Resveratrol content in wine – resveratrol biochemical properties

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Abstract

Resveratrol is one of the phenolic compounds with high antioxidant activity. It occurs in nature in two forms, cis- and trans-. The cis- form occurs in grapes, but after being processed into wine, the trans- form is now present. The presence of resveratrol has been demonstrated in more than 72 plant species, mainly in the skins and on the surface of these plants. The physiological function is not completely known, but the highest values of resveratrol have been found in plants infected with microorganisms. Resveratrol is also a phytoalexin, a plant antibiotic that has a protective function in plants. A positive effect of resveratrol has also been observed in humans. According to research, the recommended daily dose should be 12.5 mg/kg body weight, which, according to the levels of resveratrol found in food, cannot be achieved with wine or any other food. Due to very poor bioavailability, where almost three quarters of resveratrol are metabolized in the body, consumption of the recommended daily dose is highly unrealistic. Despite its very low bioavailability, resveratrol has been shown to be an important cardioprotectant, has antitumor activity, is effective in the fight against aging and diabetes, and also acts as a neuroprotective agent. The main method for determining the resveratrol content in food is by high performance liquid chromatography. The aim of the review was to make an overview about chemical properties of resveratrol found in wine.

Keywords: resveratrol, wine, antioxidant capacity, cardioprotectivity, neuroprotectivity

Introduction

In recent years there has been a growing interest in research into phenolic compounds, both in food science and medicine. Phenolic compounds are one of the main characteristics of red wine. They participate in shaping the structure, colour, transparency and stability of wine. These compounds include, in particular, catechin, epicatechin, camphoric, chlorogenic, protocatechuic, syringic and ferulic acids, resveratrol, quercetin, and myricetin. Today, the growing demand for red wines on the world market is mainly explained by high antioxidant activity. There is a positive correlation between the antioxidant effect and the amount of phenolic compounds. The higher the concentration of phenolic compounds, the higher the antioxidant effect. Their quantities in different types of wine vary considerably depending on the vine variety, the soil and atmospheric conditions, the agronomic techniques used, the ripening of the grapes and their health, and the techniques of wine processing (Kekelidze et al., 2017; Fabjanowicz et al., 2018).

The main focus of the present study is on resveratrol, a polyphenolic compound belonging to the natural antioxidants. Resveratrol occurs naturally as cis and trans isomers. The trans isomer usually predominates. The formula of the trans isomer is trans-3,4,5- trihydroxystilbene, i.e. it is a derivative of stilbene (Wiciński et al., 2018). It has been found in many species of plants and was first isolated from *Veratrum grandiflorum* (Hanzlíková et al., 2001). It occurs mainly in grapes, then in smaller concentrations in peanuts, grapefruit peel, mulberries, cocoa, blackcurrant skin, and seeds (Wiciński et al., 2018).

Resveratrol is the subject of much research, mainly for its anti-inflammatory, anti-cancer, chelating and beneficial cardiovascular effects; it also significantly reduces blood sugar (Ulakcsai, 2018).

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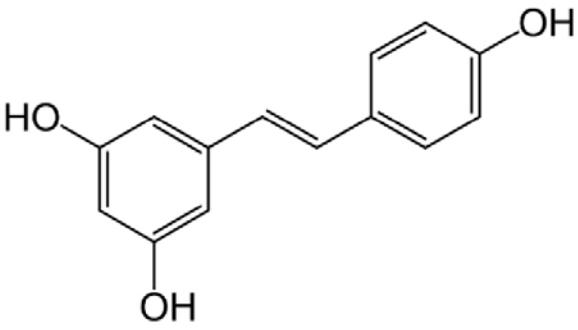


Fig. 1: *Trans* isomer of resveratrol (Hanzlíková et al., 2001)

Resveratrol analytical determination

The vinification process, which is the transformation of fruit juice into wine by fermentation, has a significant effect on the concentration of resveratrol. The resveratrol glycosides cis- and trans-resveratrol-β-d-glycopyranoside (cis- and trans-piceid) and trans-resveratrol are extracted from the grapes. Gradual fermentation decreases the concentration of piceid isomers, while resveratrol isomers increase, with trans-resveratrol having the highest final concentration. The reduction of the piceid isomer is explained by their hydrolysis. While the hydrolysis of piceid isomers increases the concentration of resveratrol, the extraction of resveratrol from grapes is caused by increased ethanol production. The extraction of trans-resveratrol from wine reaches a maximum concentration after approximately 10 days of fermentation (King et al., 2006).

With the development of technology in the 1980s it became possible to determine the content of resveratrol in grapevines, with the use of high-performance liquid chromatography (HPLC) as the most common method (Hanzlíková et al., 2001). Without the need to heat the samples to a certain temperature, the biggest advantages of this method are speed and efficiency (Švec, 2009).

Another method used to determine resveratrol is capillary electrophoresis, a highly sensitive and rapid, repeatable method (Gattu et al., 2018).

Resveratrol can also be determined immuno-enzymatically using an ELISA method that is based on the interaction between antigen and antibody. The principle is the reaction of a colourless substrate to a coloured product. Either spectrophotometric determination or fluorescence can subsequently be used (Heyduk et al., 2018).

Biochemical properties of resveratrol

Resveratrol has been found in more than 72 species of plants, which include, for example, plants from the *Brassicaceae*, *Apiaceae*, *Amaryllidaceae*, *Asteraceae*, and *Theaceae* families, as can be seen from Table 1.

In plants, resveratrol acts as a phytoalexin, which is a plant antibiotic that plants produce when attacked by pathogenic microorganisms such as fungi and bacteria (Beljanski, 2010) in response to stress and UV radiation (Willson, 2010).

Resveratrol phytoalexins are among the mechanisms of cell resistance. It has been shown that after exposure to UV radiation or after infestation of a grapevine with fungi such as *Plasmopara viticola* or *Botrytis cinerea*, these substances begin to form and accumulate rapidly (Hanzlíková et al., 2010).

Table 1: Resveratrol concentrations in commonly available foods (Hanzlíková et al., 2001)

Plant	Resveratrol [mg.kg$^{-1}_{\text{dry matter}}$]	Dry matter [%]	Resveratrol [mg.kg^{-1}]	Amount [kg] containing 1 mg of resveratrol
Chinese cabbage	9	5	0.5	2.2
Red cabbage	15	16	2.4	0.4
White cabbage	8	7	0.6	1.8
Savoy cabbage	7	14	1.0	1.0
Brussels sprouts	15	17	2.6	0.4
Broccoli	15	12	1.8	0.6
Chicory	20	5	1.0	1.0
Parsley	5	19	1.0	1.1
Carrot	4	10	0.4	2.5
Beetroot	8	22	1.8	0.6
Garlic	2	29	0.6	1.7
Yellow onion	17	9	1.5	0.7
Red onion	12	9	1.1	0.9
Peanut vine	2	94	1.9	0.5
Tea plant	1	94	0.9	1.1

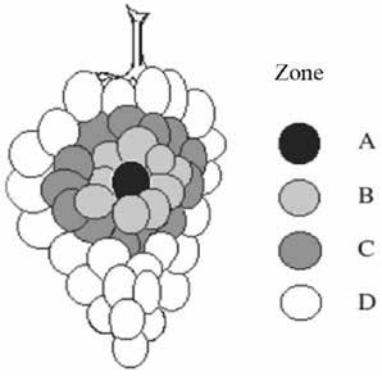


Fig. 2: Grapevine bunch infected with *Botrytis cinerea* (Hanzlíková et al., 2010)

Zone	*Trans*-resveratrol [mg·kg^{-1}]		
	Pinot	Chardonnay	Gamay
A	6	2	6
B	28	6	16
C	22	4	9
D	20	2	9

Fig. 3: Resveratrol concentration in grape skins 48 h after infection with *Botrytis cinerea* (Hanzlíková et al., 2010)

Resveratrol has also been shown to have remarkable health benefits. However, the low bioavailability of resveratrol reduces its therapeutic utility. Based on this finding, many of its derivatives have been developed by scientists. Methoxylated, hydroxylated and halogenated derivatives that show favourable therapeutic potential have been investigated (Nawaz et al., 2017).

A number of studies also point to the low bioavailability of unconjugated resveratrol in the body. At least 70 per cent of ingested resveratrol is absorbed and easily metabolised to glucuronide and sulphate derivatives (Fernández-Mar et al., 2012). The plasma concentration of resveratrol and its derivatives depends mainly on its administered dose; the maximum concentration of metabolites in plasma is usually reached 30 minutes after administration (Marier et al., 2002). Its presence has been found not only in plasma and urine, but also in the tissues of the heart, liver, and kidneys (Fernández-Mar et al., 2012).

Resveratrol interacts with proteins to bind to albumin. This bond increases in the presence of fatty acids. The hepatic cell uptake of resveratrol involves two processes, passive and carrier-mediated. Due to carrier-mediated transmission, resveratrol, although tightly bound to blood proteins, can to a large extent be delivered to body tissues (Jannin et al., 2004). Resveratrol has also been shown to synergize with both quercetin and ellagic acid in inducing apoptosis in human leukaemia cells (Mertens-Talcott, 2005). It also interacts with ethanol to inhibit iNOS expression, vitamin E to prevent lipid peroxidation, catechin to protect PC12 cells from β-amyloid toxicity, as well as with tyrosol and β-sitoesterol to modulate LDL oxidative stress and PGE2 synthesis (Fernández-Mar et al., 2012).

Studies also show the intrinsic antioxidant capacity of resveratrol, which may be related to its chemo-preventive effect (Fernández-Mar et al., 2012). This fact is an important factor in protection against oxidative stress, which occurs during the abnormal accumulation of oxygen intermediates (ROS) formed by cellular organisms. These are in particular superoxide, hydrogen peroxide, and hydroxyl radicals, which are usually detoxified by intracellular enzymes such as glutathione, superoxide dismutase, and catalase. Scientific research, based on the incubation of cultured rat aortic smooth muscle cells (ASMC) with resveratrol at low micromolar concentrations, has shown a significant induction of a range of cellular antioxidants and enzymes over concentration and/or time. Preventive treatment with resveratrol also led to a significant reduction in the intracellular accumulation of reactive oxygen species in ASMC (Li, Cao, & Zhu, 2006).

In vivo tests have shown that resveratrol increases plasma antioxidant capacity and reduces lipid peroxidation, which is strongly associated with the risk of coronary heart disease and myocardial infarction. Studies in rats, pigs, and humans suggest that resveratrol may suppress pathological increases in lipid and other macromolecule peroxidation in vivo (Fernández-Mar et al., 2012).

In their research, Baroni et al. (2012) concluded that the grape variety used for wine production is particularly important for determining the biological activity of resveratrol. They studied the effect of H_2O_2 on the yeast *Saccharomyces cerevisiae*, which was cultivated in an environment without the presence of wine and with the presence of wine. The yeast in the presence of wine showed less damage.

According to some publications, resveratrol acts as an antioxidant and antimutagen. Based on an experiment in which wine was produced in a conventional manner, by prolonged maceration and with the addition of oenological enzymes, it was found that the vinification method does not have a significant effect on the antioxidant properties of wine. None of these methods showed an increased value of antioxidant activity. In contrast to wine obtained by classical maceration and with the addition of oenological enzymes, the content of trans-piceid in wine obtained by prolonged maceration was reduced (Mulero et al., 2011).

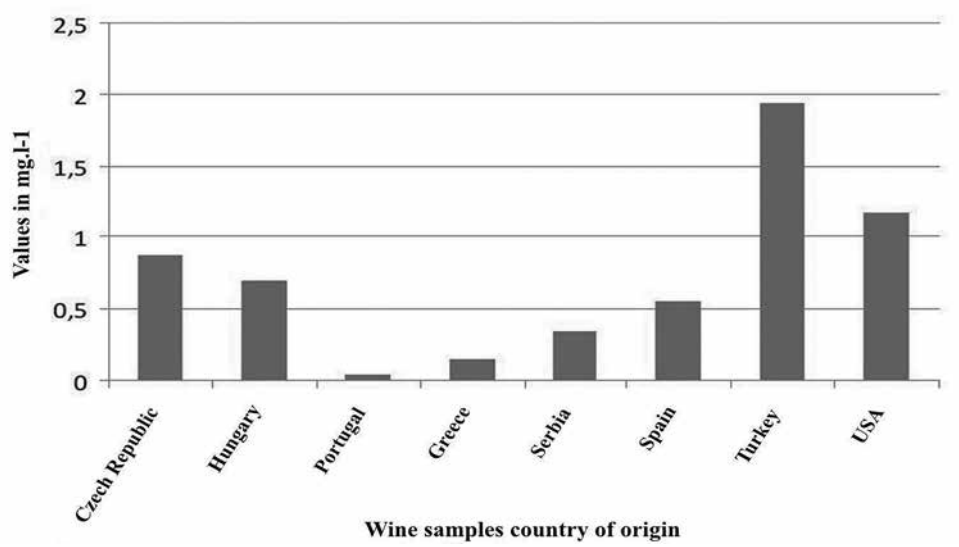
A study by Galmarini et al. (2013) monitored the effect of wine drying and the addition of maltodextrin dextrose equivalent on antioxidant activity. A slight decrease was observed during storage, which was amplified by higher temperature and lower water activity.

Resveratrol also inhibits the enzymatic activity of both forms of cyclooxygenase, reducing the risk of many cancers. It mediates anti-inflammatory effects and inhibits cyclooxygenase and hydroperoxidase functions (antipromoting activity), and it induces promyelocytic leukaemia cell differentiation (antiprogressive activity) (Fernández-Mar et al., 2012). Resveratrol has the antiproliferative effects of inducing apoptosis in cell lines of various origins, such as leukaemia and cancer of the breast, prostate, colon, and pancreas, as well as head and neck cancers. It induces Fas-dependent apoptosis in some cell lines and Fas-independent apoptosis in others (Aggarwal et al., 2004).

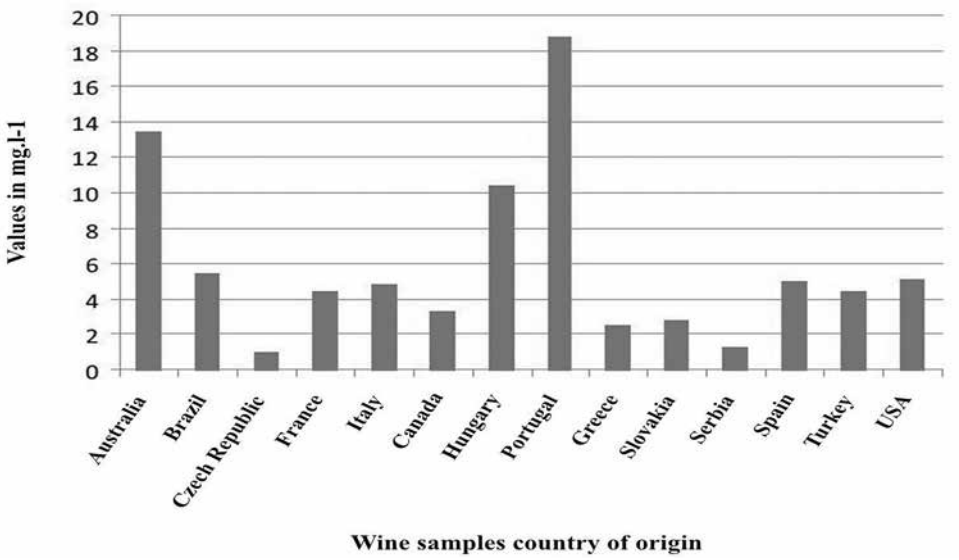
Research studies show that drinking a glass of red wine a day can reduce the risk of prostate cancer by up to half, and that the protective effect appears to be strongest against

the most aggressive forms of the disease. It has also been shown that men who consume four or more glasses of red wine a week have a 60% lower incidence of more aggressive types of prostate cancer (Fernández-Mar et al., 2012).

The tables below show that the content of trans-resveratrol is many times higher in red wines than in white wines. Large differences were also observed for wines from different countries of origin, where wines from Portugal, Australia or Hungary, for example, showed the highest values (Švajdová, 2011). The highest content in white wines was observed in wines originating from Turkey and the United States (Graph 1).



Graph 1: Content of trans-resveratrol in white wines by origin (Švajdová, 2011)



Graph 2: Content of trans-resveratrol in red wines by origin (Švajdová, 2011)

The effect of resveratrol on cardioprotectivity has also been a significant finding. At low concentrations, resveratrol inhibits apoptotic cell death, thereby providing protection against a variety of diseases, including ischemic reperfusion injury of the myocardium, atherosclerosis, and ventricular arrhythmias. In higher doses it facilitates apoptotic cell death and behaves as a chemo-preventive alternative (Das & Das, 2007; Fernández-Mar et al., 2012). One study by Frankel et al. (1993) demonstrates that trans-resveratrol inhibits copper-catalysed LDL cholesterol peroxidation. Wine diluted 1000 times, containing 10 mumol/l of total phenols, inhibited the oxidation of LDL cholesterol significantly more than alpha-tocopherol. The results of the study show that the non-alcoholic components of red wine have strong antioxidant properties against the oxidation of human LDL cholesterol (Frankel et al., 1993).

An important finding was made by Nawaz et al. (2017), who report the results of tests with hypercholesterolemic hamsters fed the methoxyl derivative of resveratrol, pterostilbene. At a dose of 25 ppm, they had a 29% lower LDL cholesterol level, a 7% higher plasma HDL cholesterol level, and a 14% lower plasma glucose level compared to the control group (Nawaz et al., 2017).

Further research confirms the effect of resveratrol on atherosclerosis. One of the main factors contributing to the process of atherosclerosis is platelet aggregation. Resveratrol prevents platelet aggregation in vitro and in vivo, and also helps reduce atherosclerotic plaque formation and restore dilatation (Fernández-Mar et al., 2012).

A study by Li et al. (2000) provides evidence that in addition to the presence of antioxidant activity, resveratrol can also stimulate channels in endothelial cells. Direct stimulation of these channels by resveratrol may be responsible for its effect on endothelial cell functional activities.

Resveratrol is able to penetrate the blood-brain barrier and show strong neuroprotective effects here even at low doses. Resveratrol has also been shown to fight neuronal dysfunction caused by Huntington's and Alzheimer's diseases via SIRT1 (sirtuin) (Fernández-Mar et al., 2012; Parker et al., 2005).

Also important is its ability to fight aging at the cellular level, which can ultimately lead to a breakthrough in geriatric and anti-aging drugs. The contribution of resveratrol to memory and the prevention of neurodegenerative diseases are well documented in laboratory animals, while in humans these data are insufficient. Resveratrol has been shown to modulate mitochondrial biogenesis by activating PGC-1α, which may eventually slow the aging process, prevent many chronic diseases, and improve muscle endurance (Smoliga et al., 2011).

Absorption and metabolism of resveratrol in the human body

Resveratrol is absorbed in the small intestine. However, a number of studies point to the low utilization of resveratrol in both animals and humans. After absorption, it is rapidly metabolised in hepatocytes into various forms. The metabolites that have been identified in the human body are resveratrol sulphate and resveratrol glucuronide. These substances then flow in the bloodstream and are transformed into sulphite derivatives, which are excreted from the body (Fabjanowicz et al., 2018).

An important amount of dihydroresveratrol is also formed in the lower segments of the intestine due to the enzymatic activities of intestinal microflora. It is usually present in blood and tissues in the form of conjugates. The question therefore remains to what extent the beneficial effects allegedly associated with this stilbenoid are attributed to metabolites rather than the parent molecule, which is found in the body only in very low concentrations (Mattarei, 2015).

The recommended amount corresponds to a dose of 12.5 mg/kg body weight, in an adult weighing 80 kg. These concentrations are justified by animal experiments, most of

which require a daily dose of 5 to 100 mg resveratrol/kg body weight to achieve a specific biological effect (Weiskirchen, 2016).

Studies in humans have shown that resveratrol is a safe drug and is reasonably well tolerated at doses up to 5 g/day. Most of these therapeutic activities have only been determined in cell culture or preclinical models (Weiskirchen, 2016).

However, these recommended daily doses are so high that it is not possible to absorb them by drinking wine or from other dietary sources. There is a need for more in-depth research to determine more accurate recommended doses of resveratrol for humans and, consequently, the amounts recommended for the treatment of specific diseases (Weiskirchen, 2016).

Oral administration of resveratrol may provide sufficient concentrations in the gastrointestinal tract to achieve therapeutic benefits. This is thought to be due to the accumulation of resveratrol in epithelial cells located in the digestive tract (Almeida at al., 2009).

Although relatively well absorbed, it has low bioavailability, leading to low plasma concentrations that allow only limited systemic distribution and concentrations that are not high enough to produce significant pharmacological effects. Therefore, other routes of delivering resveratrol to the body are being investigated. One such method is the use of other phytochemicals in conjunction with resveratrol, which is thought to improve bioavailability by protecting it from rapid metabolism. However, its effects on the human body are the subject of further research (Vries, 2018).

Conclusion

Resveratrol is a substance that is not common in nature. Nevertheless, its presence in 72 species of plants is proven, with the highest concentrations being found in grapevine. In plants, it acts as a plant antibiotic and is produced when the plant is attacked by foreign microorganisms.

Resveratrol is also one of the phenolic compounds which are characterized by high antioxidant capacity. The more phenolic compounds (and thus resveratrol) a wine contains, the higher its antioxidant capacity. Its content may vary in the wine depending on the soil, grape variety, technique used, ripening and many other biotic and abiotic factors.

From the present study, it can be concluded that of the white wine samples, wine originating in Turkey has the highest content of resveratrol, with the sample from Portugal winning the red wine category. The sample of white and red wine from the Czech Republic contained less than 1 mg/l of resveratrol, which in the case of white wine is an above-average result, unfortunately for red it is the lowest value of all analysed samples.

From the above chapters it can be concluded that resveratrol is a substance with favourable properties. However, many of the results come from tests on rodents and under modified laboratory conditions. From studies performed directly on humans, the researchers concluded that the resveratrol contained in wine has undeniable beneficial effects on the human body. However, in order for these effects to be applied in therapy or treatment, the wine would have to be consumed in high amounts. This eliminates health benefits due to the alcohol content. Many scientists are currently working to develop a suitable derivative that would increase bioavailability so that concentrated resveratrol can be used for therapeutic purposes. Even at low doses, resveratrol has been shown to be an excellent cardioprotectant, has an antidiabetic effect, neuroprotective activity, and also retards aging.

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