

## Red Wine as Resveratrol Protective System

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**Abstract:** The study describes an isomerization of *trans*-resveratrol exposed to diffused daylight in dependence on temperature and character of liquid sample (type of solvent, wine ...). These effects were investigated using the reverse-phase-HPLC method. *Trans*-resveratrol isoform was light sensitive and under daylight isomerized to *cis*-isoform very easily. The exposition of *trans*-resveratrol solution by higher temperatures did not induce isomerization reaction if that was kept in dark. The wines were analyzed immediately after opening (0 hour), and again after 48 hours and after 17 days. The concentration of resveratrol in tested red wines was stable at 4°C, under room temperature the total amount of resveratrol in all cases increased.

**Keywords:** resveratrol; *Vitis vinifera*; red wine; antioxidants; polyphenols

### INTRODUCTION

Resveratrol (*trans*-3,5,4'-trihydroxystilbene) like other fully natural polyphenolic compounds is studied extensively, owing to its positive effect on human organism. These compounds are classified as antioxidants and it is believed that their positive effect is tightly connected with lipid metabolism and consequently with incidence of cardiovascular and cancer diseases [1, 2].

Resveratrol is fully soluble in organic solvents such as ethanol, methanol, dimethylsulfoxide etc.

The most of phenolic compounds are responsible for colour and contribute to the bitter flavour of wines. Resveratrol is known to occur in wine in free and glycosidically bound form, which is known as piceid or polydatin. Both resveratrol and piceid can be found in grape products with the concentration of the glycoside usually being significantly higher than the aglycone. The relative distribution between the glycosylated and aglycone forms in wines is dependent on a number of factors influenced by fermentation and enological techniques used [3]. 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 [4]. 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 [5].

The 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, several condensation reactions, involving especially anthocyanins, catechins and procyanidins, take place and result in the formation of new polymeric compounds [6]. The chemical composition of wine is influenced by the climatic and atmospheric conditions, soil type and locality, vine cultivation and the treatment to which it is subjected and at last by winemaking process.

Resveratrol and other hydroxylated stilbenes are accumulated in plants in response to attacks by pathogens such as *Plasmopora 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 [7]. Irradiation of plant tissues with UV light has some important effects on phenolic metabolism. UVB 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 [8].

## EXPERIMENTAL

**Resveratrol.** *Trans*-resveratrol was synthesized in the crystalline form and at a purity of 99%. *Cis*-resveratrol was obtained after a 10-hour exposure of a standard *trans*-resveratrol ethanolic solution (100 mg/l) to the diffused daylight (it was examined by NMR). Under these conditions, 80% of *trans*-resveratrol was converted into the *cis*-isomer.

**Samples.** Samples of commercial red wines (harvest year between 1986 and 2002) produced in the Bohemian (Most, Velké Žernoseky, Litoměřice, Roudnice) and Moravian (Čejkovice, Hodonín, Dubňany, Lednice) vineyard regions. The samples were obtained directly from the wineries. The wines were made from varieties commonly growing in these regions (blue grapes: Blaufränkisch, Pinot Noir, Saint Lawrence, Cabernet Sauvignon, Cabernet Moravia, Zweigeltrebe, Laurot, Tintet, Neronet, Merlot; white grapes: Erilon, Rubikon, Hibernál).

**HPLC analysis.** *Trans*- and *cis*-resveratrol were determined by the HPLC method using the TSP 3500 liquid chromatograph (TSP, U.S.A.) equipped with TSP 4100 UV detector (TSP, U.S.A.) and the HP 1049 electrochemical detector with glassy working electrode (Hewlett-Packard, U.S.A.) at a potential of 0.75 V, coupled to the Apex Data Station (Apex, Czech Republic). The sample was injected with the Rheodyne valve (Cotati, U.S.A.) into the column filled with the stationary 120-5-C18 reverse-phase Nucleosil (250 × 4 mm, 5 µm; Supelco, USA), with the pre-column (10 × 4 mm) packed with the same stationary phase. The separation was carried out at room temperature. The isocratic elution at a flow rate of 1.0 ml/min used the mobile phase of 25% acetonitrile, 0.1% H<sub>3</sub>PO<sub>4</sub> and NaCl (c = 5 mmol/l) in demineralized water. After each analysis, the column was rinsed with mobile phase for 10 min. Identification and quantification of resveratrol isomers were carried out by the internal or external standard techniques.

## RESULTS AND DISCUSSION

Many authors are dealing with stability of *trans*- and *cis*-resveratrol in wines under various physical conditions. The most usual conditions are protection against light and storage at room temperature or at 4°C for various time periods. Data presented in literature are very different and not consistent.

Some special kinds of wines are after fermentation process stored in oak barrels in order to obtain specific barrique character. Such storage can lead to decrease of resveratrol approximately to 68% *trans*-resveratrol and 58% *cis*-resveratrol of initial values. It seems that precipitation, oxidation and absorption in barrels could be a cause of such decrease. Another possible reason for resveratrol decrease could be use of filtration [9].

GOLBERG *et al.* [10] performed number of experiments on resveratrol content in wines. They did not detect significant changes after 7 days for both temperatures (4°C and 20°C). However after 6 weeks the value decreased to 82% under room temperature. Meanwhile the concentration of resveratrol in wine stored under 4°C was not changed. They also proved that under these conditions exposition of wine to air during 48 hours had no harmful effect on resveratrol concentration [11]. In second experiment, performed one year later very different results were found. The concentration of *cis*-resveratrol remained the same under 4°C and under room temperature was stable for 7 days. After 6 weeks the decrease to 67% was detected under the room temperature. If the wine was stored opened, the concentration of resveratrol remained constant only 48 hours for both temperatures.

Resveratrol was stable in wine that was protected against sun exposure longer than one week under 4°C, but after 6 weeks the concentration decreased about 10 to 40%. The cause of that behaviour could be dimerization [12].

Also the age as factor that can significantly influence the content of resveratrol was extensively studied. Surprisingly it seems that resveratrol in wine is stable even though it could be suggested that it would decrease in compliance with age and partly with oxidation, as is declared for other polyphenols [13].

In our experiments, after dissolving in ethanol 50% (v/v) or methanol 50% (v/v) significant changes in *trans/cis* ratio has been observed when samples were exposed to daily-diffused light. *Trans*-resveratrol ethanolic solution isomerized to the *cis*-isomer

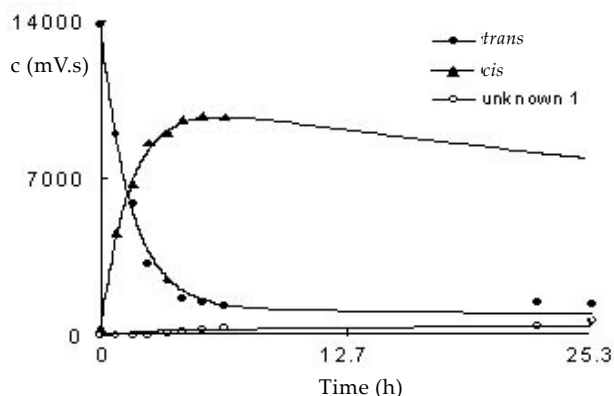


Figure 1. Changes of *trans*-resveratrol ethanolic solution during exposition to daily-diffused light

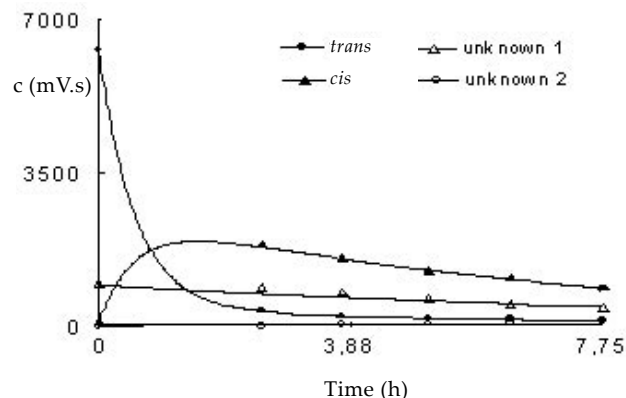


Figure 2. Changes of *trans*-resveratrol methanolic solution during exposition to daily-diffused light

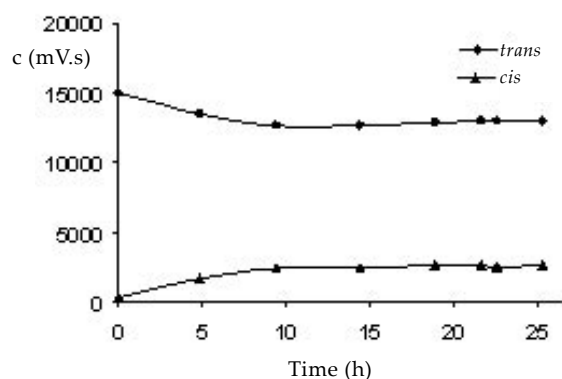


Figure 3. Changes of *cis/trans* resveratrol isomers during exposition of red wine samples to daily-diffused light

within 5 hours (Figure 1), in methanolic solution *trans*-resveratrol isomerized to the *cis*-isoform in 6 hours (Figure 2). The isomer equilibrium remained unchanged during sample standing in darkness. The isomer equilibrium changed during samples continuing exposition to daily-diffused light with the benefit of three unknown substances – probably resveratrol dimers or trimers (Figures 1 and 2).

The ratio of *trans*-resveratrol to *cis*-resveratrol in red wine samples remained broadly unchanged during exposition to daily-diffused light. The *trans*-isomer in red wine samples during exposition to daily-diffused light slightly changed towards the

higher *cis*-resveratrol content (Figure 3). The final ratio of *cis/trans* isomers differs from equilibrium of resveratrol solution in ethanol and methanol. This fact can be explained by presence of other phytoalexins and antioxidants and their mutual protective effects.

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