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CHEMISTRY

Chemical composition of red wines made from hybrid grape and common grape (*Vitis vinifera* L.) cultivars

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Abstract. Since the formulation of the "French paradox", red grape wines are generally considered to be health-promoting products rather than culpable alcoholic beverages. The total wine production, totalling an equivalent of 30 billion 750 mL bottles in 2009, only verifies the fact that global demand is increasing and that the polyphenols present in wines are accounting for a significant proportion of the daily antioxidant intake of the general population. Both statements justify the interest of new regions to be self-sufficient in the wine production.

Novel cold tolerant hybrid grape varieties also make it possible to produce wines in regions where winter temperatures fall below −30 °C and the yearly sum of active temperatures does not exceed 1750 °C. Also the greater disease resistance of hybrid grapes – which allows production with less chemical plant protection agents – attracts attention. It is understood that the new regions and varieties raise questions about the quality of these wines. Therefore, the aim of our work was to determine to which extent wines produced from hybrid grapes differ from wines vinified from common grapes regarding their phenolic, saccharidic, and acidic spectra and elemental composition.

Results demonstrate that although the polyphenolic spectra of red wines produced from hybrid grapes are generally similar to those of traditional wines, they show a wider range of anthocyanins, a balanced phenolic acid profile, qualitative differences in saccharide composition, and a very low heavy metal content.

Key words: hybrid grape wines, anthocyanins, hydroxystilbenes, metals, polyphenols, saccharides.

INTRODUCTION

Since the formulation of the "French paradox" by Renaud and De Lorgeril (1992), red grape wines have had the reputation of reducers of the incidence of heart infarction. The health benefits of red wines may be contributed to the complex of a number of different anthocyanins, catechins, proanthocyanins, hydroxystilbenes (*trans*-resveratrol in particular), and various flavonols (De Pascual-Teresa et al., 2010; Mikstacka et al., 2010; Dolinsky and Dyck, 2011; Kim et al., 2011; Smoliga et al., 2011). Although many of these polyphenols have been reported to have beneficial effects in vitro, studies in humans still show controversial results (Berger et al., 2012). Therefore, the chemical studies of wines should not only involve polyphenols, but also potentially beneficial or harmful saccharides, organic acids, metals, and so forth.

Interspecific crossing of grapevines has become a new focus for breeding programmes all around the world. Although the sensorial attributes of wines produced from these new varieties are distinct and therefore scorned by many oenologists, it is understood that the higher tolerance of hybrids for powdery mildew,

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nematodes, and even phylloxera will eventually help to reduce the pest control chemicals used for grape production and hence contribute to the global food security.

The qualitative and quantitative composition of wines made from the berries of *Vitis vinifera* L. cultivars has been extensively studied. On the contrary, according to our knowledge, until now mostly polyphenols have been studied in grape species other than *V. vinifera* (Zhao et al., 2010; Liang et al., 2012). It has been reported that the genotype affects the composition of anthocyanins (Jánváry et al., 2009); however, very little is known about the saccharidic, phenolic acid, and metal spectra of wines made from grapes of hybrid origin.

It can be hypothesized that the differences between wines made of interspecific hybrid grapes and *V. vinifera* cultivars may extend beyond polyphenols, and these differences may be extended to organic acids and saccharides. We postulate that although regarding some of these features, wines produced from hybrid cultivars may show unique properties, their general composition is similar to traditional red wines.

The aim of our work was to compare wines produced from hybrid grapes and wines made from widespread *V. vinifera* cultivars and to identify the differences in their chemical composition.

MATERIALS AND METHODS

Studied wines

Polyphenols, phenolic acids, saccharides, and microelements were identified and quantified in wines made from *Vitis vinifera* L. cultivars 'Cabernet Sauvignon', France (CSFr), Spain (CSEs), and Chile (CSCl); 'Pinot Noir', Romania (PNRo); 'Shiraz', South Africa (ShZA); 'Merlot', South Africa (MeZA); and from hybrid grape cultivars 'Rondo', Estonia (RoEt); and a blend of 'Rondo' (50%), 'Zilga', and 'Hasanski Sladki', Estonia (BlEt). Traditional wine samples were purchased from local supermarkets and were randomly chosen to represent the most popular wine grape cultivars in the world.

'Rondo' originates from a cross (1964) by Professor V. Kraus in the former Czechoslovakia by crossing 'Zarya Severa' (*V. amurensis* hybrid) and 'St. Laurent'. Wines from 'Rondo' are purplish-black in colour, show notes of black currant in their taste, and are sensorially most similar to the wines from 'Cabernet Sauvignon'. The latter is represented by three wines from different countries in this study. 'Zilga', bred by a Latvian breeder P. Sukatnieks, is a cross of 'Smuglyanka' (*V. amurensis*) × 'Dvietes Zila' (*V. labrusca*) × 'Yubilei Novgoroda' (*V. vinifera* × *V. labrusca*). 'Hasanski Sladki', also known as 'Baltica', is an interspecific hybrid of *V. amurensis*, *V. labrusca*, *V. riparia*, and *V. vinifera* (75% *V. amurensis*) bred in Russia by A. K. Bous.

Estonian wines were vinified from grapes grown in the experimental vineyard of the Estonian University of Life Sciences (58°21′25″N, 21°36′16″E) in 2009 using traditional winemaking methods. The grapes were grown using organic practices and no spraying treatment or mineral fertilizer was applied.

Chemicals used

Dibasic sodium phosphate, sodium carbonate, Folin-Ciocalteu reagent (FCR), sodium hydroxide, sodium tetraborate decahydrate, 1-butyl-3-methylimidazolium chloride, nitric acid, and hydrogen peroxide as well as standard compounds catechin, piceid (polydatin), *trans*-resveratrol, quercetin, myricetin, quercetin glucoside, kaempferol, 3,4-dihydroxybenzoic acid (3,4-DHB), caffeic acid, syringic acid, salicylic acid, ferulic acid, gallic acid, sinapinic acid, myo-inositol, arabitol, glucitol, mannitol, fucose, cellobiose, galactose, glucose, fructose, arabinose, xylose, ribose, acetic acid, were purchased from Sigma-Aldrich (Germany or USA). The stock atomic spectroscopy standard solutions (1000 mg/L) of Pb, Cd, As, Cu, Mg, K, Na, Mn, Zn, and Fe were purchased from Fluka, Switzerland. All chemicals were of analytical grade and used as received. High-purity water (Milli-Q, Millipore, USA) was used for all solutions of standards, HPLC eluents, background electrolytes, and dilution of samples. The cellulose chromatographic paper FN16 was from Whatman.

Determination of total polyphenols

The total polyphenol content in the wines was measured by a novel paper microzone-based colorimetric assay described by Vaher and Kaljurand (2012). Briefly, a 2 μL solution of 2 M (with respect to acid) FCR was manually spotted onto Whatman FN16 filter paper and 2 μL of wine samples and solutions of different concentrations of gallic acid (in the range of 0.25–10.0 mM) were later applied to the FCR spots for calibration. Then 2 μL of 20% sodium carbonate was spotted. After 10 min, an intense blue colour developed.

The whole sheet of Whatman paper containing the calibration and wine samples (diluted 10 times) was then photographed using a mobile phone camera. The picture was loaded into a personal computer, and a calibration curve was created using the freeware image processing program ImageJ. The analyses were carried out in triplicate.

HPLC analysis of polyphenols coupled with a diode array detector and an electrospray

Liquid chromatography mass spectrometer (LC-DAD-MS/MS) was carried out with a 1100 series system from Agilent Technologies (Palo Alto, USA) according to

Püssa et al. (2006). For the separation of compounds, a reversed phase HPLC column (Zorbax 300SB-C18, 2.1 mm × 150 mm; 5 μm; Agilent Technologies) was used in a stepwise mobile phase gradient of 0.1% formic acid and acetonitrile at a flow rate of 0.3 mL/min at 35 °C. The sample injection volume was 5 or 10 μL. For the detection and identification of substances, the Agilent 1100 Series UV-Vis DAD and 1100 Series LC/MSD Trap-XCT with an electrospray ionization interface were connected to an Agilent 1100 Series instrument consisting of an autosampler, a solvent membrane degasser, a binary pump, and a column thermostat. The MS^2 conditions of the negative or positive ion detection: m/z interval 50–1000; target mass 400; number of precursor ions 2; maximum accumulation time 100 ms; compound stability 100%; flow rate of the drying gas (N_2 from the generator) 10 L/min, gas temperature 350 °C; nebulizer pressure 30 psi, collision gas He pressure 6×10^{-6} mbar. The DAD was working at an interval of 200–600 nm. Prior to analysis, wine samples were centrifuged and cooled to 4 °C using an Eppendorf 5810 R centrifuge at 4000 rpm for 10 min. Samples were filtered using Sartorius Minisart RC 4 syringe filters (pore size 0.45 μm) before injection.

HPLC analyses were carried out in a single repetition to assess the qualitative differences. Therefore the data on specific polyphenolic compounds are presented without confidence intervals.

Capillary electrophoresis of phenolic acids and saccharides

Phenolic acids and saccharides were identified using an Agilent 3D capillary electrophoresis instrument (Agilent Technologies, Waldbronn, Germany), equipped with a UV/Vis DAD. Phenolic acids were identified according to the methods described by Helmja et al. (2008) and Peres et al. (2009). The measurements of saccharides were conducted according to the methods described by Rovio et al. (2011) and Vaher et al. (2011).

Uncoated fused silica capillaries (Polymicro Technologies, AZ, USA) with an internal diameter of 50 μm and a length of 71.5/80 cm (effective length/total length) were employed in the experiments. The separation voltage was adjusted to 17 kV for the saccharide analysis. The wavelength of 270 nm was used for detection. The injection pressure was set to 35 mbar and the injection time was 10 s. The analysis temperature was 15 °C.

For the separation of phenolic acids the length of capillaries was 51.5/60 cm, separation voltage 25 kV, analysis temperature 25 °C, and detection wavelength 210 nm. The injection pressure was 50 mbar and injection time 10 s. Before the measurements, new capillaries were conditioned by rinsing them sequentially with 1 M sodium hydroxide and ultrapure water. Between analyses, the capillaries were rinsed with 5% acetic acid, water, and the electrolyte solution, for 5 min with each solution.

The background electrolyte (BGE) for carbohydrates consisted of 130 mM sodium hydroxide and 36 mM disodium hydrogen phosphate (pH 12.6). For analysis of phenolic acids the BGE was 25 mM sodium tetraborate decahydrate (pH 9.3). Analyses were carried out in triplicate.

Atomic absorption spectroscopy

Spectra AA 220F and 220Z atomic absorption spectrometers (Varian, Mulgrave, Australia) equipped with a side-heated GTA-110Z graphite atomizer, a Zeeman-effect background correction, and an integrated autosampler were used. Graphite tubes with coating and platforms made of pyrolytic graphite were used throughout the work. Argon of 99.99% purity (AGA, Helsinki, Finland) was used as the purge gas. Acetylene of 99.99% purity (AGA, Helsinki, Finland) was used as the fuel gas in flame atomic absorption spectroscopy.

For the determination of total mineral element constituents 1 mL of wine sample was mineralized with 4 mL of concentrated nitric acid and 1 mL of concentrated hydrogen peroxide in teflon bombs using a microwave oven (Anton Paar Multiwave 3000, Graz, Austria) at temperatures up to 180 °C for 30 min. After cooling down, the solution in the bombs was transferred to volumetric flasks (15 mL) with ultrapure water. All the experiments were made in triplicate. For the determination of As and Cd the colloidal Pd modifier, synthesized according to the procedure described by Volynsky and Krivan (1997), was used. Other elements were detected according to the procedures described by Aceto et al. (2002).

Statistical methods and software

The HPLC 2D ChemStation Software with a ChemStation Spectral SW module was used for the HPLC process guidance. The paper micro-zone colorimetric assay was done using the ImageJ software by Wayne Rasband. Quantification procedures and ANOVA were carried out using R statistical software (version 2.14.1).

RESULTS AND DISCUSSION

Total phenolics

The content of total phenolics ranged from 1.32 to 2.08 g/L of gallic acid equivalent (Table 1). The overall average of all samples was 1.77 g/L. Higher concentrations were recorded in the wines CSCl and CSFr, both vinified from ‘Cabernet Sauvignon’. Although wines made from the hybrid grape cultivars in our experiment

Table 1. The concentration of the main anthocyanins, flavan-3-ols, flavonols, and hydroxystilbenes in the studied wines

	CSFr	CSEs	CSCl	PNRo	ShZA	MeZA	RoEt	BlEt
Total phenolics (g/L GAE)[a]	2.08±0.15	1.81±0.14	2.07±0.19	1.93±0.16	1.71±0.16	1.49±0.12	1.32±0.10	1.72±0.15
Anthocyanins (AUC[b])	320.0	419.0	268.0	287.0	514.0	333.0	261.0	256.0
Delphinidin-3-O-glucoside	4.12	3.32	1.69	3.90	2.15	1.84	–	–
Peonidin-3,5-O-diglucoside	–	–	–	–	–	–	39.24	31.49
Malvidin-3,5-O-diglucoside	–	–	–	0.21	0.38	–	34.66	34.22
Malvidin-3-O-glucoside	46.55	36.71	40.38	50.20	44.26	41.47	6.75	6.86
Malvidin-acetylglucoside	10.85	28.52	25.53	17.97	21.07	19.46	0.73	0.57
Malvidin-3-O-arabinoside	2.59	4.28	2.88	6.32	5.26	7.34	4.10	2.19
Petunidin-3-O-glucoside	6.86	7.42	4.51	5.65	4.44	3.79	0.75	0.04
Flavonols (g/L)								
Myricetin	5.7	6.3	2.6	1.0	3.5	4.2	0.7	1.0
Quercetin (q)	7.7	9.9	8.5	–	3.0	11.7	1.5	2.8
Q. glucuronide	8.9	14.6	20.8	–	8.4	20.8	8.4	12.8
Q. glucoside	11.9	15.2	13.0	–	–	1.0	11.6	15.6
Q. galactoside	1.3	1.4	1.2	–	–	1.9	1.8	2.0
Q. rhamnoside	0.4	0.3	0.4	–	1.2	0.3	0.4	0.4
Flavan-3-ols (PH[c])								
Monomers (2)	33.0	25.0	20.0	47.0	36.0	28.0	56.0	56.0
Dimers (5)	114.0	136.0	152.0	204.0	132.0	138.0	142.0	277.0
Trimers (4)	14.0	28.0	18.0	44.0	23.0	23.0	16.0	19.0
Total	161.0	189.0	190.0	295.0	191.0	189.0	214.0	352.0
Hydroxystilbenes (mg/L)								
trans-Resveratrol	1.2	1.2	1.1	1.7	1.0	1.7	1.5	1.6
cis-Resveratrol	0.9	1.0	1.1	0.9	1.2	1.3	1.0	1.3
trans-Piceid	4.3	4.1	2.8	3.2	4.5	7.9	2.3	3.1
trans-Resveratroloside	1.9	2.3	1.9	6.6	2.5	2.9	2.2	2.5
Total	8.3	8.6	6.9	12.4	9.2	13.8	7.0	8.5

[a] Values represent means of triplicate determinations ± SD.
[b] AUC – area under chromatographic curve at $\lambda = 520$ nm. Individual anthocyanins are represented as a percentage of total pigmented compounds quantified by extracted ion mass peak areas.
[c] PH – MS peak height $\times 10^{-6}$. Numbers in parentheses after flavan-3-ols show the number of counted isomers.
– not detected.

showed values below average – 1.32 g/L for RoEt and 1.72 g/L for the BlEt – there is evidence that several wild grape species may exhibit higher concentrations than *V. vinifera* cultivars (Liang et al., 2012).

It has also been noted that the polyphenol content is in a negative correlation with the fruit weight (Liang et al., 2012.). This is understandable, knowing that several wild species exhibit higher skin to pulp ratio due to their smaller fruit weight. However, in our experiment the different methods used for wine production, the vinification year, and microclimate most likely influenced the phenolics content more than the species used.

Anthocyanins

In our experiment a total of 22 pigmented compounds were detected and identified. Malvidin was discovered to be the most abundant anthocyanidin present in all wines, which is in agreement with the literature (Wrolstad, 2000). Altogether 12 compounds containing malvidin were identified, and they accounted for 57.7% to 62.3% of the pigments of wines made from hybrid grapes and 73.3% to 86.9% of the pigments found in wines made from common grape cultivars. The highest concentration of malvidin compounds was registered in the ShZA. It is interesting to note that in hybrid grape wines most malvidin was in the form of malvidin-3,5-O-diglucoside whereas in common grape wines malvidin-3-O-glucoside was found to be the most abundant form (Table 1).

The profile of anthocyanins was species- and cultivar-dependent, whereas the wines of hybrid species distinguished by their content of anthocyanidin diglucosides. In the hybrid grape wines, peonidin was found in similar concentrations as malvidin, the main form being peonidin-3,5-O-glucoside. Compared to the traditional wines, the hybrid grape wines also contained less petunidin and no delphinidin compounds were discovered.

All the anthocyanidins determined in wines from *V. amurensis* by Zhao et al. (2010) were also detected in

our experiment. Our results show that peonidin-3,5-O-diglucoside was present only in the hybrid grape wines, and except traces found in the ShZA and PNRo, malvidin-3,5-O-diglucoside was also found to be characteristic of hybrid grape wines. This is in agreement with Jánváry et al. (2009), who stated that 3,5-O-diglucosides of anthocyanidins are only found in wines made from berries of hybrid grapevine cultivars while *V. vinifera* cultivars are unable to produce diclucosides due to a double mutation in the anthocyanin 5-O-glucosyltransferase gene. Our results confirm the idea that the presence of 3,5-O-diglucosides can be used as a discriminant variable to differentiate wines produced from hybrid grapes and *V. vinifera*.

The total relative anthocyanin content was estimated by the areas under chromatograms at 520 nm. The highest total anthocyanin content was determined in the wine ShZA. The hybrid RoEt and BlEt showed total anthocyanin content values 21–23% lower than the mean, representing the lowest values in our study. It is generally understood that the total anthocyanin content in wines is affected by the grape variety, *terroir*, and winemaking practices (most importantly the duration of the on-skin fermentation). Unarguably, the amount of sunlight necessary for anthocyanin formation is lower in high latitude vineyards. To the contrary, some authors have noted higher concentrations of pigments in *Vitis* species other than *V. vinifera* (Liang et al., 2012). We must therefore conclude that shortage in light and warm temperatures in cold climate grape growing must be compensated by choosing appropriate genetics and vinification methods that utilize the anthocyanins of the grape to the fullest.

Flavonols

The flavonol group of polyphenols contained aglyconic quercetin and myricetin, traces of kaempferol as well as their various glycosides. Wines made from the hybrid grapes were characterized by a relatively higher concentration of glycosides; wines from 'Shiraz' and 'Cabernet Sauvignon', in turn, contained more flavonol aglycones (Table 1). The content of myricetin and kaempferol aglycones was found to be 5 to 7 times lower in the Estonian wines. The highest content of flavonols was established in the wines from 'Cabernet Sauvignon'. The wine PNRo had very low concentrations of flavonols in any form (Table 1). The concentration of both quercetin glucuronide and rhamnoside was quite equal in all the studied wines except the PNRo.

Flavan-3-ols

The total content of these health-promoting flavonoids (EFSA, 2012) was found to be the highest in the BlEt and in the PNRo (Table 1). Catechin and epicatechin as well as their four isomeric dimers (procyanidins) and five B-type isomeric trimers were discovered in all studied wines without any qualitative difference observed. High flavan-3-ols concentrations in the hybrid grape wines can possibly be explained by the high seed to fruit ratio of the 'Rondo' grape, as flavan-3-ols are mainly found in the grape seeds.

Vinification methods, such as on-skin maceration time, affect the flavan-3-ol concentration in wine. Therefore the sensorial properties of hybrid grape wines might benefit from shorter on-skin maceration times. This again would have a negative influence on the anthocyanin concentration of the wine. Although the high flavan-3-ol content together with high acidity and low alcohol content generally produces wines with an "unbalanced palate", it is understood that from a nutritionist's point of view wines with a high flavan-3-ol content are favoured.

Hydroxystilbenes

The total molar concentration of all the resveratrol forms was in the range of 6.9 to 13.8 mg/L. The highest content of resveratrol and its derivatives was established in the wines MeZA and PNRo, whereas the BlEt and RoEt showed results below average (Table 1). Resveratrol was found in four different forms: as an aglycone in both *trans*- and *cis*-conformations and as a glycoside (3-O-glucoside or piceid or polydatin and 4'-O-glucoside or resveratroloside). In the wines studied, resveratrol was mostly in the form of these two glucosides. Different hydroxystilbene forms in the studied wines were in the same order as the numbers published in the literature (Gambelli and Santaroni, 2004; La Torre et al., 2006). The BlEt was distinguished only by a slightly higher *trans*-piceid and *cis*-resveratrol content. The wines PNRo and MeZA were characterized by exceptionally high *trans*-piceid and *trans*-resveratrol contents, respectively.

Phenolic acids

The phenolic acid profiles of the studied wines varied substantially (Table 2). Chlorogenic acid was only found in the CSFr and BlEt. With the exception of chlorogenic acid, the CSFr had the poorest acid profile in our experiment: only the presence of salicylic, caffeic, and gallic acids was established. Sinapinic acid was only found in the MeZA and PNRo, whereas caffeic, salicylic, and gallic acids were present in all wines.

Compared to the results of the study by La Torre et al. (2006), three additional acids were found using capillary electrophoresis: sinapinic, chlorogenic, and salicylic acid. Furthermore, small amounts of citric, caftaric, 2-S-glutathionyl caftaric, coutaric, and coumaric acids were also identified in all studied wines

using the MS/MS data from chromatographic analysis (Fig. 1). Wines with exceptional acid profiles were the PNRo and ShZA. The former was exceptionally rich in gallic, sinapic, and vanillic acids, the latter in syringic, caffeic, and salicylic acids. The hybrid wine RoEt was found to have a very balanced phenolic acid profile whereas the BlEt showed only slightly more 3,4-DHB.

Titratable acidity was not measured in our experiment. According to the literature, hybrid grapes may show up to twofold higher total acid content than the grapes of *V. vinifera* (Liang et al., 2012). A high acid content influences the sensory parameters of the wine but also acts as a preservative and stabilizes the wine colour due to the chemical dehydroxylation of anthocyanins in low pH conditions. It is also well known that red wines with a relatively high acid content require less sulphur dioxide to preserve the wine from oxidation and bacterial spoilage.

Saccharides and sugar alcohols

Altogether 12 saccharides and sugar alcohols (glucose, galactose, fructose, arabinose, xylose, ribose, fucose, cellobiose, glucitol, mannitol, arabitol, and myo-inositol) were identified using capillary electrophoresis. The total monosaccharide concentration in the studied wines varied significantly and ranged from 0.5 to 54.8 g/L. It is evident that glucose and fructose are responsible for most of the variation (Table 3). If the principal grape sugars were not taken into account, differences between wines were much more modest with concentrations ranging from 0.39 to 2.55 g/L.

The hybrid grape wines generally showed the lowest concentrations of all the above-mentioned saccharides. Surprisingly, arabinose and arabitol were not present in the wines made from hybrid grapes. Also, the cellobiose content was found to be at least 8 times lower than the average of our experiment (Table 3).

The content of myo-inositol, a polyol related with bacterial spoilage in wines, ranged from 0.26 to 1.43 g/L. While the highest concentration was observed in the CSEs, the wines from hybrid grapes showed the lowest values in our experiment: 0.26 and 0.39 g/L for BlEt and RoEt, respectively. Also the content of mannitol was found to be mostly lower in the wines made from hybrid grapes. Xylose and ribose were only detected in the CSFr. It was noted by Noe et al. (1999) that the increases of xylose and ribose as well as arabinose, rhamnose, and galactose can be directly related to the enzymatic treatment of wine.

It is understood that vinification methods strongly affect the sugar content. To ensure products with optimal sugar and alcohol levels, most producers use methods to artificially stop fermentation. This process can greatly affect the concentration of the principal sugars, but does not affect the concentration of arabinose, rhamnose, ribose, xylose, and galactose (Rovio et al., 2011). Therefore, the general low concentrations of monosaccharides can be explained by the genetic differences of hybrid grapes and the short growing season characteristic of the high geographic latitude of the experimental vineyard.

Metals

The total concentration of metals ranged from 1047 to 1340 mg/L, with potassium accounting for 87.5–92.3% of the total concentration. When the K content was not taken into account, the total concentration of metals ranged from 101.5 to 150.2 mg/L.

Hybrid grape wines excelled in their low concentrations of potentially harmful heavy metals. The content of lead, cadmium, and copper in wines from hybrid grapes was found to be from 39% to 58% below the average of our experiment (Table 4). The amount of lead in wine is restricted in several countries by law to guarantee consumer health protection (Aceto et al., 2002). In our experiment higher concentrations of Pb were detected in the PNRo and the CSFr, lower concentrations in wines from Chile and Estonia. For arsenic, the RoEt showed an exceptionally low concentration – only 1.92 μg/L. It is noteworthy that the CSCl in our experiment contained almost 31 times more arsenic than the hybrid RoEt from Estonia.

The concentration of iron was also found to be lower in the Estonian wines, being 14- to 18-fold lower than the average. It has been shown that iron interacts with red wine phenolics during in vitro digestion, decreasing their antioxidant capacity (Argyri et al., 2006). According to the authors' opinion, a low iron content in red wine would therefore be desirable.

Generally, the mineral composition of grapes and wine originates from the vineyard soil, and is not specified by the genetics of the grape. Other sources may include the spray treatments (Cu from the Bordeaux mixture) and soil dust on grape skins (Baxter et al., 1997). Our results confirm the idea that the variations in metal contents may also be used as fingerprints to determine the origin (or authenticity) of a wine. The idea of being able to distinguish wines by their chemical composition has been expressed by many authors, but successful separation only by polyphenolic determinations is difficult and imprecise (Gambelli and Santaroni, 2004). Trace elements can be considered as good indicators of the geographical origin of wines because the chemical elements are not metabolized or modified during the fermentation process (Kallithraka et al., 2001).

Table 2. Phenolic acids in the studied wines

	CSFr	CSEs	CSCl	PNRo	ShZA	MeZA	RoEt	BlEt
Chlorogenic acid	249.95±13.22	–	–	–	–	–	–	84.11±5.04
Ferulic acid	–	–	153.44±8.09	135.53±7.27	35.61±1.95	63.04±3.40	72.18±3.79	53.90±2.96
Vanillic acid	–	–	–	151.95±9.28	95.32±5.81	–	60.07±3.57	–
Salicylic acid	10.71±0.65	8.44±0.42	20.73±1.45	21.75±1.42	71.22±4.88	19.61±1.27	25.05±1.55	26.86±1.87
Syringic acid	–	25.71±1.47	21.42±1.19	9.08±0.58	44.63±2.21	9.09±0.49	20.37±1.12	7.31±0.37
3,4-Dihydroxybenzoic acid	–	8.87±0.62	–	–	–	12.15±0.85	–	6.89±0.48
Caffeic acid	39.31±2.01	48.30±2.41	29.79±1.51	25.45±1.37	69.61±3.58	37.49±1.88	29.34±1.47	39.04±1.98
Gallic acid	295.29±17.51	246.70±14.91	215.62±12.98	385.55±23.13	273.57±16.21	312.03±18.72	194.49±11.67	294.11±17.65
Sinapinic acid	–	–	–	75.01±4.50	–	48.47±2.81	–	–

Values represent means of triplicate determinations ± SD; – not detected.

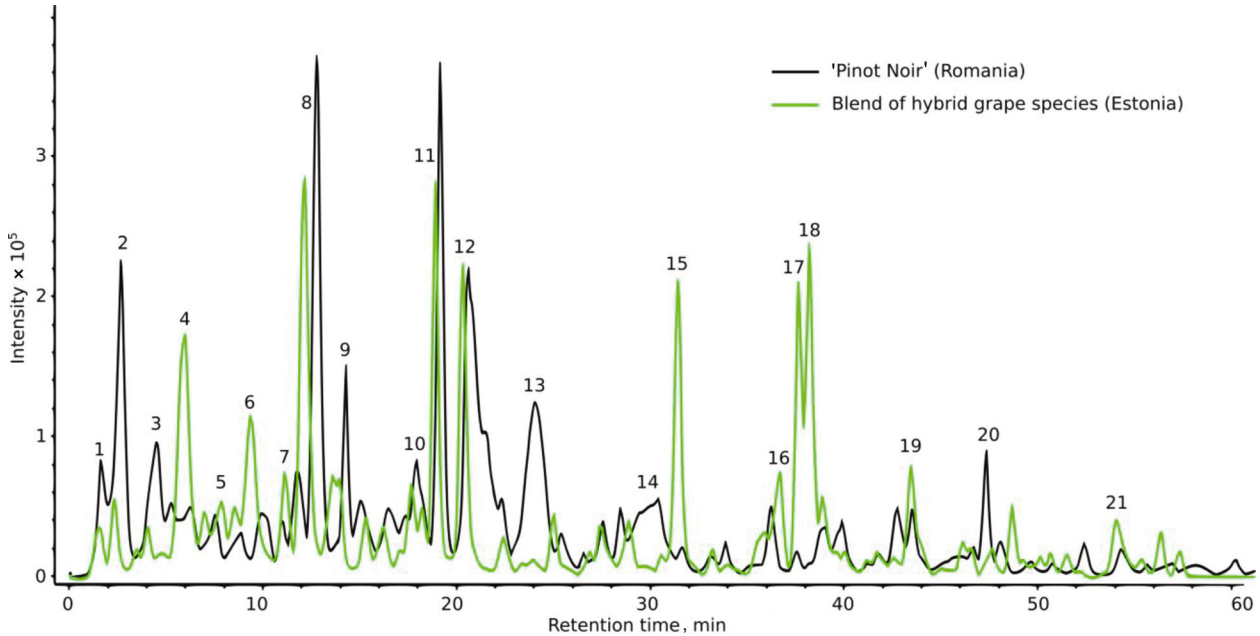

Fig. 1. Base peak chromatograms (negative mode) of the PNRo and BlEt. 1 – citric acid (191); 2 – gallic acid (169); 3 – unknown (657); 4 – caftaric acid (311); 5 – 2-S-glutathionyl caftaric acid (grape reaction product (GRP)) (616); 6 – coutaric acid (295); 7 – procyanidin B1 (577); 8 – catechin (289); 9 – coumaric acid glycoside (325); 10 – syringic acid (197); 11 – procyanidin B4 (577); 12 – epicatechin (289); 13 – unknown (427); 14 – piceid hydroxybenzoate (509); 15 – myricetin glucoside (479); 16 – quercetin galactoside (463); 17 – quercetin glucuronide (477);18 – quercetin glucoside (463); 19 – piceid (389); 20 – quercetin derivative (507); 21 – quercetin (301). The number in parentheses indicates the precursor ion m/z.

Table 3. Composition of saccharides and their concentration (g/L) in the studied wines

Wine	Myo-inositol	Arabitol	Glucitol	Mannitol	Fucose	Cellobiose	Galactose	Glucose	Fructose	Arabinose	Xylose	Ribose	Total
CSFr	0.34±0.03	0.02±0.00	0.09±0.01	0.08±0.01	0.04±0.00	0.07±0.01	0.05±0.01	0.95±0.91	0.94±0.09	0.08±0.01	0.03±0.00	0.02±0.00	2.72±0.35
CSEs	1.43±0.15	0.08±0.01	0.17±0.02	0.33±0.03	0.16±0.02	0.21±0.02	0.18±0.02	27.18±2.68	25.02±2.31	–	–	–	54.75±10.08
CSCl	0.49±0.05	0.05±0.01	0.15±0.01	0.17±0.02	0.08±0.01	0.06±0.01	0.07±0.01	2.25±0.22	2.25±0.22	0.16±0.01	–	–	5.74±0.84
PNRo	0.46±0.04	0.12±0.01	0.03±0.00	0.12±0.01	–	0.02±0.00	0.01±0.00	12.6±1.18	5.25±0.49	–	–	–	18.61±3.79
ShZA	0.38±0.03	0.11±0.01	0.11±0.01	0.14±0.01	0.07±0.01	0.09±0.01	0.05±0.01	0.50±0.06	0.69±0.07	0.18±0.02	–	–	2.31±0.22
MeZA	0.33±0.03	0.12±0.01	0.11±0.01	0.14±0.01	0.02±0.00	0.06±0.01	0.05±0.01	1.11±0.10	1.01±0.09	0.28±0.02	–	–	3.21±0.38
RoEt	0.39±0.04	–	0.14±0.01	0.04±0.00	0.02±0.00	0.01±0.00	0.01±0.00	0.05±0.00	0.02±0.00	–	–	–	0.69±0.11
BlEt	0.26±0.03	–	0.08±0.01	0.02±0.00	0.01±0.00	–	0.02±0.00	0.02±0.00	0.04±0.00	–	–	–	0.45±0.07
Average	0.51	0.08	0.11	0.13	0.06	0.08	0.05	5.58	4.4	0.18			

Values represent means of triplicate determinations ± SD; – not detected.

Table 4. Metal content (mean of triplicate determinations ± SD) of the wines studied

Wine	Pb	Cd	As	Cu	Mg	K	Na	Mn	Zn	Fe
	μg/L				mg/L					
CSFr	16.58±0.15	8.62±0.09	27.75±0.27	333.3±3.1	87.8±0.9	1237.5±4.4	10.28±0.10	0.46±0.01	0.61±0.01	3.57±0.04
CSEs	7.83±0.08	6.58±0.08	10.42±0.11	333.3±3.0	87.2±0.9	945.8±9.4	11.02±0.11	0.93±0.01	0.50±0.01	1.59±0.02
CSCl	5.08±0.05	8.94±0.08	58.75±0.58	25.0±0.3	99.7±0.9	1067.9±10.8	12.15±0.12	1.36±0.01	1.11±0.01	2.56±0.03
PNRo	19.50±0.19	6.69±0.04	41.92±0.39	225.0±2.2	109.8±1.0	1045.0±10.0	35.52±0.34	1.22±0.01	0.60±0.01	2.76±0.03
ShZA	13.08±0.10	6.20±0.05	8.5±0.08	275.0±2.6	113.0±1.0	997.1±9.9	23.45±0.23	1.01±0.01	0.61±0.01	1.65±0.02
MeZA	15.08±0.13	8.42±0.06	10.42±0.10	158.3±1.6	111.5±1.0	1109.2±10.9	24.08±0.23	1.41±0.01	0.54±0.01	2.15±0.02
RoEt	7.17±0.06	4.62±0.06	1.92±0.02	175.0±1.7	89.3±0.9	1089.2±10.8	13.75±0.14	0.53±0.01	0.97±0.01	0.13±0.00
BlEt	4.75±0.04	4.09±0.03	15.25±0.14	125.0±1.3	91.3±0.9	1213.2±12.0	12.8±0.12	0.63±0.01	0.88±0.01	0.10±0.00
Average	11.2	6.8	21.9	206	99	1088	17.9	0.9	0.7	1.8

CONCLUSIONS

It is evident that despite minor differences, the composition of wines vinified from *Vitis vinifera* L. cultivars and wines produced from hybrid grape varieties are reasonably similar. The specific findings include:

1. Wines made from hybrid grapes contained significantly less toxic metals such as Cd, Pb, As, and Cu. The low content of Fe in the hybrid grape wines in comparison with the wines from *V. vinifera* is also remarkable.
2. Arabinose and arabitol were not present in the wines made from hybrid grapes. Also the concentrations of myo-inositol, mannitol, and cellobiose were found to be lower in hybrid grape wines.
3. Phenolic acid profiles of wines made from hybrid cultivars were similar to those of common grape cultivars, showing only little more of 3,4-DHB and less of vanillic acid.
4. Wines made from hybrid grapes contained significantly more flavan-3-ols, probably due to the higher seed to fruit ratio of hybrid grapes. These constituents are of particular future interest due to their recent health claim by the European Food Safety Authority.
5. It was confirmed that the presence of anthocyanidin diglucosides is characteristic only for hybrid grape wines.

Our work on distinguishing the chemical differences between traditional red wines and hybrid grape wines will continue. As wines made from hybrid grape cultivars differ in their chemical composition, more research should also be conducted to find suitable technological and agricultural practices to bring forth the full health benefits of cold climate grapes. Unquestionably, from a chemists' perspective, hybrid cultivars deserve attention as a potential source of physiologically active compounds, and may be of great future value for producing wines with an alternative chemical composition.

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Hübriidviinamarjadest valmistatud veinide biokeemiline koostis võrrelduna *Vitis vinifera*'st valmistatud veinide omaga

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Uued liikidevahelised hübriidviinamarjasordid võimaldavad valmistada veine aladel, kus talvised temperatuurid langevad alla –30 °C ja aktiivsete temperatuuride summa ei ületa 1750 °C. Uued veiniregioonid ja sordid tõstatavad aga küsimusi nendest sortidest tehtud veinide kvaliteedi kohta. Käesoleva töö eesmärgiks oli välja selgitada, kas hübriidviinamarjadest valmistatud punased veinid erinevad oma keemilise koostise poolest maailmas laialt levinud harilikest viinamarjadest valmistatud veinidest. Esimesed tulemused näitavad, et kuigi hübriid- ja harilikest viinamarjadest valmistatud veinide polüfenoolide profiil on üldjoontes sarnane, on kohalikest hübriidviinamarjadest valmistatud veinides suurem valik antotsüaane, rohkelt tervisele kasulikke flavaan-3-oole ning märkimisväärselt vähem raskmetalle. Kvalitatiivseid erinevusi on ka sahhariidide ja fenoolsete hapete sisalduses.