

Resveratrol – Occurrence in Wine Originating from Czech Vineyard Regions and Effect on Human Health*

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Abstract

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Resveratrol is a substance of a natural character, naturally present in some foods, namely in wine. The grape peels of *Vitis vinifera* contain about 50 to 100 mg of resveratrol/g and the average resveratrol concentration in red wines produced in the world fluctuates between 1.0 to 3.0 mg/l. A few biological activities were discovered with resveratrol in the last few years such as positive action against some cardiovascular diseases described as so called French paradox and anti-cancer chemo-preventive activity. The denominators of those activities are anti-oxidizing and free radical scavenging features of resveratrol molecule. As expected, red wines from vines originating in the Czech vineyard regions appear to contain relatively high levels of resveratrol (from 1.4 to 9.4 mg/l).

Key words: resveratrol; anti-oxidants; polyphenols; red wine; French paradox; anti-cancer chemo-preventive activity

Resveratrol is a substance of a natural character occurring in the fruit of grapevine (*Vitis vinifera* L.) and consequently in red wine, on which most information has been published. It is assumed that resveratrol could be one of the active substances that decrease the risk of cardiovascular diseases through a reasonable consumption of red wine (RENAUD & DE LORGERIL 1992). Chemically, it is a substance of a polyphenolic character from the group of phytoalexins – stilbenes (3,4,5-trihydroxystilbene) and it exists in *cis*- and *trans*-isomer forms. In natural sources, there are more *trans*-isomers. As a natural polyphenolic substance, it shows a whole range of biological activities, such as anti-oxidizing and anti-microbial features (and namely anti-mold features), the ability to absorb free radicals, effect blood sedimentation rate (agglutination of blood cells). Recently, *trans*-resveratrol has also been attributed anti-mutagen and chemo-protective features against tumor proliferation (JANG *et al.* 1997).

Resveratrol has been found in more than 72 herbal species belonging into 31 genera and 12 families. Many of these are a normal part of the human diet such as mulber-

ries, peanuts and grapes. Quite a rich and rampant resource of resveratrol is the grapes of *Vitis vinifera*. Grape peels contain about 50 to 100 mg of resveratrol/g and the average concentration in red wines fluctuates between 1.0 to 3.0 mg/l.

The physiological function of resveratrol in herbs is not still quite clear. Creation of phytoalexins (*trans*-resveratrol and ϵ -viniferine) is one of the cell's resistance mechanisms. Resveratrol is known as a compound synthesized by an herbal cell as a reaction to stress or pathogenic invasion (damage, infection, UV radiation, ozone etc.). The quick creation and accumulation of phytoalexines after invasion of the herbal cell by the gray mold *Botrytis cinera* or *Plasmopora viticola* or after exposure to UV radiation are parts of a defensive complex of protective substances of the grapevine. The maximum concentration of *trans*-resveratrol is reached after 24 to 96 hrs of exposure. Then, its value decreases and after about 16 days, it reaches its original state before exposure (BAVARESCO *et al.* 1997). During this abiotic stress, induction of stilbene synthetase activity occurs.

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More systematic information on resveratrol and especially on its presence has been available since the 1980's. Since then, its concentrations in red wine have been monitored. One might say its presence and contents in wine from world famous vineyard regions have been mapped quite well. For instance GOLDBERG *et al.* (1996) analyzed almost 700 red wines and discovered high concentrations of *trans*-resveratrol in samples from Bordeaux, Burgundy, Switzerland and Oregon. Lower values were typical of the Mediterranean areas (such as Italy, Spain and Portugal), South Africa, America and Australia. It is only logical that our work was focused on methodology and determination of resveratrol concentrations in red wine of Bohemian and Moravian origin.

Some studies expect that, based on the discovery that red wine contains considerably higher concentrations of *trans*-resveratrol than white wine, the red or blue species of grapevines contain concentrations of *trans*-resveratrol many times higher than the white species. In some cases, the ratio of *trans*- and *cis*-isomers might be completely reversed in favour of the white varieties (SOLEAS *et al.* 1995). The information about the presence of *cis*- and *trans*-resveratrol and/or other phenolic substances cannot be generalized. Since it depends not only on the genetic predispositions of the particular grapevines but also on many other factors such as locality, climatic conditions, agricultural techniques used, techniques of wine production (GOLDBERG *et al.* 1996). These factors cause a large difference in absolute quantities and relative values of concentrations of polyphenolic compounds contained in wine.

In ripe grapes the synthesis of *trans*-resveratrol present in the form of a free compound or bound compound like glycoside, called piceid (3- β -glucoside of resveratrol), must be somehow initiated (JEANDET *et al.* 1995). Many of the factors stated above and the mechanical damage to the peels of berries induce, even with non-infected grapes, the synthesis of a small amount of phytoalexins. It is possible that the source of *trans*-resveratrol in wine is the polyphenolic substances – constitutive stilbenes – extracted from the peel and the remainders of stalks during alcohol fermentation and decomposed by following processes in the new wine, such as by malolactic fermentation due to enzymatic activity of these bacteria. Another potential source might be ϵ -viniferin which is formed by a similar trigger mechanism like as *trans*-resveratrol. During the maturation (fermentation) process of grapes, it is released from the stem and transformed to *trans*-resveratrol during alcohol fermentation (GOLDBERG *et al.* 1996).

The wave of interest in monitoring the presence of resveratrol in wine was caused by 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 coronary artery diseases. This epidemiological fact led to the assumption that the consump-

tion of wine may act against the effects of a high fat diet and reduce the extent of coronary artery diseases (RENAULD & LORGEREIL 1992). This resulted in further interest in resveratrol leading to the monitoring of its biological effects *in vivo* and *in vitro*. First, attention was focused on the examination of its anti-oxidizing substances. Information on the effects of resveratrol on the oxidation of LDL lipoprotein blood plasma fraction (BELGUENDOZ *et al.* 1997) is available. There are medical studies discussing the relation between resveratrol and cardiovascular diseases, mainly in relation to the metabolism of lipids (FRANKEL *et al.* 1995). In general, it holds true that natural phenolic substances showing anti-oxidizing effects might be applied as substances related to fatal tumor proliferation (JANG *et al.* 1997).

Another wave of interest in *trans*-resveratrol as a biologically active substance synthesized mainly by the grapevine grew abruptly with the discovery of the fact that it could be one of the main active components used in traditional oriental herbal medicine to treat heart and tumor diseases. It is a compound present in herbal extracts with benign effects on liver lipids (LDL fraction), regulation of leukocyte synthesis (leukocyte eicosanoid synthesis) with animal models (KIMURA *et al.* 1985).

Later studies made with preparations of pure resveratrol proved its capacities to inhibit oxidation of low-density lipoproteins (LDL) and restrict the occurrence of thrombosis, leading to arteriosclerosis and cardiovascular diseases resulting in heart attack. It is therefore expected that it plays an important role in preventing cardiovascular diseases.

Resveratrol is a compound with which anti-cancer chemo-protective activities affecting three main stages of carcinogenesis were proved. Chemical prevention, cancer prevention by the effects of chemical substances that decrease the risk of tumor proliferation, is one of the direct methods leading to the reduction of growth rate and cancer death rate. The process of chemical carcinogenesis can be split into three general stages (WATTENBURG 1993). Resveratrol inhibits cases in the cell related with the initiation of cancer proliferation, with its continuance and growth. Resveratrol, like other chemo-preventive substances, inhibits hydroperoxidase and cyclooxygenase enzymes. The cyclooxygenase catalyzes the conversion of arachidonic acid to substances that might stimulate tumor proliferation and might restrict the immunity capacities of the body. Resveratrol acts as an antioxidant and antimutagen. It inhibits the formation of free radicals, induces the activity of enzymes such as quinonoreductase and thus contributes to metabolic detoxification of carcinogens – anti-initiating activity.

No information on the development of the toxicity of resveratrol has been published. The option of modulating the oxidative damage caused by many teratogens seems interesting. The development of toxicity of xenobiotics depends on their bioactivation after the influence of em-

brionic cytochromes P450, prostaglandin H synthesis (PHS) and lipoxygenases (LPOs). Released intermediates, like electrophilic compounds and free radicals, are capable to form a covalent bond with biomolecules (DNA, proteins and lipids etc.) or to oxidize them. This oxidative damage of macromolecules plays an important role in the cell proliferation and has a strong teratogenic effect (WELLS *et al.* 1997).

A part of cell reaction to oxidative stress and DNA damage is the induction of genes inscribed as GADD (Growth Arrest DNA Damage). Recently, the induction of GADD 153 and 43 as markers of oxidative damage in mammal cultures of embryonic cells was determined and published (QU *et al.* 1997). During DNA damage, blocking of the cell cycle and apoptosis induction occurs. It results in abnormal morphology or embryonic death.

MATERIAL AND METHODS

Resveratrol: *Trans*-resveratrol in the crystal form was synthesised by Dr. J. Šmírkal in purity approx. 99%. *Cis*-resveratrol was obtained by 10-hour exposure of *trans*-resveratrol standard solution (100 mg/l) to daily light. At these conditions 90% of *trans*-resveratrol was converted into the *cis*-isomer.

The structure of resveratrol isomers is shown in Fig. 1.

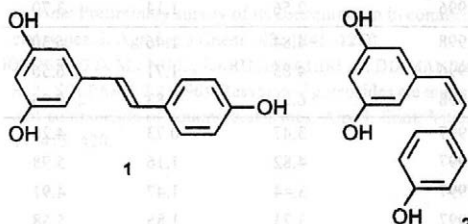


Fig. 1. Structures of the *trans*-isomer (1) and *cis*-isomer (2) of resveratrol

Samples: The samples of commercial red wines from Bohemian (Most, Velké Žemoseky, Litoměřice, Roudnice) and Moravian (Čejkovice, Hodonín, Dubňany) vineyard regions from harvest years 1986–1998 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, André, Zweigeltrebe and Dornfelder) commonly growing in these regions.

Resveratrol Analysis: *Trans*- and *cis*-resveratrols from wine were determined by HPLC method using TSP 3500 liquid chromatograph (U.S.A.) equipped with HP 1049 electrochemical detector with glassy working electrode (Hewlett-Packard, U.S.A.), coupled to Apex Data Station (Czech Republic). The sample of wine was filtered through microfilter (0.2 μ m, 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 \times 4 mm, 5 μ m) with pre-column (10 \times 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 5 mmol NaCl in demineralized 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.

RESULTS AND DISCUSSION

During the past decade natural antioxidants have evoked the widest interest in human diet as protective compounds with significant biological activities. Resveratrol has been one of them.

It was also necessary to screen contents of resveratrol or of other stilbenes from the natural sources potentially available in the Czech Republic and to establish appropriate analytic methods for it. The focus should be on the screening of resveratrol contents in selected red wines and then, after taking into account, the technological impacts on transfer of resveratrol from grapes to wine, on screening of its contents in selected species of grapevine.

The HPLC method that we have developed is a modification of previously published methods for resveratrol and flavonols (JEANDET *et al.* 1995). The main advantages of *trans*- and *cis*-resveratrol assays have been a possibility of the direct injection of wine sample without any pre-concentration steps, easy feasible isocratic elution and their very sensitive and specific response in electrochemical detector. The detection limit for both resveratrol isomers in sample was 10 μ g/l. The appropriate analytical HPLC method with electrochemical detection of resveratrol was developed and routinely used for screening of resveratrol contents in selected domestic red wines.

The *trans*- and *cis*-resveratrol contents were determined in a collection of 38 red wines originating from Bohemian and Moravian vineyard regions and made by local wineries (Table 1). 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 5.6 mg/l, *cis*-resveratrol from 0.7 to 5.1 mg/l and total amount from 1.4 to 9.4 mg/l. Czech red wines had 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 are among 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. It does not always enable full sunlight to reach the grapes and sufficiently warm weather during the vegetation period. The resveratrol content in grapes probably reflects climatic conditions and microbiological (fungal) pressure that are higher in a cooler area in northern Bohemia. From

Table 1. Resveratrol content in red wine originating from Bohemian and Moravian vineyard regions

Vineyard region <i>Locality</i>	Variety of vine ^a	Year of harvest	Resveratrol		
			<i>trans</i> -	<i>cis</i> -	total
Mostecká	Svatovavřinecké (I)	1998	0.71	0.71	1.42
Most	Svatovavřinecké (II)	1998	1.02	0.78	1.80
	Svatovavřinecké	1996	2.29	1.28	3.57
	Zweigeltrebe (I)	1998	0.77	1.18	1.95
	Zweigeltrebe (II)	1998	0.87	1.17	2.04
	Zweigeltrebe (III)	1998	0.95	1.13	2.08
	Zweigeltrebe (kosher I)	1998	0.91	1.25	2.16
	Zweigeltrebe (kosher II)	1998	0.57	1.16	1.73
	Zweigeltrebe	1996	0.92	1.01	1.93
	Rulandské modré (I)	1998	0.82	1.53	2.35
	Rulandské modré (II)	1998	1.31	2.09	3.40
	Rulandské modré (kosher)	1998	1.20	1.24	2.44
	Rulandské modré	1996	1.39	1.26	2.65
	Rulandské modré	1995	5.55	0.94	6.49
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	Zweigeltrebe	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 Labem	Svatovavřinecké	1996	4.85	1.71	6.56
	Modrý portugal	1998	6.23	2.83	9.06
Mutěnická	Svatovavřinecké	1997	3.47	0.73	4.20
Hodonín	Frankovka	1997	4.82	1.16	5.98
	André	1997	3.44	1.47	4.91
	Cabernet sauvignon	1997	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
	Dornfelder	1997	2.56	1.93	4.49
	Dornfelder	1993	2.23	1.98	4.21

^ain brackets the different vineyard position and the kosher product are indicated

the point of view of resveratrol content in wine another important factor is the winemaking technology, namely initial steps in which resveratrol is released from the grape skin. Czech wineries use as a classical technology so a modern method 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 we favour the view that climate is the predominant factor.

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

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Souhrn

MELZOCH K., FILIP V., BUCKIOVÁ D., HANZLÍKOVÁ I., ŠMIDRKA J. (2000): Výskyt resveratrolu v révových vínech pocházejících z českého a moravského vinařského regionu a jeho účinek na lidské zdraví. *Czech J. Food Sci.*, **18**: 35–40.

Resveratrol je látka přírodního charakteru vyskytující se zejména v hroznech vinné révy (*Vitis vinifera*) a následně v červeném víně. Předpokládá se, že resveratrol by mohl být jednou z aktivních složek přispívající při „přiměřené“ konzumaci červeného vína ke snížení rizika kardiovaskulárních chorob. Chemicky se jedná o látku polyfenolického charakteru ze skupiny fytoalexinů –tilbenů (3,4',5-trihydroxystilben), která se vyskytuje ve formě *cis*- a *trans*-isomeru. V přírodních zdrojích převládá *trans*-isomer. Jako polyfenolická látka přírodního původu vykazuje celou řadu biologických aktivit – byly prokázány vlastnosti antioxidantní, antimikrobiální (zejména protiplísňové), schopnost pohlcovat volné radikály, ovlivňuje srážlivost krve (aglutinaci krevních destiček), v poslední době jsou *trans*-resveratrolu přisuzovány i protimutagenní a chemoprotektivní vlastnosti proti nádorovému bujení. Poměrně bohatým a rozšířeným zdrojem resveratrolu jsou právě hrozny *Vitis vinifera*. Slupka hroznů obsahuje kolem 50 až 100 mg resveratrolu/g a jeho průměrná koncentrace v červených vínech světové produkce se pohybuje mezi 1,0–3,0 mg/l. Analyzovaná červená vína pocházejí z českého a moravského vinařského regionu z oblastí mostecké (Most), žernosecké (Velké Žernoseky, Litoměřice) a roudnické (Roudnice nad Labem) v Čechách a na Moravě z oblasti mutěnické (Hodonín, Čejkovice a Dubňany). Celkový obsah resveratrolu se ve vzorcích červených vín pohyboval v rozmezí 1,4–9,4 mg/l, což odpovídá hodnotám, které je možné vzhledem k jeho koncentracím v červených vínech světové produkce považovat za mírně nadprůměrné. Toto zvýšení obsahu resveratrolu pravděpodobně souvisí s drsnějšími klimatickými podmínkami v našich vinařských regionech, které patří mezi nejseverněji položené oblasti, kde se vinná réva pěstuje. Vinná réva je vystavena, zejména v období dozrávání, zvýše-