

**DETERMINATION OF trans-RESVERATROL IN SOME
ROMANIAN WINES AND SPIRITS**

Lavinel G. Ionescu ^{a,b} and André A. Souto ^b

SCIENCO Scientific Consulting Services ^a
Huntington Beach, California, USA

and

Departamento de Química Pura ^b

Faculdade de Química

Pontifícia Universidade Católica do Rio Grande do Sul

Porto Alegre, RS, BRASIL

ABSTRACT

Resveratrol, a phytoalexin of the hydroxystilbene group, is widely beneficial to health. It has been shown to possess anti-oxidative, anti-carcinogenic, anti-tumor and anti-viral properties. The concentration of trans-resveratrol in some Romanian wines and spirits has been measured using a direct injection isocratic UV-HPLC (Ultra Violet – High Liquid Pressure Chromatography) method. The experimental values obtained ranged from 0,10 mg/L for Cognac Murfatlar to 2,32 mg/L for Cabernet Sauvignon from the Dealu Mare Region.

KEYWORDS: trans-resveratrol, Romanian wines

RESUMO

Resveratrol, uma fitoalexina do grupo do hidroxiestilbeno, é altamente benéfico para a saúde. Foi demonstrado que o resveratrol possui propriedades anti-oxidativas, anti-carcinogênicas, anti-tumorais e anti-virais. A concentração de trans-resveratrol foi determinada em alguns vinhos e cognacs da Romênia usando método de injeção isocrática direta e Cromatografia Líquida de Alta Pressão (CLAP). Os valores experimentais obtidos, variaram de 0,10 mg/L para Cognac Murfatlar até 2,32 mg/L para Cabernet Sauvignon da Região de Dealu Mare.

INTRODUCTION

Resveratrol, a phytoalexin of the trans-stilbene group, is found in grapes, peanuts, berries, pines, many medicinal and non-medicinal plants and in red wines.

The beneficial effects of wines, in particular red wines, have been known since ancient times and are probably due to a combination of the large number and variety of chemical compounds present in grapes and wine, including amino acids, hydrocarbons, alcohols, aldehydes, esters, sugars, glucosides, organic acids, phenols, polyphenols, vitamins, enzymes, tannins, anthocyanins, flavins, flavans, inorganic salts, etc. ¹⁻⁵

Recently, resveratrol has been pointed out as the component of wine more beneficial to health, has been used to explain the so-called "French paradox" and has been shown to possess anti-oxidative, anti-tumor, anti-carcinogenic and anti-viral properties.

The compound of particular interest is trans-resveratrol (trans-3,4',5-trihydroxystilbene), whose structure is illustrated in Figure 1. As can be seen, it can be considered a precursor of diethylstilbestrol, estradiol and other hormones.

Aryl derivatives of trans-stilbene have been shown to be good liquid scintillators, in addition to having physiological properties. ^{6,7}

In a simple-minded approach, trans-resveratrol has been recently begun to be considered a miraculous compound, "The Chateau Hormone", responsible for good health, longevity, a cure-all panacea and a possible explanation of the "French paradox", very much alike the elixir of long life or the philosopher's stone of Alchemy.

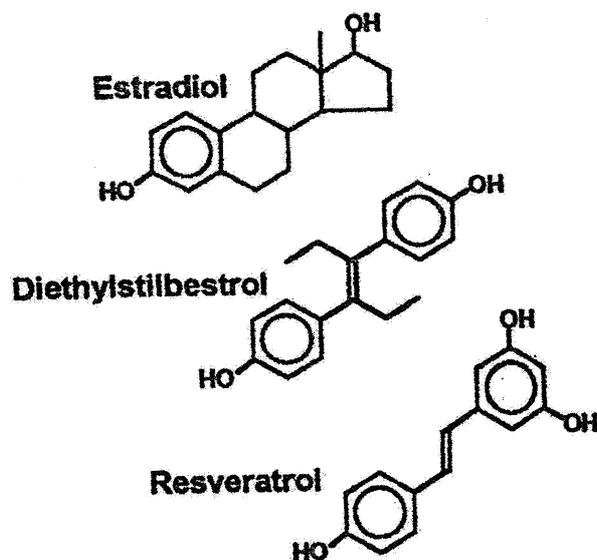


Figure 1. Structure of trans-resveratrol and related compounds

The population of many countries of Southern Europe, including France, Italy, Spain, Portugal, Croatia, Greece, Albania, Bulgaria and Romania suffers little heart disease, in spite of a diet that is relatively rich in fat. This fact has been explained by the regular consumption of red wine in moderate amounts and has been called the “French paradox”.⁹⁻¹²

S. Renaud and M. De Lorgeril⁹ studied the relationship between wine, alcohol, platelet function and coronary heart disease, while D. M. Goldberg and coworkers¹⁰ suggested the apparent ability of moderate consumption of red wine to reduce the risk of cardiovascular disease.

B. D. Gehm, J. M. McAndrews P.Y. Chien and J. L. Jameson¹¹ showed that resveratrol found in grapes and wine is an agonist for the estrogen receptor, concluded that resveratrol is a phytoestrogen, that its estrogenic actions broaden the spectrum of its biological actions and that it may be relevant to the reported cardiovascular benefits of drinking red wine.

On the other hand, P. Kopp¹², after analyzing a variety of studies done with resveratrol, concluded that whether resveratrol is indeed implicated in the explanation of the “French paradox” remains to be shown. According to him, currently there is no information on the serum concentration of resveratrol after

red wine consumption, nor its exact fate after ingestion and we can only speculate how much and which vintage of "Château Hormone" we should drink in order to benefit from the effect of resveratrol, while avoiding the potentially detrimental actions of ethanol.

More recent studies by M. Buluk and E. Demirel-Yilmaz¹³ have shown that resveratrol decreases calcium sensitivity of vascular smooth muscle, enhances cytosolic calcium increase in the endothelium and causes independent relaxation of the vascular smooth muscle.

The chemopreventive activity of resveratrol in several types of cancer and the protective effects of polyphenols in oxidative stress have also been reported. M. Alkhalif and A. M. El-Mowafy have demonstrated that resveratrol activates adenylyl cyclase in human breast cancer cells and that it also has a potent antiproliferative effect on human osteosarcoma.¹⁴

R. Rodrigo and G. Rivera have studied in detail the protective effects of red wine in renal damage mediated by oxidative stress.¹⁵

EXPERIMENTAL PROCEDURE

The experimental procedure used was essentially described by A. A. Souto and coworkers.¹⁶ The HPLC system employed was composed of a Perkin – Elmer 785A UV-Vis detector adapted for stopped-flow scanning (190-360 nm), a PE Series 200 pump, a PE 900 Series Interface and a PE Series 200 vacuum degasser. An octadecyl column 250 mm long was used, with 5µm particle diameter and 4.5mm inside diameter (Brownlee, Norwalk, USA). After filtration using a 0.45mm membrane, the samples were diluted six times with eluent and directly injected through a 20 µL loop into a C18 guard column. Reversed phase HPLC was performed with an isocratic elution (1.5 mL/min) using a solution of water-acetonitrile (75:25) as the eluent. The signal was monitored at 306 nm. The trans-resveratrol standards were supplied by Pharma Science, Inc., Montreal, Quebec, Canada. The wines and spirits analyzed were obtained from commercial suppliers in Romania. For quantification, an external standard calibration curve was done ranging from 0.10 to 10.0 mg/L of trans-resveratrol. The square

regression coefficient of the analytical curve was near unity ($r^2 = 0.9986$) Data were processed by Turbo Chrom 4.0 software.

RESULTS AND DISCUSSION

A representative HPLC chromatogram is illustrated in Figure 2.

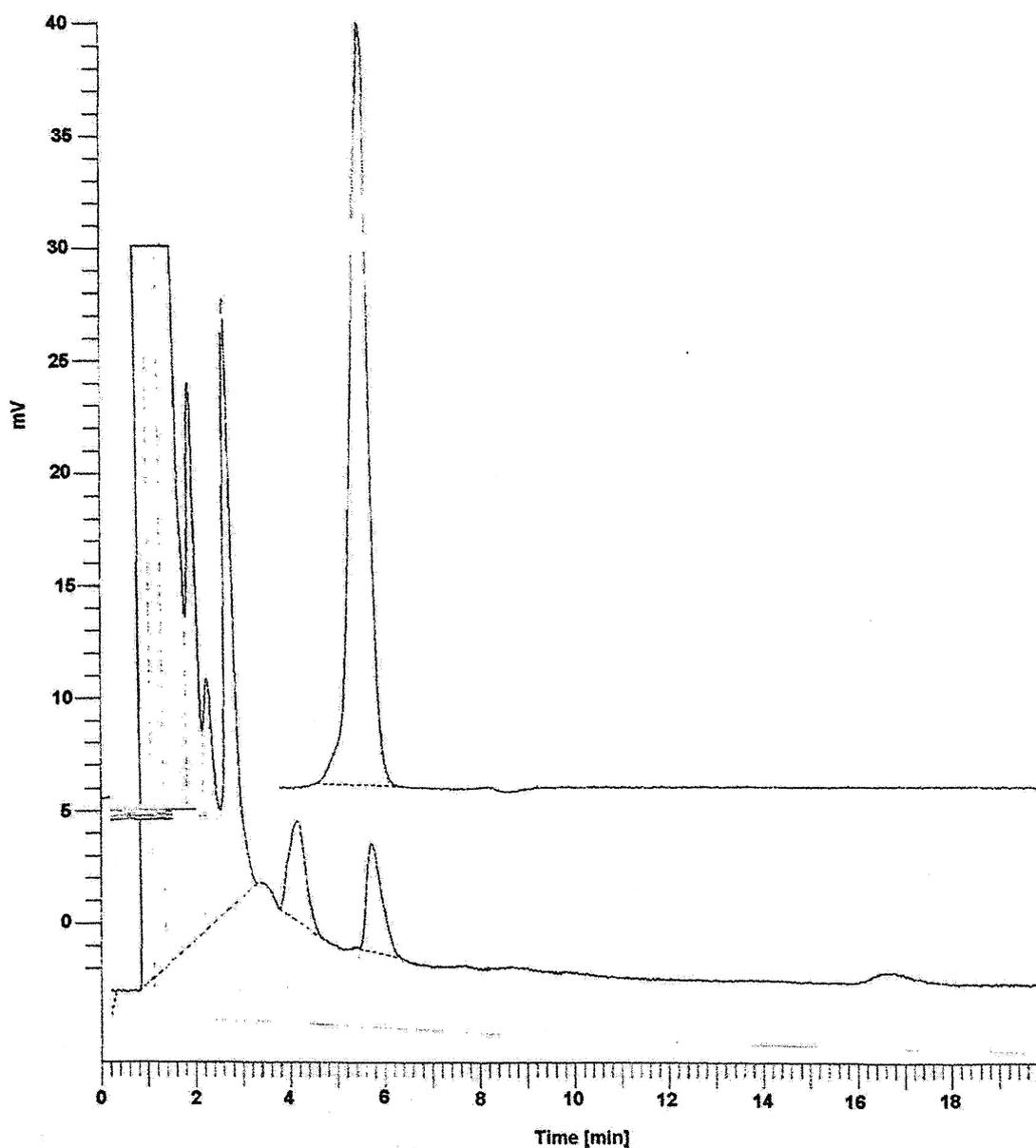


Figure 2. Typical HPLC chromatogram of a trans-resveratrol standard solution (top) and a diluted wine sample of Cabernet Sauvignon from Dealu Mare.

Some typical results obtained for the chromatographic analyses for the different Romanian wines and spirits are shown in Table I.

Table I. Concentration of *trans*-Resveratrol in Some Romanian Wines and Spirits.

Wine	Year	Conc. mg/L
Cabernet Sauvignon (Dealu Mare)	1984	2.32
	1991	2.02
Cabernet Sauvignon (Murfatlar)	1997	1.38
	1998	1.42
Dacia-Feteasca Albă (Cotnari)	1998	0.52
	1993	0.40
	1999	0.33
Pinot Gris (Murfatlar)	1992	0.10
	1999	0.09
Muscat Otonel (Murfatlar)	1997	0.14
Sampanie –Silvania-Sec (Salaj-Zalau)	1991	0.12
Cognac (Murfatlar)	1998	0.12
	1999	0.14
Apricot Brandy (Murfatlar)	1997	0.10
Palinca (Alba Iulia)	1992	0.11
Cognac Fortuna (Focsani)	1995	0.05
Rachiu (Alibunar-Banat- Yugoslavia)	1992	0.14

The concentration of *trans*-resveratrol determined in Romanian white wines and spirits varied from 0.10 to 0.52 mg/L. These values were expected and are typical of white wines that are known to contain lesser amounts of resveratrol. The highest value measured for a white wine was for Feteasca Alba from the Cotnari Region.

The cognacs and brandies analyzed showed small amounts of resveratrol (~0.10 mg/L), as expected.

The red wines presented values that ranged from 1.38 to 2.32 mg/L. The highest value was observed for Cabernet Sauvignon from Dealu Mare (1984 Vintage). The value of 2.32 mg/L can be considered significant, when compared to

other red wines from around the world (0.20 -5.06 mg/L) and taking into account the age of the wine.

ACKNOWLEDGMENT. Financial support received from Sarmisegetusa Research Group, Santa Fe, New Mexico, USA, is gratefully acknowledged.

REFERENCES

1. L. G. Ionescu, "Química do Vinho", Laboratório de Química de Superfícies – LQS, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil, 1980, 45 pp.
2. A. Dinsmoor Web, "Chemistry of Winemaking, American Chemical Society, Washington, D.C. USA, 1974, 311pp.
3. C.S. Ough, *Chem. Eng. News*, 65(1), 19-28 (1967).
4. R. Coordonnier, *Rev. Fr. Enol.*, 50, 15-26 (1952).
5. M.A. Gerasimov, *Vinodelie & Vinogradstvo*, 12(7), 9-11 1952).
6. L.G. Ionescu, "Liquid Scintillators. Some 3-Aryl 1,2-Dihydronaphthalenes, 3-Aryl-1,2-Dihydro-4-Methylnaphthalenes, 3-Aryl-1,2-Dihydrophenanthrenes and 3-Aryl-1,2-Dyhidro-4-Methylphenanthrenes. Steric Effects of the Methyl Group. Master's Thesis, The University of New México, Albuquerque, N. M., USA, 1965, 112pp.
7. G.H. Daub, F. N. Hayes, D.W. Holty, L. G. Ionesu and J. S. Schornick, *Molecular Crystals and Liquid Crystals*, 4, 343-355 (1968).
8. N. Boyce, *New Scientist*, 16, (1997).
9. S. Renaud and M. De Lorgeril, *Lancet*, 339, 1523-1526 (1992).
10. D.M. Goldberg, S.E. Hahn and J. G. Parker, *Clin. Chim. Acta*, 237, 155-187 (1995).
11. B. D. Gehm, J. M. McAndrews, P.Y. Chien and J. L. Jameson, *Proc. Natl. Acad. Sci. USA*, 94, 14138-14143 1997).
12. P. Kopp, *Eur. J. Endocrinol.*, 138, 619-620 1998).
13. M. Bulic and E. Demirel Yilmaz, "Possible Mechanism for Depression of Smooth Muscle", in "Advances in Recent Cardiovascular Research", A. Vavo and A. Vegh, Eds., Monduzzi Editore, Bologna, Italy, 2002, p. 55-59.

14. A. M. El Mowafy and M. Alkhalif, *Carcinogenesis*, **24**, 869-873 (2003)
15. R. Rodrigo and G. Rivera, *Free Radic. Biol. Med.*, **33**, 409-422 (2002).
16. A. A. Souto, M. C. Carneiro, M. Seferin, M. J. Senna, A. Conz and K. Gobbi, *J. Food Comp. Anal.*, **14**, 441-445 (2001).