Resveratrol in Parts of Vine and Wine Originating from Bohemian and Moravian Vineyard Regions

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SUMMARY

Chemically, resveratrol is a substance of a polyphenolic character from the group of phytoalexins – 3,5,4'- trihydroxystilbene – and exists in *cis* and *trans*-isomer forms. In natural sources *trans*-isomer is more common. As a natural polyphenolic substance, it shows a whole range of biological activities, such as anti-oxidizing and anti-microbial features (namely anti-fungal activities), the ability to absorb free radicals, affects blood sedimentation rate etc. Recently, *trans*-resveratrol has also been attributed anti-mutagen and chemo-protective features against cancer proliferation. It is assumed that resveratrol could be one of the active substances contributing to the health benefits, namely it decreases the risk of cardiovascular diseases through a reasonable consumption of red wine. Grapes of *Vitis vinifera* and especially red wine represent its main source in human diet. Grape peels contain about 0.5 to 2.0 mg of resveratrol/g of dry weight and the average concentration in red wines of world provenience fluctuates between 1.0 and 3.0 mg/l. Resveratrol was determined by HPLC method with electrochemical detection after direct injection of wine or plant extracts. As expected, red wines from vines originating in the Bohemian and Moravian vineyard regions appeared to contain relatively high levels of resveratrol (from 1.3 to 15.4 mg/l) and *trans*/*cis* ratio ranged from 0.5 to 4.8, excess of *cis*-resveratrol to *trans*-isomer was typical for red wine growing in Most region (northern Bohemia) where vineyards are exposed to higher environmental stress due to frequent air pollutions in this area. In addition, resveratrol determined in different parts of grapevine (leaves, rachis) varied from 6 to 490 mg/kg of the dry matter. Cluster stems were found as the richest source of resveratrol.

KEY WORDS

Resveratrol, *Vitis vinifera*, wine, antioxidants, polyphenols

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INTRODUCTION

Resveratrol (*trans* - 3,5,4´-trihydroxystilbene) is a natural phenolic compound, which belongs to phytoalexins. It is found in many plants, such as mulberries, grapes and peanuts (Sobolev and Cole, 1999; Zhu et al., 2000). Increasing interest in monitoring the presence of resveratrol in wine was caused by so-called the "French paradox". It has been found that in some parts of France, the death rate caused by coronary artery diseases was lower in spite of high consumption of fats. The consumption of wine was one of the dietary factors that may partially explain the low mortality caused by atherosclerosis (Renauld and De Lorgeril, 1992).

Phytoalexins are benzo-γ-pyronderivatives which are ubiquitous in photosynthesizing cells. They have been used for centuries in folk medicine to treat human diseases such as inflammation, allergy, headache, parodentosis, virus and fungal infection, stomach or duodenal ulcers and even cancer (Adrian et al., 2000; Palomino et al., 2000). Resveratrol has been proven as remarkably efficient in preventing from skin tumours in mice and in inhibiting the replication in vitro of human leukemia cells (Jang et al., 1997).

Resveratrol possesses antibacterial and antifungal activities and induces platelet hypoaggregation in rats; it protects liver from the lipid peroxidation and inhibits the oxidation of low density lipoproteins. More recently, its canceropreventive activity has been proven (Palomino et al., 2000).

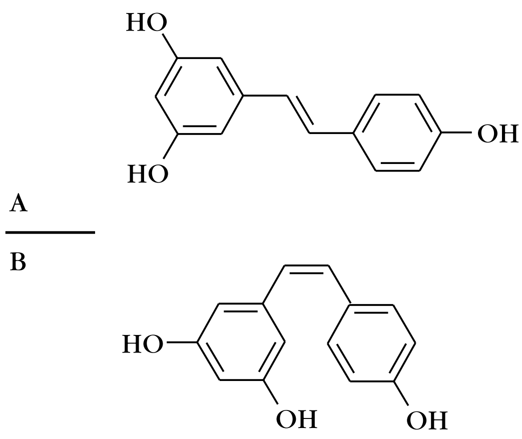

FIGURE 1. Structures of the *trans* isomer (A) and *cis*-isomer (B) of resveratrol

Phenolic compounds are responsible for colour of wines and contribute to their bitter flavour. Resveratrol is known to occur in wine in free (Figure 1) and glycosidically bound form, which is called as piceid or polydatin. Piceid can be found in grape products in the concentration usually significantly higher than the aglycone. The relative distribution between the glycosilated and aglycone forms in wines is dependent on a number of factors, especially on viticultural and enological techniques used (Gu et al., 2000). Free *trans*- and *cis*-resveratrols are commonly present in a concentration range of 0.2 - 13 mg/l in red wines and 0.1 - 0.8 mg/l in white wines, respectively. Concerning bound forms of resveratrol, the piceid concentrations are reported to be in a range of 0.3 - 9.0 mg/l in red and 0.1 – 2.2 mg/l in white wines (Vinas et al., 2000). Red wines contain in total 1500 - 2500 mg/l phenolics, although their presence and structures are affected by a number of factors including grape variety, sun exposure, vinification techniques and aging (Frankel et al., 1993).

Occurrence of complex phenolic substances in wines is not only a consequence of their extraction from grapes during winemaking. When grapes are crushed before the beginning of alcoholic fermentation, a lot of condensation reactions, involving especially anthocyanins, catechins and procyanidins, take place and result in the formation of new polymeric compounds (Revilla and Ryan, 2000). Chemical composition of wine is influenced by the climatic and atmospheric conditions, soil type and locality, vine cultivation and at last by winemaking process.

Resveratrol and hydroxylated stilbenes are accumulated in plants in response to attacks by pathogens such as *Plasmospora viticola* or *Botrytis cinerea* (fungal infections) the causal agents for downy mildew and grey mould, respectively, or abiotics such as UV light, mechanical injury or aluminium chloride (Adrian et al., 2000). Irradiation of plant tissues with UV light has some important effects on phenolic metabolism. UV-B light irradiation seems to be associated with an increase in the enzymes responsible for flavonoid biosynthesis, as these compounds can act as UV screens preventing the UV-induced damage in the genetic material of plant cells (Dong et al., 1995; Cantos et al., 2000).

HPLC techniques have become the dominant and more frequently used method of resveratrol determination. LC methods with gradient elution and UV or fluorimetric detection have been reported for the determination of resveratrol and piceid in biological samples, especially in grape berries and wines. Recently resveratrol has been assayed by the direct injection without purification and pre-concentration steps. LC with electrochemical detection has been shown to be selective and sensitive enough for the determination of resveratrol and other phenolic compounds in natural sources (Zhu et al., 2000; Melzoch et al., 2000).

MATERIAL AND METHODS

Resveratrol

Trans-resveratrol in the crystalic form was synthetised in purity of 99%. *Cis*-resveratrol was obtained by 10-hour exposure of *trans*-resveratrol standard in 50% (v/v) alcoholic solution (100 mg/l) to daylight. At these conditions the 80% of *trans*-resveratrol was converted into the *cis*-isomer.

Samples

The samples of commercial red and white wines from Bohemian (Most, Velké Žernoseky, Litoměřice, Roudnice) and Moravian (Čejkovice, Hodonín, Dubňany, Lednice) vineyard regions from harvest years 1986 - 1999 were obtained directly from wineries. These wines were made from varieties of red or blue grapes (Frankovka, Rulandské modré, Svatovavřinecké, Modrý portugal, Cabernet sauvignon, Cabernet moravia, Zweigeltrebe, Laurot, Tintet, Neronet, Merlot) and of white grapes (Erilon, Rubikon, Hibernal) commonly growing in these regions.

Extraction

The samples of grape berries, rachises and leaves were extracted in 80% (v/v) ethanol solution. The plant material was used in the fresh state after grinding, the published results were related to the dry matter. The extraction took 24 hours at 20 ^{0}C in the darkness and afterwards liquid phase was separated from solids by filtration.

HPLC analysis

Trans- and *cis*-resveratrols were determined by HPLC method using TSP 3500 liquid chromatograph (U.S.A.) equipped with HP 1049 electrochemical detector with glas working electrode (Hewlett-Packard, U.S.A.) at the potential of 0.75 V, coupled to Apex Data Station (Czech Republic). The sample of wine was filtered through membrane filter (pore diameter of 0.2 μm, Millipore, U.S.A.) and permeate was injected with Rheodyne valve (U.S.A.) onto the column filled with the stationary reversed phase Nucleosil 120-5-C18 (250 x 4 mm, 5 μm) with pre-column (10 x 4 mm) with the same material. The isocratic elution at a flow rate of 1.0 ml/min used a mobile phase of 25 % acetonitrile, 0.1 % H_3PO_4 and NaCl (c = 5 mmol/l) in demineralised water. Identification and quantification of resveratrol isomers were carried out by comparing the retention times and the peak areas with resveratrol standard values or by addition of resveratrol standard directly into the sample (spiking of *trans-* and *cis*-resveratrols).

RESULTS AND DISCUSION

During the past decade natural antioxidants have evoked the widest interest in human diet as protective compounds with significant biological activities and resveratrol has become one of the most popular substance. It was interesting to screen contents of resveratrol and other stilbenes from the natural sources potentially available in the Czech Republic and to establish appropriate analytic methods for their determination. Our interest was focused on the resveratrol content screening in selected red and white wines (Table 1), parts of *Vitis vinifera* (Table 2) and to technological impacts on transfer of resveratrol from grapes into wine, as well.

The appropriate analytical HPLC method with electrochemical detection of resveratrol was devel-

TABLE 1. Resveratrol content in red wines originating from Bohemian and Moravian vineyard regions

Vineyard region *Locality*	Variety of vine	Year of harvest	Resveratrol (mg/l) *trans-*	*cis-*	total
Mostecká	Svatovavřinecké	1998	1.02	0.78	1.80
Most	Svatovavřinecké	1996	2.29	1.28	3.57
	Zweigeltrebe	1998	0.91	1.25	2.16
	Zweigeltrebe	1996	0.92	1.01	1.93
	Rulandské modré	1998	1.31	2.09	3.40
	Rulandské modré	1996	1.39	1.26	2.65
Žernosecká	Svatovavřinecké	1998	1.07	0.71	1.78
Velké Žernoseky	Svatovavřinecké	1995	1.74	1.01	2.75
	Modrý portugal	1998	1.99	1.48	3.47
Žernosecká	Svatovavřinecké	1998	5.47	1.23	6.70
Litoměřice	Zveigeltrebe	1998	2.11	1.37	3.48
	Rulandské modré	1996	2.56	1.14	3.70
Roudnická	Svatovavřinecké	1998	4.84	1.46	6.30
Roudnice nad	Svatovavřinecké	1996	4.85	1.71	6.56
Labem	Modrý portugal	1998	6.23	2.83	9.06
Mutěnická	Svatovavřinecké	1998	3.47	0.73	4.20
Hodonín	Frankovka	1998	4.82	1.16	5.98
	Cabernet sauvignon	1998	3.73	1.85	5.58
Mutěnická	Frankovka	1998	1.72	1.39	3.11
Čejkovice	Frankovka	1997	2.16	1.15	3.31
	Frankovka	1996	1.35	1.52	2.87
	Frankovka	1995	1.50	1.35	2.85
	Frankovka	1992	2.97	2.14	5.11
	Frankovka	1986	2.18	0.68	2.86
Mutěnická	Rulandské modré	1997	4.22	5.14	9.36
Dubňany	Frankovka	1994	3.91	4.51	8.42
	Cabernet moravia	1996	1.80	1.83	3.63

TABLE 2. Resveratrol content in vine leaves, rachises, grape berries and wines originating from vineyard region "Mikulovská" from vintage 1999

Variety of vine	Vine leaf resveratrol			rachis resveratrol			grape berry resveratrol			wine resveratrol		
	trans-	*cis-*	total	*trans-*	*cis-*	total	*trans-*	*cis-*	total	*trans-*	*cis-*	total
		mg/kg d.m.			mg/kg d.m.			mg/kg berry			mg/l	
Cabernet sauvignon	5.00	1.30	6.30	7.00	traces	7.00	0.72	traces	0.72	3.19	0.94	4.13
Pinot noir	1.60	1.20	2.80	13.00	traces	13.00	2.34	traces	2.34	10.53	4.87	15.41
Laurot	4.40	1.60	6.00	15.00	2.00	17.00	5.80	traces	5.80	5.21	2.28	7.49
Tintet	3.60	traces	3.60	440.00	6.80	446.80	0.30	traces	0.30	3.85	1.54	5.39
Neronet	9.90	traces	9.90	209.00	2.30	212.30	0.70	traces	0.70	0.67	0.65	1.33
Merlot	7.10	3.00	10.10	15.00	1.80	16.80	0.70	traces	0.70	1.31	0.61	1.93
Erilon*	44.20	2.20	46.20	482.00	9.90	491.90	0.44	traces	0.44	0.48	0.09	0.57
Rubikon*	14.60	2.60	17.20	6.00	traces	6.00	0.20	traces	0.20	0.16	0.06	0.22
Hibernal*	5.40	1.30	6.70	63.00	3.40	66.40	0.32	traces	0.32	0.71	0.22	0.93

*white grapevine varieties

oped as a modification of previously published assays for resveratrol and flavonols (Jeandet et al, 1995) and routinely used for screening of resveratrol contents in selected domestic wines and *Vitis vinifera* samples. The main advantages of *trans*- and *cis*-resveratrol assays have been a possibility of the direct injection of wine sample or alcoholic extracts of vine without any purification and pre-concentration steps, easy feasible isocratic elution and their very sensitive and specific response in electrochemical detector. The detection limit for both free resveratrol isomers in sample was less then 10 µg/l.

The *trans*- and *cis*- resveratrol contents were determined in a set of nearly a hundred wines originating from Bohemian and Moravian vineyard regions and made by local wineries, see Table 1 and 2. Only free forms of resveratrol isomers were assayed. There were relatively large variations in the resveratrol contents of individual red wine samples, *trans*-resveratrol concentration varied from 0.7 to 10.5 mg/l, *cis*-resveratrol from 0.6 to 5.1 mg/l and total amount from 1.8 to 15.4 mg/l. Generally, resveratrol concentrations in the white wines were significantly lower comparing to red ones. Total amount of resveratrol did not exceed value of 0.9 mg/l. To answer the question "Why it is?" one should have to find differences in winemaking technology for production of white and red wines, especially differences in the initial phase of fermentation when in the first case fresh grapes are immediately pressed and clarified must is fermented and in the second case crushed berries lay in fermenting mash for several days. Then resveratrol is gradually released from grapes into liquid mash during this maturation.

Czech red wines have got relatively higher resveratrol concentrations than the other world wines made from grapes grown in a good climate in sunny and southern localities such as California, Spain, Italy (Goldberg et al., 1995). The Bohemian and Moravian vineyard regions belong to the northernmost localities for vine growing in Europe. That is why the grapes do not usually achieve the same degree of ripeness as the more southern ones. The resveratrol content in grapes very probably reflects climatic conditions during the vegetation period and microbiological (fungal) pressure that is higher in a cooler and wet area in northern Bohemia.

In *Vitis vinifera* plant resveratrol is not present only in grape berries (content of *trans*-resveratrol varied up to 5.8 mg/kg $_{berry}$), especially in the grape skin from which is released during the maturation. Czech wineries use as a classical technology as modern methods of vinification, including recent reductive technologies, addition of enzyme for improving yield of must and extraction of colouring and aromatic compounds, etc. We did not find any relationship to the age of wines (to the vintage year), but the predominant factor affecting resveratrol amount was the climate and likewise the significant role played the winemaking technology, namely initial steps in which resveratrol is released from the grape skin.

Other parts of *Vitis vinifera* contain resveratrol in the very different concentrations. Vine leaves contain relatively high amount of resveratrol, varied from 2.8 to 46.2mg/kg$_{d.m.}$, nevertheless rachises were recognised as the richest source of resveratrol nearly reaching up to 500 mg per kilogram of dry matter. For grape berries and other plant parts were typical very high values of the ratio of *trans* to *cis*-resveratrols in comparison with completed wines, it means that very low *cis*-resveratrol concentration was present in natural plant matter (Table 2). This fact proves that *trans*-resveratrol formation has got a priority in the vine metabolism compared with *cis*-isomer which is formed by isomeration (e.g. photoisomerization of *trans*-resveratrol). Another source of free resveratrol forms were piceids, those are easily extracted from plant samples into mash due to their higher hydrophility and successively can be enzymaticly hydrolysed by yeast β-glucosidase. The general distinction concerning resveratrol content in white and red wine is in the different wine technology, because the resveratrol concentrations in vine leaves, rachises and grape berries for the white, red and blue varieties of *Vitis vinifera* were comparable.

On the basis of resveratrol concentrations, red wines of Czech origin are considered as a significant source of resveratrol and therefore can contribute to health benefits of consumers.

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