and Khikhvi prepared by Kakhetian and European wine making method was investigated by Glonti (2010a). Glonti and Glonti (2013) also published extensive study where total phenolics, sulfur, various volatile compounds, amino acids, and minerals were quantified in wines fermented in kvevri and by standard process.

In our study, we investigated antioxidant activity (using ORAC and DPPH method), total phenolic content, total sulfite content and quantified 14 phenolic compounds (by HPLC-UV/Vis method) including phenolic acids, flavonoids and stilbenes in Georgian red (Alexandrouli, Saperavi and cuvee of Saperavi and Saperavi Budeshuriseburi) and white wines (Rkatsiteli, and cuvee of Rkatsiteli and other local cultivars). Central and West European red (Cabernet Moravia, Cabernet Sauvignon, Pinot Noir) and white wines (Chardonnay, Sauvignon Blanc) were assayed with the aim to compare the differences between regions and cultivars.

2. Materials and methods

2.1. Wines

Red and white wine samples were acquired from different regions of Georgia, Czech Republic, France, Italy, and Austria, and were provided by local producers or purchased from supermarkets or wine stores. A total of 26 red wines of the cultivars Pinot Noir (n = 5), Cabernet Sauvignon (n = 7), Cabernet Moravia (n = 2), Seperavi (n = 9), cuvee of Saperavi and Saperavi Budeshuriseburi (n = 2), and Alexandrouli (n = 1) and 13 white wine samples of the cultivars Chardonnay (n = 6), Sauvignon Blanc (n = 3), Rkatsiteli (n = 2), and cuvee of Rkatsiteli and other local cultivars (n = 2) were assayed. Detailed information about tested wines (cultivar, vintage, producer, origin and wine type) is given in Table 1.

2.2. Winemaking methods

The majority of red and white wines included in this study were made based on the common "European methods". However, white wine samples RK 1 and RK 4 from Georgia, were made by the Kakhetian method, which is one of method elaborated in Georgia. This style of wine is based on long period (up to 5 month) maceration and fermentation of must with usage of 100% of grape pomace (skin, seeds, stems) in clay vessel called "kvevri" buried under ground (Gagunashvili, 2006; Glonti, 2010a,b).

2.3. Reagents, solvents and standards

2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH, purity 97.0%), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,6-di-*tert*-butyl-4-methylphenol (BHT, \geq 99.0%), (\pm)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox, 97.0%), analytical grade quality solvents, Folin-Ciocalteu reagent, fluorescein sodium salt (FL, 93.3%), fluorosalicylic acid (97.0%), standards of phenolic acids: 3,4-dihydroxybenzoic (purity \geq 97%.0), caffeic (\geq 98%.0), chlorogenic (94.9%), gallic (97.9%), ferulic (99.0%), *p*-coumaric (\geq 98.0%), syringic (\geq 95.0%) and vanillic acid (\geq 97.0%); flavonoids: epigallocatechin gallate (\geq 95.0%), kaempferol (\geq 97.0%), myricetin (\geq 96.0%), quercetin (\geq 95.0%) and rutin (\geq 95.0%), and stilbene resveratrol (99.0%) were obtained from Sigma–Aldrich (Prague, Czech Republic). Inorganic salts of p.a. grade used for buffer preparation and total phenolic content assessment were purchased from Lach-ner (Brno, Czech Republic).

2.4. Antioxidant activity

2.4.1. DPPH radical-scavenging assay

The antioxidant effect of wine and positive control (trolox) was tested using DPPH assay according to the slightly modified method previously described by Sharma and Bhat (2009). Two-fold serial dilution of each sample was prepared in absolute methanol (175 μ L) in 96-well microtiter plate. Subsequently, 25 μ L of freshly prepared 0.4 mM DPPH solution in methanol was added to each well (final volume 200 μ L), creating a range of concentration 50–0.78125 μ L mL⁻¹ for red wines and 500–7.8125 μ L mL⁻¹ for white wines. Range of concentration for trolox (used as a reference antioxidant) was settled at 80–1.25 μ g mL⁻¹ and calibration curve was thereafter established. Mixture was kept in dark at room temperature for 30 min. Absorbance was measured at 517 nm using Infinite 200 reader (Tecan, Männedorf, Switzerland). Results were expressed as trolox equivalents (g TE L⁻¹ wine).

2.4.2. Oxygen radical absorbance capacity (ORAC) assay

Slightly modified method previously described by Ou et al. (2001) was used. Firstly, outer wells of black 96-well microtiter plates were filled with 200 μL of distilled water, in order to provide better thermal mass stability. Stock solution of AAPH radical and FL were prepared in 75 mM phosphate buffer (pH 7.0). Each sample (25 μL) was diluted in 150 μL FL (48 nM) and incubated in 37 °C for 10 min. Reaction was started by application of 25 μL AAPH (153 mM) yielding final volume of 200 μL and final dilution at 1:4000 for red wines and 1:800 for white wines (in phosphate

 Table 1

 Information on variety, producer, origin and type of studied wines.

Wine code	Cultivar and vintage	Producer	Origin	Wine type
PN 1	Pinot Noir, 2008	VINIUM Velké Popovice	Moravia, Czech Republic	Red dry
PN 2	Pinot noir, 2010	Augustinian cellar Neoklas	Moravia, Czech Republic	Red dry
PN 3	Pinot noir, 2007	Gsellmann & Hans	Burgenland, Austria	Red dry
PN 4	Pinot noir, 2008	Virely Arcelain	Bourgogne, France	Red dry
PN 5	Pinot noir, 2007	André Goichot	Bourgogne, France	Red dry
CS 1	Cabernet Sauvignon, 2010	Famiglia Cielo	Venetia, Italy	Red dry
CS 2	Cabernet Sauvignon, 2010	Folonari	Venetia, Italy	Red dry
CS 3	Cabernet Sauvignon, 2010	Brise de France	N/A ^a , France	Red dry
CS 4	Cabernet Sauvignon, 2011	Jean D?Aosque	Languedoc, France	Red dry
CS 5	Cabernet Sauvignon, N/Aa	Víno Mikulov	Moravia, Czech Republic	Red dry
CS 6	Cabernet Sauvignon, 2008	Templar cellars Čejkovice	Moravia, Czech Republic	Red dry
CS 7	Cabernet Sauvignon, 2005	Teliani valley PLC	Teliany, Georgia	Red dry
CM 1	Cabernet Moravia, N/Aª	Templar cellars Čejkovice	Moravia, Czech Republic	Red dry
CM 2	Cabernet Moravia, 2007	Vinselekt Michlovský	Moravia, Czech Republic	Red dry
SA 1	Saperavi, 2008	P.E. Givi Nikolashvilli	Gurjaani, Georgia	Red dry
SA 2	Saperavi, 2007	Teliani valley PLC	Tsinandali, Gerogia	Red dry (aged in oak, unfiltered)
SA 3	Saperavi, 2005	Teliani valley PLC	Napareuli, Georgia	Red dry
SA 4	Saperavi, 2006	Teliani valley PLC	Mukuzani, Georgia	Red dry
SA 5	Saperavi, 2004	Teliani valley PLC	Kidzmarauli, Georgia	Red semi-sweet
SA 6	Saperavi, 2006	Geovan Wine	Kakheti, Georgia	Red dry
SA 7	Saperavi, 2010	Tbilvino	Kakheti, Georgia	Red dry
SA 8	Saperavi, 2009	Kindzmarauli Marani	Kakheti, Georgia	Red dry
SA 9	Saperavi, 2007	Kindzmarauli Marani	Kakheti, Georgia	Red dry (barrel aged)
SA 10	Kvareli (Saperavi + Saperavi Budeshuriseburi), 2005	Kindzmarauli Marani	Kakheti, Georgia	Red dry
SA 11	Saperavi (95%)+Saperavi Budeshuriseburi (5%), N/A ^a	Kindzmarauli Marani	Kakheti, Georgia	Red dry (fermented 25–30 days)
AL 1	Alexandrouli, 2007	Chrebalo Wine Factory	Ambrolauri district, Georgia	Red dry
CH 1	Chardonnay, 2011	VINIUM Velké Pavlovice	Moravia, Czech Republic	White dry
CH 2	Chardonnay, N/A ^a	Chateau Valtice	Moravia, Czech Republic	White dry
CH 3	Chardonnay, 2010	Folonari	Venetia, Italy	White dry
CH 4	Chardonnay, 2010	Delibori	Venetia, Italy	White dry
CH 5	Chardonnay, 2011	Joseph Castalan	Pays d?oc, France	White dry
CH 6	Chardonnay, 2010	André Goichot	Bourgogne, France	White dry
SB 1	Sauvignon Blanc, 2010	Famiglia Cielo	Venetia, Italy	White dry
SB 2	Sauvignon Blanc, 2011	Brise de France	N/A ^a , France	White dry
SB 3	Sauvignon Blanc, 2008	VINIUM Velké Pavlovice	Moravia, Czech Republic	White dry
RK 1	Rkatsiteli, 2010	Institute of Horticulture,	Kakheti, Georgia	White dry (fermented by
		Viticulture and Oenology (IHVO)	-	Kakhetian meathod)
RK 2	Rkatsiteli, 2009	Tbilvino	Kakheti, Georgia	White
RK 3	Rkatsiteli + Mtsvane, 2008	Geovan Wine	Kakheti, Gerogia	White dry
RK 4	Rkatsiteli (50%)+ Mtsvane Kakhuri (20%)+ Khikhvi (15%)+ Kisi (15%), N/A ^a	Kindzmarauli Marani	Kakheti, Georgia	Amber (fermented by Kakhetian method)

a Information not available.

buffer). Trolox was tested at range of concentration $4-0.5~\mu g~mL^{-1}$ and calibration curve was thereafter established. Fluorescence changes were measured in one minute intervals for 120 min with emission and absorbance wavelengths were set at 494 nm and 518 nm, respectively. Antioxidant capacity was calculated as area under the calibration curve as proposed by (Cao and Prior, 1998). Results were expressed as trolox equivalents (g TE L^{-1} wine).

2.4.3. Total phenolic content (TPC) assay

Total phenolic compounds were measured using modified method previously described by Singleton et al. (1998). Sample in volume of 100 μ L (dilution: red wine:water 1:19; white wine:water 1:1) was added to 96-well microtiter plate. Range of concentration of gallic acid (used as a reference compound) was settled at 16.7–0.008 μ g mL $^{-1}$. Thereafter, 25 μ L of pure Folin-Ciocalteu reagent was added. Plate was inserted in orbital shaker at 100 rpm for 10 min. Reaction was started by addition of 75 μ L 20% Na₂CO₃. Mixture was kept in dark at 37 °C for 2 h and then absorbance was measured at 700 nm. Results were expressed as gallic acid equivalents (g GAE L $^{-1}$ wine).

2.4.4. Total sulfite content assay

Commercially available kit EnzytecTM Color SO₂-Total kit (R-Biopharm AG, Darmstadt, Germany) was used for the quantification

of total sulfites in wine samples. The test was performed according to manufacturer's instructions with slight modifications. Briefly, sample solutions were prepared by mixing 950 μL of buffer with 50 μL of each sample. Sample solution (200 µL) was added to 96-well microtiter plate and measured using Infinite 200 reader with absorbance set at 340 nm. Thereafter, 40 µL of chromogen was added and 200 µL of this solution was transferred to new 96-well microtiter plate and measured at 340 nm again. Calibration curve was constructed by using calibrator (SO₂) in concentration range between 300 and 0 mg L^{-1} . Difference in optical densities (ΔA) was calculated as $\Delta A = (A2 - Rf \times A1)_{sample or calibrator} - (A2 - Rf \times A1)_{blank}$, where A2 is the absorbance value acquired from the second measurement and A1 absorbance value of samples before application of the chromogen. Rf refer to diffraction factor which was settled at 0.952 according to manufacturer's instructions. Total sulfite content was further calculated according to following equation: $C_{\text{sample}} = C_{\text{calibrator}}/\Delta A_{\text{calibrator}} \times \Delta A_{\text{sample}}$. Results were expressed as $mg SO_2 mL^{-1}$ wine.

2.5. High performance liquid chromatography (HPLC) analysis

2.5.1. Extraction procedure

Each wine sample (4.75 mL) was treated by addition of 150 μ L BHT (200 μ g dissolved in 1 mL of methanol) to prevent oxidation

of phenolic compounds. As inner standard 100 μ L of fluorosalicylic acid (200 μ g dissolved in 1 mL of methanol) was used. Each sample was acidified to pH 2.8 by adding 10 μ L of 35% HCl. Remaining alcohol (ethanol and methanol) presented in each wine was further evaporated by inserting sample to refrigerated CentriVap concentrator (Labconco, Kansas City, MO, USA) for 2 h under 50 °C. Extraction procedure was performed by liquid–liquid extraction using 5 mL of diethylether. Wine:diethylether solution was vortexed for 60 s. Separation of the ether layer was facilitated by centrifugation at 3500 rpm for 5 min. Extraction procedure was repeated three times and provided about 15 mL of ether extract which was further evaporated to dryness on rotary evaporator R210 (Büchi, Flawil, Switzerland). Residue was dissolved in 500 μ L acetonitrile:water (50:50) solution and poured into HPLC vial glass for further analysis.

2.5.2. HPLC-UV/Vis

Apparatus consisted of autosampler Midas (Spark, Emmen, Netherlands), thermostat (Midas, Spark, Emmen, Netherlands) and pump (Q-Grad, Watrex, Prague, Czech Republic). System was coupled on-line to UV6000 LP detector (SpectraSystem, Thermo-Finnigan, Waltham, MA, USA) and was controlled by software Clarity (DataApex, Prague, Czech Republic) and EZ-Chrom Elite (Thermo-Finnigan, Waltham, MA, USA). For identification of simple phenolic acids, stilbenes and flavonoids, Kinetex (2.6 μ m) PFP, 100 A (150 \times 4.6 mm) column was used (Phenomenex, Torrance, CA, USA). Gradient elution was carried out employing mobile phase

A (water with 0.5% acetic acid) and B (acetonitrile with 0.5% acetic acid) as follows: 0 min, 96:4 (A:B); 10 min, 85:15; 14 min, 79:21; 25 min, 78:22, 34 min, 59:41; 38 min, 0:100; 48 min, 0:100; 51 min, 96:4; 61 min, 96:4. Injection volume was settled at 10 μ L, flow rate at 1 mL/min and thermostat temperature at 33 °C.

2.5.3. Quantitative analysis

UV absorption was monitored at wavelengths between 194 and 500 nm. Quantification was done under 260 and 300 nm (chromatogram of wine sample SA 8 and chromatogram of standard solution is shown in Fig. 1). Evaluation of acquired data was performed in software Clarity (DataApex, Prague, Czech Republic) and EZ-Chrom Elite (ThermoFinnigan, Waltham, MA, USA). Standard calibration curves were obtained in a concentration range of $100-0.2~\mu g~mL^{-1}$ with nine concentrations levels ($100,50,20,10,5,2,1,0.5,0.2~\mu g~mL^{-1}$). UV peak areas of the external standards (at each concentration) were plotted against the corresponding standard concentrations ($\mu g~mL^{-1}$) using weighed linear regression to generate standard curve. Retentions times and linear equations for each standard are given in Supplementary Table S1. Amount of compounds were finally expressed as $\mu g~mL^{-1}$ wine.

2.5.4. Method validation

Linearity of calibration based on regression analysis was obtained at all measured concentration levels. The coefficients of determination (R^2) for particular compounds are shown in Supplementary Table S1.

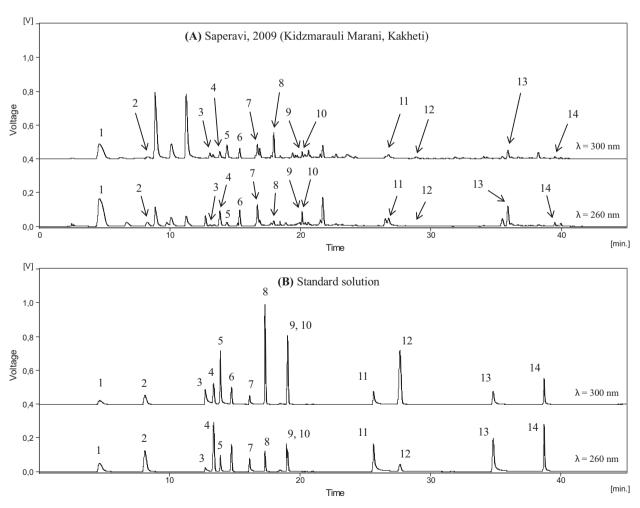


Fig. 1. Chromatogram of wine sample SA 8 (A) in comparison to standard solution (B) at concentration of 50 μg mL⁻¹. 1 = gallic acid; 2 = 3,4-dihydroxybenzoic acid; 3 = chlorogenic acid; 4 = vanillic acid; 5 = caffeic acid; 6 = syringic acid; 7 = epigallocatechin gallate; 8 = *p*-coumaric acid; 9 = rutin; 10 = ferulic acid; 11 = myricetin; 12 = resveratrol; 13 = quercetin; 14 = kaempferol.

Limit of detection (LOD) as well as limit of quantification (LOQ) were calculated from the standard additional curves as a 1:3 and 1:10 signal-to-noise ratio, respectively. For the measured compounds, LOD and LOQ ranged between 0.010–0.398 and 0.012–0.790 μg mL⁻¹, respectively (for details see Supplementary Table S1).

The precision of the HPLC measurement expressed as relative standard deviation (RSD, %) was evaluated by five replicate injections of standard solution and randomly selected wine sample during the same day. The precision for the standards and samples ranged between 2.1–9.7% and 1.3–14.7%, respectively. The detailed data for all analyzed compounds are listed in Supplementary Table S1.

Accuracy of measurement was determined as the average deviation between computed and obtained concentrations of standards in spiked samples. The recovery for the measured compounds varied from 2.6 to 10.9% (for details see Supplementary Table S1).

Repeatability of the extraction method was determined as recovery of spiked standards solved in freshly prepared wine samples. Recoveries of gallic acid, 3,4-hydroxybenzoic acid, chlorogenic acid, vanillic acid, caffeic acid, syringic acid, epigallocatechin gallate, p-coumaric, rutin, ferulic acid, myricetin, resveratrol, quercetin and kaempferol were determined at 50 μ g mL $^{-1}$. The recovery was expressed as percentage of corresponding determined concentration of pure standards. The value was computed as an average of five technical replicates. Recovery values of analyzed compounds ranged from 82.7 to 105.7% (Supplementary Table S2).

2.6. Statistical analysis

In vitro antioxidant experiments were performed in three separate experiments, each in duplicate. Results were expressed as mean values with standard deviations (mean \pm SD). Extraction and HPLC/UV-Vis analyses were performed two times for each sample. HPLC data (concentration of studied compounds) were expressed as mean.

For further statistical analyses, wine samples were grouped according to color (red and white) and further into three groups according to their production area: Georgia, Central Europe and Western Europe. Georgian group consisted, up to one exception (CS 7 - Cabernet Sauvignon), of Georgian autochthonous cultivars Saperavi, Alexandrouli, and Saperavi Budeshuriseburi only (present only in the cuvee with Saperavi) in the case of red wines and Rkatsiteli, Mtsvane, Khikhvi, and Kisi (last three cultivars present only in the cuvee with Rkatsiteli). Central European group consisted from Cabernet Sauvignon, Pinot Noir, Cabernet Moravia cultivars from Czech Republic and Austria in the case of red wines and Chardonnay and Sauvignon Blanc from Czech Republic in the case of white wines. West European group consisted from Cabernet Sauvignon and Pinot Noir cultivars from France and Italy in the case of red wines and Chardonnay and Sauvignon Blanc from France and Italy in the case of white wines.

Values recorded in DPPH, ORAC, TPC, and SO_2 assay and concentrations of 14 compounds were analyzed using one way analysis of variance (ANOVA) followed by Tukey's test in order to ascertain possible significant differences between groups. Linear correlation coefficients (r) between total sulfite content and antioxidant assays DPPH and ORAC were established using Pearson product moment correlation. For all statistical tests STATISTICA 8.0 (StatSoft Inc., Tulsa, OK, USA) software was used.

3. Results and discussion

3.1. Antioxidant activity

Georgian red wines possessed significantly higher antioxidant activity (complete results for each sample are summarized in Table 2; average values for samples separated according to

Table 2Antioxidant activity measured by DPPH and ORAC, total phenolic content (TPC) and total sulfite content of tested wines (mean ± standard deviation).

total suffice content of tested wifes (mean ± standard deviation).								
Wine code	DPPH [gTE ^a L ⁻¹ wine]	ORAC [gTEL ⁻¹ wine]	TPC [g GAE ^b L ⁻¹ wine]	Total SO ₂ [mg SO ₂ L ⁻¹ wine]				
DNI 4								
PN 1	3.06 ± 0.41	8.59 ± 1.01	1.56 ± 0.02	24.63 ± 2.14				
PN 2	3.12 ± 0.34	10.49 ± 1.55	2.08 ± 0.03	56.73 ± 4.44				
PN 3	1.88 ± 0.24	8.67 ± 1.55	1.57 ± 0.05	11.35 ± 1.14				
PN 4	4.22 ± 0.38	10.01 ± 1.48	3.31 ± 0.13	2.44 ± 0.33				
PN 5	3.38 ± 0.46	9.19 ± 1.73	2.46 ± 0.04	73.18 ± 4.09				
CS 1	2.37 ± 0.26	6.93 ± 1.45	1.55 ± 0.04	59.60 ± 4.47				
CS 2	2.95 ± 0.39	6.70 ± 1.37	1.89 ± 0.05	58.43 ± 3.99				
CS 3	3.31 ± 0.41	7.11 ± 1.33	2.03 ± 0.07	59.60 ± 4.47				
CS 4	2.82 ± 0.29	7.09 ± 1.1	1.86 ± 0.06	36.06 ± 4.52				
CS 5	2.78 ± 0.22	7.61 ± 1.54	1.69 ± 0.04	25.23 ± 0.29				
CS 6	1.97 ± 0.42	7.02 ± 0.76	1.11 ± 0.01	93.85 ± 4.49				
CS 7	4.58 ± 0.33	$\textbf{8.42} \pm \textbf{1.39}$	2.49 ± 0.07	18.17 ± 4.13				
CM 1	1.97 ± 0.31	8.29 ± 0.91	1.40 ± 0.04	34.65 ± 3.80				
CM 2	2.12 ± 0.20	$\textbf{8.77} \pm \textbf{1.63}$	1.50 ± 0.03	32.46 ± 1.73				
SA 1	5.89 ± 0.63	10.39 ± 1.31	2.78 ± 0.08	18.22 ± 1.59				
SA 2	$\boldsymbol{5.27 \pm 0.46}$	10.81 ± 1.68	2.74 ± 0.07	10.79 ± 0.37				
SA 3	$\boldsymbol{5.03 \pm 0.72}$	11.16 ± 1.75	2.42 ± 0.05	$\boldsymbol{8.38 \pm 0.49}$				
SA 4	4.79 ± 0.32	10.56 ± 2.11	2.61 ± 0.08	9.13 ± 2.06				
SA 5	$\boldsymbol{2.08 \pm 0.14}$	$\boldsymbol{7.29 \pm 0.81}$	$\boldsymbol{1.59 \pm 0.03}$	60.69 ± 2.94				
SA 6	$\boldsymbol{4.50 \pm 0.43}$	$\boldsymbol{9.99 \pm 2.14}$	$\boldsymbol{2.67 \pm 0.07}$	25.59 ± 5.34				
SA 7	$\boldsymbol{4.70 \pm 0.34}$	10.92 ± 2.00	$\boldsymbol{3.05 \pm 0.07}$	26.68 ± 2.77				
SA 8	8.59 ± 0.50	11.37 ± 2.01	4.46 ± 0.18	39.45 ± 3.80				
SA 9	5.21 ± 0.45	$\boldsymbol{9.82 \pm 1.48}$	3.22 ± 0.13	21.36 ± 2.38				
SA 10	5.01 ± 0.47	11.71 ± 1.65	$\boldsymbol{3.87 \pm 0.10}$	29.28 ± 1.89				
SA 11	$\boldsymbol{5.43 \pm 0.48}$	12.14 ± 2.15	$\boldsymbol{3.39 \pm 0.08}$	20.95 ± 5.78				
AL 1	1.91 ± 0.18	$\boldsymbol{5.87 \pm 0.66}$	1.36 ± 0.04	0.00				
CH 1	0.10 ± 0.02	$\boldsymbol{0.48 \pm 0.07}$	$\textbf{0.21} \pm \textbf{0.01}$	103.61 ± 6.98				
CH 2	$\boldsymbol{0.24 \pm 0.03}$	1.12 ± 0.19	$\boldsymbol{0.29 \pm 0.01}$	91.81 ± 2.80				
CH 3	0.14 ± 0.02	$\boldsymbol{0.84 \pm 0.12}$	$\boldsymbol{0.22 \pm 0.01}$	98.24 ± 2.54				
CH 4	$\boldsymbol{0.13 \pm 0.01}$	$\boldsymbol{0.85 \pm 0.14}$	$\boldsymbol{0.22 \pm 0.001}$	91.09 ± 2.26				
CH 5	$\boldsymbol{0.34 \pm 0.04}$	$\boldsymbol{1.12 \pm 0.21}$	$\boldsymbol{0.28 \pm 0.01}$	121.57 ± 9.47				
CH 6	$\boldsymbol{0.12 \pm 0.01}$	$\boldsymbol{0.73 \pm 0.05}$	$\boldsymbol{0.23 \pm 0.01}$	108.2 ± 3.54				
SB 1	$\boldsymbol{0.12 \pm 0.01}$	$\boldsymbol{0.74 \pm 0.14}$	$\boldsymbol{0.22 \pm 0.01}$	92.94 ± 2.88				
SB 2	$\boldsymbol{0.15 \pm 0.01}$	$\boldsymbol{1.04 \pm 0.2}$	$\boldsymbol{0.23 \pm 0.01}$	115.14 ± 2.34				
SB 3	$\boldsymbol{0.06 \pm 0.01}$	$\boldsymbol{1.27 \pm 0.11}$	$\boldsymbol{0.20 \pm 0.01}$	202.19 ± 18.68				
RK 1	$\textbf{0.51} \pm \textbf{0.04}$	$\boldsymbol{1.67 \pm 0.25}$	$\boldsymbol{0.40 \pm 0.01}$	2.66 ± 0.10				
RK 2	$\boldsymbol{0.17 \pm 0.02}$	$\boldsymbol{0.99 \pm 0.13}$	$\textbf{0.24} \pm \textbf{0.01}$	86.96 ± 2.15				
RK 3	$\boldsymbol{0.29 \pm 0.02}$	$\boldsymbol{1.64 \pm 0.24}$	$\boldsymbol{0.29 \pm 0.01}$	95.39 ± 2.65				
RK 4	$\boldsymbol{2.90 \pm 0.27}$	4.01 ± 0.70	$\boldsymbol{1.88 \pm 0.04}$	$\textbf{38.21} \pm \textbf{4.48}$				

^a Trolox equivalency.

geographical regions are shown in Table 3) than West and Central European samples in DPPH (p=0.01), ORAC (p=0.05) as well as in TPC (p=0.01) assay. The highest antioxidant effect in all three assays was recorded in wines from Georgian cultivar Saperavi and cuvee Saperavi + Saperavi Budeshuriseburi (range of DPPH, ORAC and TPC values: $8.59-2.08~{\rm g}$ TE L $^{-1}$ wine; $12.14-7.29~{\rm g}$ TE L $^{-1}$ wine and $4.46-1.59~{\rm g}$ GAE L $^{-1}$ wine, respectively), followed by Pinot Noir ($4.22-1.88~{\rm g}$ TE L $^{-1}$ wine; $10.49-8.59~{\rm g}$ TE L $^{-1}$ wine and $3.31-1.56~{\rm g}$ GAE L $^{-1}$ wine) and Cabernet Sauvignon ($4.58-1.97~{\rm g}$ TE L $^{-1}$ wine; $8.42-6.70~{\rm g}$ TE L $^{-1}$ wine and $2.49-1.11~{\rm g}$ GAE L $^{-1}$ wine). In contrast to our results, Chkhikvishvili et al. (2008) recorded

Table 3 Antioxidant activity of red wine samples grouped according to their origin (mean \pm SD).

Region	DPPH (gTE/L wine)**	ORAC (gTE/L wine) *	TPC (g GAE/L wine)**
Georgia (n=13) Western Europe (n=6)	$4.84 \pm 1.64^a \\ 3.18 \pm 0.63^{ab}$	$10.04 \pm 1.82^a \\ 7.84 \pm 1.40^b$	$\begin{array}{c} 2.82 \pm 0.83^{a} \\ 2.18 \pm 0.62^{ab} \end{array}$
Central Europe (n=7)	2.42 ± 0.55^b	8.50 ± 1.09^{ab}	1.56 ± 0.29^b

Different letters within each column showed significant differences (ANOVA with Tukey post hoc test; $p \le 0.05$, $p \le 0.01$).

b Gallic acid equivalency.

comparable or lower antioxidant activity of Georgian Saperavi wines prepared by European method in comparison with French wine. However, in mentioned study was only one sample of French wine for comparison. Higher antioxidant effect of Pinot Noir than antioxidant activity of Cabernet Sauvignon wines is in the agreement with the study of Hosu et al. (2011) and Landrault et al. (2001). Other representatives of red wines (Alexandrouli, Cabernet Moravia) were regarded as samples with moderate antioxidant efficacy (values ranging as follows: DPPH = 2.13–1.92 g TE L $^{-1}$ wine; ORAC = 8.77–5.87 g TE L $^{-1}$ wine and TPC = 1.50–1.36 g GAE L $^{-1}$ wine).

Comparison of antioxidant activity among regions in the frame of one cultivar (Cabernet Sauvignon and Pinot Noir) indicates that red wines produced in Western Europe were more effective than those from Central Europe. The activity of Cabernet Sauvignon from Western Europe ranged from 3.31 to 2.37 TE $\rm L^{-1}$ while samples from Central Europe ranged from 2.79 to 1.97 TE $\rm L^{-1}$. Similarly, the activity of West European Pinot Noir ranged from 4.22 to 3.39 TE $\rm L^{-1}$ while Central European samples ranged from 3.12 to 1.87 TE $\rm L^{-1}$ in DPPH. However, for statistical analyses and reliable conclusions more samples for each cultivar and geographical region would be needed.

White wines were significantly less effective in antioxidant assays than the red wines. Only exception was sample RK 4 (cuvee of Rkatsiteli), which was fermented by Kakhetian method. Wine RK 4 exhibited antioxidant efficacy comparable to some red wines (DPPH, ORAC and TPC values $2.90 \,\mathrm{g}$ TE L⁻¹ wine; $4.01 \,\mathrm{g}$ TE L⁻¹ wine and $1.88 \,\mathrm{g}\,\mathrm{GAE}\,\mathrm{L}^{-1}$ wine, respectively). Also another representative of white wine (RK 1) prepared by Kakhetian method showed higher antioxidant effect in comparison to the rest of tested white wines. However, its antioxidant activity (values ranging as follows: DPPH = 0.51 g TE L^{-1} wine; ORAC = 1.67 g TE L^{-1} TPC = $0.40 \,\mathrm{g}\,\mathrm{GAE}\,\mathrm{L}^{-1}$ wine) was not as strong as in case of RK 4 sample. Positive effect of Kakhetian technology (more than two times better than in case of European method) on antioxidant activity of white wines was recorded also by Shalashvili et al. (2007). This could be explained by the higher content of phenolics in wines fermented with pomace. Rest of tested white wines (Chardonnay, Sauvignon Blanc and Rkatsiteli made by common method used in Europe) were regarded as weak antioxidants (range of values for DPPH, ORAC and TPC were $0.34-0.07 \,\mathrm{g}\,\mathrm{TE}\,\mathrm{L}^{-1}$ wine; $1.64-0.48 \,\mathrm{g}\,\mathrm{TE}\,\mathrm{L}^{-1}$ wine and 0.29–0.20 g GAE L⁻¹ wine, respectively) without significant differences between regions of origin.

3.2. Total sulfite content

In the framework of this study the content of total sulfite was measured in wine samples. Generally, white wines contained higher quantities of mg SO₂ L⁻¹ (sulfite content in all samples is shown in Table 2). Two Sauvignon Blanc SB 3 (202.19 mg SO₂ L⁻¹ wine) and SB 2 (115.14 mg $SO_2 L^{-1}$ wine) samples and one Chardonnay sample CH 5 (121.57 mg SO₂ L⁻¹ wine) were most abundant samples on total SO₂ level. Sulfites in red wines ranged from 73.18 to $0 \text{ mg SO}_2 \text{ L}^{-1}$ wine. Wines with strongest antioxidant activity (SA 8, SA 10 and SA 11) contained low sulfite levels 39.45, 29.28 and 20.95 mg $SO_2 L^{-1}$ wine, respectively. Total sulfite content in tested wines was similar to values recorded by Zúñiga et al. (2014) and Comuzzo et al. (2013). We also ascertained if artificially added sulfites could possibly alter the antioxidant potential of wine in ORAC and DPPH assay. SO2 possessed EC_{50} = 289 mg L^{-1} in DPPH assay. Since the highest concentration of SO₂ in wine samples was 202 mg L⁻¹ and highest tested concentration of white wine was 0.5 mL mL⁻¹, the maximal concentration of SO_2 in sample was 101 mg L^{-1} . It means that in the sulfite most rich sample SO₂ level does not reach its EC₅₀. In the case of red wines the highest tested concentration was 0.05 mL mL⁻¹ which means that concentration and impact of SO₂ was negligible. In ORAC assay the effect of SO₂ was even lower. While 1 mL of weakest white wine was equal to activity of 0.48 mg trolox, the activity of 200 µg SO₂ (contained in 1 mL of most SO₂ rich wine) was equal only to 1.5 µg of trolox in ORAC assay. According to recorded results, no positive correlation between SO₂ and antioxidant potential (r = -0.77 for ORAC and r = -0.82 for DPPH) was revealed. These results demonstrate that content of sulfites had negligible impact on the antioxidant capacity of wines tested using ORAC and DPPH assays which is in the agreement with study of Zúñiga et al. (2014). In white wines, three times higher average concentration of total sulfites was observed in comparison to red wines (97.0 and 32.5 mg $SO_2 L^{-1}$ for white and red wines, respectively). Since white wines do not contain as much quantities of phenolic compounds as red wines, higher concentrations of sulfites must be added in order to prevent degradation (Comuzzo and Zironi, 2013).

3.3. HPLC analysis

Individual concentrations of 14 phenolic compounds presented in each wine sample were quantified by HPLC-UV/Vis analysis with the aim to find differences between wines produced in Georgia, Central and Western Europe (concentrations of compounds in all wine samples is shown in Table 4). Order of mean content of presented compounds in all investigated samples were as follows: gallic acid > epigallocatechin gallate > caffeic acid > p-coumaric acid > myricetin > syringic acid > vanillic acid > 3,4-dihydroxybenzoic acid > quercetin > chlorogenic acid > resveratrol > rutin > ferulic acid > kaempferol.

Georgian wine samples contained significantly higher concentrations of quercetin (p = 0.01), kaempferol (p = 0.05), and syringic acid (p = 0.05), while content of *trans*-resveratrol was considerably lower (p = 0.01) in Georgian wines than in West and Central European samples (Table 5). The highest quantities of quercetin, kaempferol and syringic acid were recorded in wines prepared from Saperavi cultivar (quercetin = $14.44-1.07 \mu g mL^{-1}$; kaempferol = $1.68-0.03 \, \mu g \, mL^{-1}$ and syringic acid $12.59-4.72 \, \mu g \, mL^{-1}$), followed by Pinot Noir (quercetin = $9.64-0.00 \,\mu g \, mL^{-1}$; kaempferol = 0.60–0.00 μg mL $^{-1}$ and syringic acid = 12.02–5.17 μg mL $^{-1}$) and Cabernet Sauvignon cultivars (quercetin = $8.51-0.00 \mu g \, mL^{-1}$; kaempferol = $0.63-0.00 \mu g \, mL^{-1}$ and syringic acid = 8.00-4.28 μ g mL⁻¹). On the contrary, Pinot Noir was the richest samples in resveratrol content, followed by representatives of Cabernet Sauvignon (concentration range 8.71–2.43 and 7.41–1.13 μ g mL⁻¹, respectively). In contrast to our results, McDonald et al. (1998) recorded higher content of quercetin in Cabernet Sauvignon (2.6–4.6 μg mL⁻¹) than in Pinot Noir (0.9–2.6 $\mu g\ mL^{-1}$) wines from France. However, our results are in the agreement with the study of Nikfardjam et al. (2006) where Hungarian Pinot Noir wine samples contained higher concentrations of quercetin and resveratrol (7.5 and 3.2 μg mL⁻¹, respectively) than Cabernet Sauvignon samples (5.5 and 2.8 μg mL⁻¹, respectively). Our data recorded for trans-resveratrol in Czech Pinot Noir samples (2.43 and 8.71 $\mu g \, m L^{-1}$) are similar or slightly higher than those of Kolouchova-Hanzlikova et al. (2004) which were in range from 1.322 to $6.253 \,\mu g \, mL^{-1}$. Wines belonging to Saperavi cultivar contained only moderate to low concentration of resveratrol, ranging from 5.11 to 0.32 μg mL⁻¹. In comparison, Chkhikvishvili et al. (2008) recorded values in range $0.69-1.17 \mu g mL^{-1}$ for Saperavi wines.

Although the differences were observed in red wines grouped according to geographical origin, it seems possible that cultivar played also important role because Georgian group included only two wines from different cultivars than Saperavi (one Cabernet Sauvignon and one Alexandrouli sample). Several comparative studies dealing with differences in phenolic composition across wine cultivars, vintage, and production area revealed elevated

 Table 4

 Concentration of phenolic compounds in studied wines (expressed as mean of two measurements).

Wine code	Compound ^a /(µg mL ⁻¹)													
	3,4-DB	CAF	COUM	CHL	FER	GAL	SYR	VAN	RESV	EGCG	KAEM	MYR	QUER	RUT
PN 1	7.15	13.84	9.32	2.11	1.45	117.84	9.16	7.06	8.71	52.25	ND ^{b,f}	5.95	0.97	ND ^{b,i}
PN 2	1.43	10.39	3.27	$ND^{b,c}$	1.53	64.08	5.17	5.06	2.43	36.31	$ND^{b,f}$	3.34	$ND^{b,h}$	0.89
PN 3	10.62	6.77	4.45	3.77	0.58	38.00	12.02	9.12	7.66	19.89	$ND^{b,f}$	6.94	0.32	$ND^{b,i}$
PN 4	2.60	26.40	4.65	2.53	1.33	104.65	5.69	5.17	3.50	58.87	0.47	3.17	7.95	3.26
PN 5	3.19	15.46	5.40	3.98	$ND^{b,d}$	99.59	5.48	4.51	5.75	54.83	0.60	6.66	9.64	1.99
CS 1	4.40	5.69	5.04	2.43	$ND^{b,d}$	70.48	5.33	3.73	1.95	31.97	0.17	5.31	1.12	1.52
CS 2	5.40	3.62	2.92	1.98	0.77	85.44	5.46	3.12	1.13	49.16	0.07	7.46	3.48	1.50
CS 3	3.25	9.15	6.18	2.24	1.31	43.77	5.54	2.58	2.05	24.13	0.22	14.50	8.51	4.46
CS 4	6.27	8.39	7.45	1.93	0.65	51.32	5.57	3.07	2.93	26.80	$ND^{b,f}$	11.34	0.58	2.94
CS 5	3.49	27.05	14.06	1.38	1.31	63.09	6.67	4.88	7.41	30.36	$ND^{b,f}$	7.64	0.45	$ND^{b,i}$
CS 6	5.23	6.31	7.34	2.97	1.06	47.38	4.28	3.39	2.50	19.42	$ND^{b,f}$	4.04	$ND^{b,h}$	$ND^{b,i}$
CS 7	4.28	12.55	7.87	1.91	0.59	96.74	8.00	3.82	1.17	50.19	0.63	9.21	7.62	$ND^{b,i}$
CM 1	0.68	11.02	11.14	1.67	1.12	24.79	8.57	5.64	4.50	17.89	$ND^{b,f}$	8.90	0.69	$ND^{b,i}$
CM 2	1.80	6.42	9.23	1.90	0.36	39.65	8.85	4.14	3.35	14.21	$ND^{b,f}$	7.09	1.58	$ND^{b,i}$
SA 1	6.87	15.51	13.89	2.01	1.74	85.72	8.42	8.52	3.79	44.59	0.36	13.39	12.57	2.73
SA 2	3.25	8.10	15.93	2.04	1.28	61.81	9.09	4.81	5.11	30.57	1.19	18.67	14.44	2.61
SA 3	3.28	4.98	9.56	2.20	0.88	74.03	8.44	3.97	1.36	32.59	0.54	11.27	8.71	$ND^{b,i}$
SA 4	1.86	19.29	16.53	2.01	1.77	43.27	8.14	4.46	3.29	27.40	0.80	15.30	9.70	2.45
SA 5	3.75	7.59	10.94	2.41	0.76	34.68	6.12	3.16	0.34	15.04	0.03	7.04	3.48	$ND^{b,i}$
SA 6	3.62	6.32	14.40	3.04	1.01	52.00	12.37	5.11	1.14	29.12	0.38	10.94	6.19	2.96
SA 7	2.13	3.94	5.24	3.92	0.52	41.50	4.72	2.83	1.24	24.12	1.68	14.46	12.18	2.37
SA 8	1.86	3.15	2.79	3.34	0.29	43.65	4.73	3.06	0.32	26.91	0.76	9.92	7.56	4.13
SA 9	9.06	7.99	9.08	2.82	0.65	101.38	12.59	6.89	0.85	52.60	0.27	8.79	3.53	$ND^{b,i}$
SA 10	7.15	6.63	12.70	3.49	1.79	91.70	12.59	9.63	1.12	56.28	0.24	3.98	1.28	$ND^{b,i}$
SA 11	6.72	4.81	3.62	1.79	0.45	97.57	10.24	8.05	0.43	53.18	0.22	2.63	1.07	1.82
AL 1	8.93	9.10	4.57	1.40	0.95	60.40	11.53	8.52	0.13	26.94	$ND^{b,f}$	$ND^{b,g}$	$ND^{b,h}$	$ND^{b,i}$
CH 1	0.32	1.11	0.84	0.79	0.32	4.10	0.10	0.15	$ND^{b,e}$	0.61	$ND^{b,f}$	$ND^{b,g}$	$ND^{b,h}$	$ND^{b,i}$
CH 2	1.00	2.77	0.99	1.82	0.17	7.22	0.25	0.25	$ND^{b,e}$	2.24	$ND^{b,f}$	$ND^{b,g}$	$ND^{b,h}$	$ND^{b,i}$
CH 3	1.21	2.40	1.13	1.50	0.37	8.24	0.40	0.37	ND ^{b,e}	1.93	$ND^{b,f}$	$ND^{b,g}$	$ND^{b,h}$	$ND^{b,i}$
CH 4	1.28	3.38	1.35	1.57	0.48	15.66	0.25	0.62	ND ^{b,e}	3.74	$ND^{b,f}$	$ND^{b,g}$	$ND^{b,h}$	$ND^{b,i}$
CH 5	1.44	8.24	3.47	1.55	0.52	3.23	0.37	0.33	ND ^{b,e}	1.23	$ND^{b,f}$	$ND^{b,g}$	$ND^{b,h}$	$ND^{b,i}$
CH 6	1.59	6.77	2.43	1.64	0.32	2.11	0.25	0.23	ND ^{b,e}	0.99	$ND^{b,f}$	$ND^{b,g}$	$ND^{b,h}$	$ND^{b,i}$
SB 1	0.81	1.41	0.76	1.94	0.25	15.02	0.27	0.33	$ND^{b,e}$	3.38	$ND^{b,f}$	$ND^{b,g}$	$ND^{b,h}$	$ND^{b,i}$
SB 2	0.94	4.09	2.43	1.47	0.46	1.99	0.17	0.20	$ND^{b,e}$	1.02	$ND^{b,f}$	$ND^{b,g}$	$ND^{b,h}$	$ND^{b,i}$
SB 3	1.06	1.33	1.76	1.35	0.30	5.77	0.22	0.28	$ND^{b,e}$	1.36	$ND^{b,f}$	$ND^{b,g}$	$ND^{b,h}$	$ND^{b,i}$
RK 1	1.29	7.41	0.84	3.22	0.21	9.93	0.21	0.35	0.32	1.68	$ND^{b,f}$	$ND^{b,g}$	$ND^{b,h}$	$ND^{b,i}$
RK 2	0.76	5.25	0.96	2.84	0.30	7.14	0.16	0.18	ND ^{b,e}	1.39	$ND^{b,f}$	$ND^{b,g}$	$ND^{b,h}$	$ND^{b,i}$
RK 3	2.70	7.34	3.32	2.49	0.57	10.31	0.51	0.55	ND ^{b,e}	2.00	$ND^{b,f}$	$ND^{b,g}$	$ND^{b,h}$	$ND^{b,i}$
RK 4	3.21	0.91	0.73	2.04	0.17	38.48	0.25	0.46	ND ^{b,e}	22.91	$ND^{b,f}$	$ND^{b,g}$	$ND^{b,h}$	$ND^{b,i}$

a 3,4-DB=3,4-hydroxybenzoic acid; CAF=caffeic acid; COUM=p-coumaric acid; CHL=chlorogenic acid; FER=ferulic acid; GAL=gallic acid; SYR=syringic acid; VAN=vanillic acid; RESV=resveratrol; EGCG=epigallocatechin gallate; KAEM=kaempferol; MYR=myricetin; QUER= quercetin; RUT=rutin.

content of flavonoids (especially quercetin, myricetin, and kaempferol), whereas decreased levels of *trans*-resveratrol in local grape varieties in comparison to commonly cultivated (Pinot noir, Cabernet Sauvignon) (McDonald et al., 1998; Landrault et al., 2001; Atanacković et al., 2012).

Cabernet Moravia were relatively rich in resveratrol and syringic acid content (concentration ranging between 4.50–3.35 $\mu g \ mL^{-1}$ and 8.85–8.57 $\mu g \ mL^{-1}$, respectively). Nevertheless, representatives of Cabernet Moravia had low quantities of

quercetin (range of concentrations were $1.58-0.69 \, \mu g \, mL^{-1}$). Kaempferol was not detected in Cabernet Moravia samples. Cabernet Sauvignon and Pinot Noir samples from Western Europe contained higher levels of kaempferol, quercetin and rutin then those from Central Europe. For example, quercetin concentrations reached from 0.58 to $9.64 \, \mu g \, mL^{-1}$ in West European samples while in Central European samples quercetin reached from 0.00 to $0.97 \, \mu g \, mL^{-1}$. Also Goldberg et al. (1998) observed higher levels of quercetin in samples from warmer climates.

Table 5Content of syringic acid, *trans*-resveratrol, quercetin, and kaempferol in red wine samples grouped according to their origin (mean ± SD).

Region	Syringic acid [*]	Resveratrol**	Quercetin**	Kaempferol [*]
Georgia (n=13) Western Europe (n=6) Central Europe (n=7)	$\begin{array}{c} 9.00 \pm 2.77^a \\ 5.51 \pm 0.12^b \\ 7.82 \pm 2.64^{ab} \end{array}$	$\begin{array}{c} 1.56\pm1.53^{a} \\ 2.88\pm1.63^{ab} \\ 5.22\pm2.65^{b} \end{array}$	$\begin{array}{c} 7.36 \pm 4.43^a \\ 5.21 \pm 3.98^{ab} \\ 0.80 \pm 0.50^b \end{array}$	$\begin{aligned} 0.59 \pm 0.47^a \\ 0.31 \pm 0.22^{ab} \\ 0.00 \pm 0.00^b \end{aligned}$

Different letters within each column showed significant differences (ANOVA with Tukey post hoc test; $p \le 0.05$, $p \le 0.01$).

^b Compound not detected.

^c Limit of detection (LOD) = $0.398 \mu g \, mL^{-1}$.

 $^{^{}d}\ LOD = 0.070\,\mu g\,mL^{-1}.$

 $^{^{}e}$ LOD = 0.072 μ g mL⁻¹.

f LOD = $0.010 \,\mu g \, mL^{-1}$.

 $^{^{\}rm g}$ LOD = 0.220 μ g mL⁻¹ h LOD = 0.074 μ g mL⁻¹.

i LOD = $0.042 \,\mu g \, m L^{-1}$.

None statistical differences between white wines grouped according to their origin were revealed. Quercetin and kaempferol were under detection limits in representatives of white wines. Resveratrol was only detected in sample RK 1 (0.32 μ g mL⁻¹). In comparison to other white wines, RK 4 differed in high gallic acid and epigallocatechin gallate content (38.48 and 22.91 μ g mL⁻¹, respectively). Both RK 1 and RK 4 wine samples were prepared by Kakhetian method. Higher content (5.82 μ g mL⁻¹) of resveratrol in Saperavi wine prepared by Kakhetian method than in Saperavi wines prepared by European method $(0.69-1.17 \,\mu g \,mL^{-1})$ was recorded also by Chkhikvishvili et al. (2008). Also the higher content of epigallocatechin gallate is in concordance with study of Shalashvili et al. (2007) where the white wines manufactured by Kakhetian method where significantly richer in total catechines. Further, our observations are in the agreement with conclusions made by Goldberg et al. (1999) that enological techniques have higher impact than climatic factors on the content of phenolic compounds in the case of white wine.

4. Conclusion

In summary, Georgian red wines showed higher antioxidant potential than red wines from Central and Western Europe. Red wines prepared from Georgian native cultivar Saperavi and cuvee Saperavi + Saperavi Budeshuriseburi showed highest antioxidant effect followed by Pinot Noir and Cabernet Sauvignon. Further, Georgian red wines differed from Central and West European red wines by higher content of quercetin, kaempferol, and syringic acid and by lower content of trans-resveratrol. No statistical differences among cultivars and regions were revealed in the case of white wines. However, white wines prepared by Kakhetian winemaking method possessed increased antioxidant activity and contained higher amounts of phenolic compounds. Our results indicate that antioxidant effect and content of phenolics in red wines was influenced by geographical origin and by cultivar while winemaking technology was the main factor influencing antioxidant activity and concentration of phenolic compounds in studied white wines. This study showed interesting potential of Georgian wines and therefore we suggest further detailed research of grapevine cultivars originated from Caucasus region because it could bring interesting results regarding chemical composition and biological activity connected with health-promoting effects.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jfca.2014.12.029.

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