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Polyphenolic composition and free radical scavenging activity of red wines available in the Latvian market

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Besides the medical properties, phenolic compounds represent one of the most important quality parameters of wine since they contribute to the organoleptic characteristics such as colour, astringency and bitterness. The aim of the investigation was to evaluate the phenolic composition and free radical scavenging activity of wines available in the Latvian market. Sixteen wine samples from different grape varieties and of different origin were analysed. The total phenol content was determined spectrophotometrically according to the Folin–Ciocalteou method, and the composition of phenols was detected using HPLC. The free radical scavenging activity of wine was determined using the DPPH$^{\bullet}$ (2,2-diphenyl-1-1-picrylhydrazyl radical) assay. The total content of phenolic compounds in red wine samples ranged within 1569–1765 mg l^{-1} chlorogenic acid equivalents (CAE), whereas in white wine samples it was only 98.97–92.12 mg l^{-1} CAE. Free radical scavenging activity was expressed as DPPH$^{\bullet}$ inhibition. The inhibition varied within 71–84% for red, and 62 and 68% for white wines. The content of five phenolic compounds (caffeic acid, catechin, epicatechin, gallic acid, vanillin) was determined in all wine samples. From the identified phenolic compounds, the main in red wines were catechin (22.27–95.17 mg l^{-1}), gallic acid (13.86–60.83 mg l^{-1}), caffeic acid (0.35–13.26 mg l^{-1}). White wines contain smaller amounts of phenols; for example, the content of catechin ranged from 4.65 to 11.56 mg l^{-1}.

Introduction

The constituents of red wine are factors of particular interest due to the intrigue created by the "French paradox" [1]. Recent studies indicate that a moderate consumption of red wines reduces the incidence of coronary heart diseases [2], arthrosclerosis, platelet aggregation and also has an anticarcinogenic effect [3]. These properties are mainly due to the presence of a large amount of polyphenols in wines. Phenolic composition is also one of the essential contributors to wine sensory properties such as colour, flavour, astringency, bitterness. Procyanidin concentration was the major factor responsible for the mouthfeel differences [4]. Astringency can be very well linearly predicted from the phenolic composition, probably under a certain saturation threshold, whereas colour properties appear to be not linearly linked to pigment contents and present a perception saturation for deep-coloured wines [5]. The phenolic composition of wines can be strongly affected, both quantitatively and qualitatively, by the particular grape cultivar [6], grape ripeness [7], environmental factors and technological procedures [8, 9]. Phenolic compounds play a key role as antioxidants due to the presence of hydroxyl substituents and their aromatic structure, which enables them to scavenge free radicals. A lot of research has been done on the analysis of red wine polyphenols and the relationship between polyphenolic the content and antioxidant activity. A correlation betweeen antioxidant properties and content of flavan-3-ols [10], anthocyanins [11], tannic acid [12], and total phenols [13] has been established.

The aim of the present investigation was to evaluate the phenolic composition and free radical scavenging activity of wines available in the Latvian market.

Materials and methods

WINES. A list of all sixteen commercially available wines made from different grape varieties originating from different countries and analysed in this study is presented in Table 1.

Table 1. List of analysed wine samples

Variety	Country	Vintage	Alcohol content, vol %	Type of wine
Cabernet Sauvignon	Chile	2006	14.0	Dry
Cabernet Sauvignon	California, USA	2001	11.0	Dry
Cabernet Sauvignon	Argentina	2007	13.5	Dry
Cabernet Sauvignon	France	2007.	12.5	Dry
Cabernet Sauvignon	Spain	2006	12.5	Dry

Variety	Country	Vintage	Alcohol content, vol %	Type of wine
Shiraz	Chile	2007	14.0	Dry
Syrah	France	2007	12.5	Dry
Shiraz	South Africa	2007	14.5	Dry
Shiraz	Australia	2006	13.5	Semi-dry
Merlot	Chile	2006	14.0	Dry
Merlot	France	2007	13.5	Dry
Merlot	Italy	2007	12.0	Dry
Pinot Noir	Moldova	2007	11.0	Semi-dry
Pinot Noir	France	2007	12.5	Dry
Chardonnay	California, USA	2006	13.0	Semi-dry
Chardonnay	Spain	2007	13.0	Dry

DETERMINATION OF THE TOTAL POLYPHENOLIC CONTENTS BY THE FOLIN–CIOCALTEU METHOD. The total phenolic concentration was determined spectrophotometrically according to the Folin–Ciocalteu colometric method. Wine was diluted with distilled water (1 : 5 v / v), 1 ml of aliquot was mixed with Folin–Ciocalteu reagents (diluted with water 1 : 2), after 20 seconds 10 ml 5% Na_2CO_3 was added. The absorbance was measured after 30 seconds at 730 nm. Total phenols were expressed as chlorogenic acid equivalents (mg l^{-1}). Each determination was performed in triplicate, and the results are expressed as mean ± SD.

DETERMINATION OF FREE RADICAL-SCAVENGING ACTIVITY BY DPPH. The ability of wines to scavenge diphenyl picryl hidrazyl (radical) (DPPH*) free radical was determined. Wine was diluted with distilled water (1 : 25 v / v), and 5 ml of wine solution was added to 5 ml of 1×10^{-4} M methanolic DPPH* solution. Samples were kept for 20 minutes in the dark, and the absorbance was measured at 517 nm. Each determination was performed in triplicate, and the results were expressed as mean ± SD. Inhibition of DPPH* in percentage (I%) of each wine sample was calculated from the decrease of the absorbance according to the relationship:

$$I\% = \frac{A_{blank} - A_{sample}}{A_{blank}} \cdot 100,$$

where A_{blank} is the absorbance of the control reaction (methanol–water with DPPH*), and A_{sample} is the absorbance of a wine sample.

DETERMINATION OF INDIVIDUAL POLYPHENOLS BY HPLC. The concentration of all individual polyphenols was determined by *HPLC (*Shimadzu LC-20 Prominence) with a diode array detector (SPD-M20A). After injecting 10 µl of a sample, separation was performed in an *Perkin-Elmer C18* 4.6 × 250 mm column (thermostated at 20 °C). For detection and quantification of compounds, the chromatograms were recorded at 278 nm. Table 2 shows the solvent gradient used for separation.

The different phenolic compounds analysed were tentatively identified according to their order of elution, retention times of pure compounds (catechin, epicatechin, gallic acid, caffeic acid and vanillin.

Table 2. Mobile phase gradient of the HPLC method

Time, min	Flow rate, ml/min	Mobile phase, %	
		A[a]	B[b]
Inital	1.3	0	100
3	1.3	5	95
17	1.3	20	80
20	1.3	20	80
30	1.3	25	75

A[a] – methanol, B[b] – acetic acid : water (2 : 98 v / v).

STATISTICAL ANALYSIS. Analysis of variance was performed by the ANOVA procedure, and $p < 0.05$ was considered statistically significant. A linear correlation analysis was performed employing the SPSS 14.00 software for Windows in order to identify phenolic compounds most strongly influencing the antioxidant activity of the wines.

Results and disscusion

The content of phenolic compounds in wines is determined by several factors such as grape variety, climate, soil, technological parameters. Since in our study commercial wines were analysed, it is impossible to know the exact production technology of each wine. Therefore, two factors affecting polyphenolic content were taken into account – grape variety and the country of origin. The red wines conteined significanty higher amounts of total phenols, and these data are in agreement with those available in literature [14, 15]. The reason is the greater grape skin and seed contact time and the higher temperture of the fermentation process for red wines. The content of total phenols varied from 1429 to

1765 mg l^{-1}, averaging 1570 mg l-1, for the red wines and 99 and 92 mg l^{-1} for white wines (Table 3). *Cabernet Sauvignon* is the premier red wine grape in the world and the dominant grape in the Bordeaux region of France and has spread to all other major growing regions. The content of total polyphenols in wines made from *Cabernet Sauvignon* grapes grown in different wine regions varied. *Cabernet Sauvignon* produced in Argentina had the highest content of polyphenols, whereas in other *Cabernet Sauvignon* wines it was significantly lower. Other authors reported different contents of phenols in *Cabernet Sauvignon* wines. Kondrashov [16] investigated *Cabernet Sauvignon* wines from different counties available in Czech market, and depending on the content of polyphenols they were ranked as follows: France (2912 mg l^{-1}) > Spain (2414 mg l^{-1}) > Chile (2365 mg l^{-1}) > California (1453 mg l^{-1}). These results can be explained by differences in production technology and the time of aging. Young *Cabernet Sauvignon* from Spain contained only 1386 mg l^{-1} [17], whereas the vine aged in oak barrels contained 3430 mg l^{-1} [18]. *Syrah* in France and *Shiraz* in Australia produce different wines depending on the climate. In warmer climates like Australia, the grape produces wines that are sweeter, but in cooler climates like the Rhone valley of France, it often has a more peppery and spicy aroma. Also, the content of polyphenols differed significantly: the highest content was found in the *Syrah* wine produced in France, and it was lower in wines from Australia and Chile. Espinoza [19] reported on the Chile *Shiraz* wine with a significantly higher polyphenol content – 2370 mg l^{-1}. Wines from *Merlot* grapes are also very popular, and some of the best come from Bordeaux, California, various parts of Italy and Chile. The highest content of polyphenols were found in *Merlot* from Italy, followed by Chile and France. Comparing the obtained data with the literature, both higher and lower contents of polyphenols in *Merlot* wines can be found. Kondrashov [16] reported on *Merlot* from Italy with the total phenol content 1447 mg l^{-1}, which is lower as compared to wine analysed in our study, whereas *Merlot* from France contained 2100 mg l^{-1} [16] and *Merlot* from Chile 2590 mg l^{-1} [19] of polyphenolic compounds, i.e. significantly more than the wines analysed in our study.

Pinot Noir is popular in France (Burgundy) and also in various regions around the world including California, New Zealand, Australia, Germany, Italy and Moldova. In both red and white wines analysed in our study the content of total phenols did not differ significantly and were are among the lowest in our analysis. Cliff&Dever [20] reported a wide range of polyphenolic content in *Pinot Noir* wines (718–2893 mg l^{-1}).

Chardonnay is a white wine and contains a lower amount of polyphenols than red wine – on average 150 to 336 mg l^{-1} [20]. *Chardonnay* wines from Spain and Califrornia contained even less polyphenols than reported in literature (below 100 mg l^{-1}).

One of the important characteristics of polyphenolic compounds is their antiradical properties. Different methods are used for the determination of antioxidant capacity, but because wine is a food product of hydroalcoholic nature,

Table 3. Total phenolic compounds (mg l^{-1}) and DPPH$^{\cdot}$ scavening activity of wines

Variety and country	Total phenols, mg l^{-1} CAE [a]	DPPH$^{\cdot}$, I (%)
Cabernet Sauvignon, Argentina	1765 ±11 ^{h}	79 ±2 ^{de}
Cabernet Sauvignon, Spain	1640 ±21 ^{g}	82.04 ±1 ^{fghi}
Cabernet Sauvignon, California	1624 ±13 ^{fg}	87.22 ±1.18 ^{j}
Cabernet Sauvignon, Chile	1577 ±9 ^{de}	81 ±1 ^{efgh}
Cabernet Sauvignon, France	1569 ±4 ^{d}	83 ±2 ^{hi}
Shiraz, South Africa	1634 ±8 ^{g}	82 ±1 ^{ghi}
Syrah, France	1572 ±52 ^{d}	80 ±2 ^{def}
Shiraz, Chile	1569 ±15 ^{d}	84 ±2 ^{i}
Shiraz, Australia	1429 ±10 ^{b}	72 ±1 ^{c}
Merlot, Italy	1632 ±16 ^{g}	71 ±1 ^{c}
Merlot, Chile	1601 ±10 ^{ef}	71 ±2 ^{c}
Merlot, France	1431 ±13 ^{b}	78 ±1 ^{d}
Pinot Noir, Moldova	1472 ±14 ^{c}	80 ±1 ^{defg}
Pinot Noir, France	1472 ±11 ^{c}	82 ±1 ^{hi}
Chardonnay, Spain	99 ±2 ^{a}	68 ±2 ^{b}
Chardonnay, California	92 ±6 ^{a}	62 ±1 ^{a}

[a]Values in the table are the average of three measurements. Different letters in one column represent significant differences ($p < 0.05$).

its antioxidant activity must be determined by methods based on water-soluble species, such as the DPPH$^{\cdot}$ assay [21]. *Cabernet Sauvignon* from California showed the highest antiradical activity. A significantly lower antiradical activity was found for white *Chardonnay* wines. A positive linear correlation between total phenol and DPPH$^{\cdot}$ radical scavening activity was observed ($r = 0.73$), and it is in agreement with some data found in the literature [21].

Different data exist regarding the correlation between the antioxidant activity and the polyphenolic contents of wine. It is possible to identfy several trends:

1. A linear correlation exists between antioxidant capacity and the contents of total polyphenols [11, 22].
2. Statistic correlation is relevant between total polyphenols and the flavonoid but not the non-flavonoid fraction [15].
3. Anti-radical activity is due to the flavan-3-ol fraction and not to anthocyanins [23].

A possible reason for the above-mentioned results reported by Majo [22] could be the influence of the different flavonoid and non-flavonoid subgroups on the antioxidant capacity, degree of polymerization and the ratio between monomeric and polymeric forms, the possible synergy or antagonism among the different classes of polyphenols, radical molecules contained in wine.

It could be concluded that antiradical activity of wines could be affected by the content of certain phenolic compounds. In the HPLC analysis of wines, five individual phenolic contents were quantified: gallic acid, caffeic acid, catechin, epicatechin, vanillin (Table 4).

Table 4. Phenolic compounds of wines, mg l^{-1}

Variety and country	Gallic acid	Caffeic acid	Catechin	Epicatechin	Vanilin	Total
Cabernet Sauvignon, Chile	35.43	4.31	52.47	3.51	0.31	96.02
Cabernet Sauvignon, California	31.46	1.54	43.00	4.43	0.20	80.63
Cabernet Sauvignon, Argentina	59.94	2.48	69.33	6.46	0.17	138.38
Cabernet Sauvignon, France	21.63	2.23	56.22	4.42	0.14	84.64
Cabernet Sauvignon, Spain	34.16	12.85	49.54	5.61	0.25	102.42
Shiraz, Chile	18.52	7.47	30.55	3.64	0.49	60.66
Syrah, France	21.59	13.26	22.27	4.86	0.64	62.62
Shiraz, South Africa	22.84	3.68	30.19	4.19	0.46	61.36
Shiraz, Australia	60.83	4.91	43.90	5.50	2.62	117.76
Merlot, Chile	30.42	4.71	47.49	3.87	0.98	87.48
Merlot, France	13.86	0.35	45.03	3.54	1.94	64.71
Merlot, Italy	32.81	1.18	47.49	4.19	0.00	85.68
Pinot Noir, Moldova	34.09	1.43	45.91	4.88	0.86	87.17
Pinot Noir, France	45.03	1.87	95.17	8.57	0.16	150.79
Chardonnay, California	0.81	1.83	4.65	0.15	0.00	7.45
Chardonnay, Spain	0.91	1.70	11.56	0.23	0.00	14.40

From the identified phenolic compounds, in red wines prevailed catechin (22.27–95.17 mg l^{-1}), gallic acid (13.86–60.83 mg l^{-1}), caffeic acid (0.35–13.26 mg l^{-1}). White wines contained lower amounts of phenols; for example, the content of catechin ranged within 4.65–11.56 mg l^{-1}. The qualitative difference between white and red wines is that red wines contain anthocyanins, pigment molecules and large amounts of catechins (low molecular weight flavans as well as polymerized tannins). Among the analysed wines, *Pinot Noir* from France contained the highest amount of catehin. Polymers of catechin would not be expected to be significant antioxidants, but low molecular weight catechins, especially monomers and dimers, could contribute much to the antioxidant strength of red wine [15]. In red wines, gallic acid was the second important polyphenol compound (highest content in *Shiraz* wine from Australia and *Cabernet Sauvignon* from Argentina), while it was detected in very low amounts in white wines. The presence of high levels of gallic acid in red wines would be expected, since this phenolic acid is principally formed by the hydrolysis of flavonoid gallate esters which are mostly absent in white wine due to the lack of skin extraction [24]. Different data exist on the epicathenin content of wines. For example, *Cabernet Sauvignon* from Italy contains only 1.2 mg l^{-1} [22], whereas from Greece 40.2 mg l^{-1} [25]. Caffeic acid in the largest quantities was found in *Syrah* wine from France and *Cabernet Sauvignon* from Spain, and the amounts of caffeic acid are in agreement with those available in literature [25, 26]. Vanillin was reported as an indicator of wines kept in oak barrels [27]. The level of organoleptic perception of vanillin is 0.5 mg l^{-1} [28]. This level was reached in five samples: *Shiraz*, Australia; *Merlot*, France; *Merlot*, Chile; *Pinot Noir*, Moldova; *Syrah*, France.

The strongest correlation was found between epicatechin, catechin and DPPH activity. Katalinic [15] reported a significant correlation between catechins and the antioxidant capacity of wines, confirmed by the FRAP, DPPH and BCB methods. High amounts of catechins in selected red wines and a significant reducing strength of pure (+)-catechin confirm the importance of catechin flavonoids in the antioxidant capacity of red wines. The antioxidant capacity of polyphenolic compounds reported in the literature [29]: epicatechin > gallic acid > catechin > caffeic acid.

Conclusions

Analysis of the phenolics and free radical scavenging activity of selected wines found in the Latvian market showed differencies in total polyphenol content depending on the variety and the country of origin. The highest content of polyphenols among red wines was determined in *Cabernet Sauvignon* produced in Argentina, whereas the lowest in *Shiraz* produced in Australia. The white wines had significantly lower levels of phenols. Wines showed a free radical scavenging activity. A positive linear correlation between antiradical activity and total phenol content was found. Among the analysed individual phenolic compounds, catechin prevailed, and its highest level was found in *Pinot Noir* from France.

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LATVIJOS RINKOJE ESANČIŲ VYNŲ FENOLIŲ SUDĖTIS IR LAISVŲJŲ RADIKALŲ SURIŠIMO AKTYVUMAS

Santrauka

Ankstesniais tyrimais nustatyta, kad nedaug vyno maisto racione mažina širdies nepakankamumo, aterosklerozės ir trom-

bocitų agregacijos riziką dėl jame esančių polifenolių poveikio. Be gydomųjų savybių, fenolių dariniai yra svarbiausios vyno kokybę nulemiančios medžiagos, gerinančios juslines produkto savybes, tokias kaip spalva, skonis.

Šio darbo tikslas – nustatyti fenolių sudėtį ir išanalizuoti laisvųjų radikalų surišimo gebą Latvijos rinkoje esančiuose vynuose. Tirta šešiolika vynų, pagamintų iš skirtingų veislių vynuogių įvairiuose auginimo regionuose. Bendrasis fenolių kiekis nustatytas spektrofotometriniu metodu (Folin-Ciocalteu), fenolių sudėtis – efektyviosios skysčių chromatografijos (ESC), laisvųjų radikalų surišimo geba – DPPH$^{\cdot}$ metodu. Tyrimų rezultatai parodė, kad fenolių raudonuosiuose vynuose buvo 1569–1765 mg l^{-1} (chlorogeno rūgšties ekvivalentais (CAE)), baltuosiuose – 99–92 mg l^{-1}. Laisvųjų radikalų surišimo geba buvo išreikšta sujungtu DPPH$^{\cdot}$ kiekiu (I%), kuris kito nuo 71 iki 84 % raudoniesiems vynams ir nuo 62 iki 68 % – baltiesiems. Penkių fenolio junginių (kavos rūgšties, katechino, epikatechino, galo rūgšties, vanilino) kiekis nustatytas visuose vynuose: raudonuosiuose vyrauja katechinas (22,3–95,2 mg·l^{-1}), galo (13,8–60,8 mg l^{-1}) ir kavos (0,4–13,3 mg l^{-1}) rūgštys; baltuosiuose vynuose fenolio junginių daug mažiau, pavyzdžiui, katechino buvo 4,6–11,6 mg l^{-1}.