

Effects of fining agents on *trans*-resveratrol concentration in wine

R.T. THRELFALL¹, J.R. MORRIS^{1,3}, and A. MAUROMOUSTAKOS²

¹Institute of Food Science and Engineering, University of Arkansas, 272 Young Avenue Fayetteville, AR, 72704, USA

²Agricultural Statistics Laboratory, University of Arkansas, AGRX-101, Fayetteville, Arkansas 72701, USA

³ Corresponding author: Dr Justin Morris, FAX I 501 575 2165; e-mail: jumorris@comp.uark.edu

Abstract *Resveratrol has been identified as a wine component related to moderate wine consumption and a reduction in serum cholesterol levels. Processes such as wine fining that result in loss of resveratrol during winemaking are therefore of interest, and led to these present studies. A number of agents were compared and were found to lower resveratrol levels in all wines to some extent. Results from two studies (1996 and 1997) are reported. The standard addition method was used in combination with High Pressure Liquid Chromatography to calculate resveratrol levels.*

In Study 1 (1996), recommended maximum levels of all fining agents (Level 3), bentonite, egg white, gelatin + kieselsol and polyvinylpyrrolidone (PVPP), lowered resveratrol levels significantly compared to controls (Level 0). Nevertheless, addition of fining agents at Level 0 resulted in resveratrol levels that were significantly higher than those at Level 3, but resveratrol levels in wine from Level 0 were not significantly different from Level 1. In Study 2 (1997), carbon + egg white, and gelatin + kieselsol fining was studied, and their effects differed according to grape variety. Least removal of resveratrol by carbon fining occurred in wine from Cabernet Sauvignon (*Vitis vinifera*) whereas most removal occurred in wines from Cynthiana (*Vitis aestivalis*) and Noble (*Vitis rotundifolia*). Resveratrol levels of control wine were significantly higher than resveratrol levels of wine treated with recommended maximum addition of fining agent in all varieties.

Taken overall, any addition of any fining agent lowered resveratrol levels in all wines to some extent, but complex interactions between fining agent and wine variety resulted in different regression trends. While recognizing the constraints set by our particular data that could reflect unique circumstances, we are nevertheless able to infer from these trends that low levels of fining agents can be used without statistically significant loss of resveratrol.

Introduction Wines contain phenolic compounds that are responsible for their overall colour and flavour. Resveratrol is one of the phenolic compounds related to health benefits associated with wine consumption. Moderate wine consumption has been shown to lower coronary artery disease and to have positive effects on lipid levels in humans (Bisson et al. 1995, Blond et al. 1995, Demrow et al. 1995, Fitzpatrick et al. 1993, 1997, Frankel et al. 1993, Fuhrman et al. 1995, Rimm et al. 1991, Seigneur et al. 1990). More recently, the chemopreventive activity of resveratrol on certain cancers was established (Ebeler et al. 1997, Jang et al. 1997).

Cabernet Sauvignon (*Vitis vinifera*), Cynthiana (*Vitis aestivalis*), and Noble (*Vitis rotundifolia*) grapes are an important part of the Arkansas wine industry. These varieties are grown in Arkansas and other regions with long growing seasons and produce good quality fruit and wine. An important step in winemaking is addition of fining agents to adsorb undesirable products of fermentation such as odour and colour, and to aid wine stability (Moorehead 1996, Zoecklein et al. 1995). Polymerised tannins, anthocyanins, pigments, other phenolics, and heat-unstable proteins are removed by fining agents (Robinson 1994). The classification of fining agents is by basic constituents but more common fining agents include bentonite, carbon, egg white, gelatin, and polyvinylpyrrolidone (PVPP). Each fining agent has specific binding properties that may reduce resveratrol levels of wine. A brief outline follows, but see Zoecklein et al. (1995) for full details on attributes of fining agents.

Bentonite is a mainly negatively-charged clay with complex hydrated aluminum silicate components. Bentonite adsorbs positively-charged molecules, such as proteins and anthocyanins, and aided by gravity, particles then sink to the bottom of the container. Bentonite is not reactive towards small phenolic compounds but binds large phenolic compounds such as anthocyanins, and may also bind phenolic compounds complexed with proteins.

Carbon has particles with high internal porosity and thus a high surface area to mass relationship. Active carbon is nonspecific but tends to bind with weakly polar molecules, especially compounds containing a benzene ring. Two types of carbon are commonly used in winemaking, viz. decolourising carbon and deodourising carbon which differ according to pore size. Addition of carbon during winemaking can cause undesirable carbon-induced oxidation.

Egg white is a further example of a fining agent with a surface positive charge that removes excess negatively-charged tannins. The albumen of egg white is composed of peptide linkages that form hydrogen bonds with the hydroxy groups of the tannins. Upon neutralization, the particles agglomerate and settle to the bottom due to increased mass.

Gelatins are also positively charged and are used for removal of excess tannins from wine. Gelatin binds with larger molecules with more phenolic groups that allow more hydrogen bonding sites. Subsequent counterfining with a silica solution such as kieselsol is recommended to prevent haze formation. Kieselsol also forms a network of fibres that aids

removal of tannins and negatively-charged particles. This interaction depends on particle size, particle shape, surface characteristics and particle size distribution within the suspension. Particle charge density is an additional factor which is in turn determined by the number of surface hydroxyl groups available for binding (Zoecklein et al. 1995).

PVPP is a synthetic polymer that complexes with phenolic and polyphenolic wine components by hydrogen bond formations. PVPP has an affinity for low molecular weight phenolics due to the rigidity of the cross-linked PVPP structure. Because PVPP is insoluble, phenolic molecules adsorbed on its surface are precipitated.

PVPP is known to reduce levels of resveratrol in both red and white wine significantly (Sieman and Creasy 1992). Making broader comparisons, Soleas et al. (1995) and Goldberg et al. (1997) applied other fining agents in winemaking. For example, bentonite, egg white, and diatomaceous earth had no major effect on recovery of cis- or trans-resveratrol from wine samples. Gelatin and silica gel removed resveratrol slightly but not significantly. High levels of activated carbon were found to absorb both isomers of resveratrol. Vrhovsek et al. (1997) determined that PVPP greatly reduced resveratrol levels in Pinot Noir wine, but gelatin was without effect.

Given such equivocal experience with such broad comparisons, further research is clearly needed with respect to particular fining agents as well as overall effects of fining agent levels on resveratrol levels in specific wines. We therefore undertook two studies during successive years (1996 and 1997) as follows:

Study 1 (1996): Effects of bentonite, egg white, gelatin + kieselsol, and PVPP at different levels on resveratrol levels in wine from Cabernet Sauvignon grapes.

Study 2 (1997): Effects of carbon, egg white, and gelatin + kieselsol at different levels on resveratrol levels in wine from Cabernet Sauvignon, Cynthiana, and Noble grapes.

Materials and methods

Chromatography equipment and procedure

Analyses were conducted on wines obtained commercially and those produced at the University of Arkansas Food Science Department. Resveratrol levels were determined with High Pressure Liquid Chromatography (HPLC) instrumentation (Hitachi L-4200 UV VIS Detector, L-6200 Intelligent Pump, and D-2000 Chromato-Integrator). A Phenomenex Bondclone 10 μ Phenyl 300 x 3.9 mm column was used with a 20 μ L injection loop. A gradient of water adjusted to pH 2.5 by 0.6 M perchloric acid and methanol was used for analysis (Ector et al. 1996). The time procedure was modified slightly from the original to accommodate for pressure changes within the column.

A commercial standard of *trans*- resveratrol was purchased from Sigma Chemical Co. (St Louis, Missouri) and used as a standard for identification. Resveratrol was measured with a detector at a wavelength of 310 nm.

Standard Addition Method

The Standard Addition Method (Nielsen 1994) was used for sample analysis. A series of 10 mL flasks was used and a constant volume of the unknown (V_U) was added to the flask to determine the analyte concentration (C_U). A known volume (V_S) of the standard solution of concentration C_S was added to each flask, so that each flask received a unique volume of standard. One mL of the known standard (0.5, 1 and 2 mg/L) dissolved in methanol was added to each flask as part of this standard addition method. The flasks had the same total volume (V_T). Each sample in the flask was analysed. In accordance with Beer's Law, measured peak height of each flask was proportional to total analyte concentration as follows:

$$A = k(V_S.C_S + V_U.C_U) / V_T$$

where: V_S = volume of standard, C_S = concentration of standard, V_U = volume of unknown, C_U = concentration of unknown, V_T = total volume, k = proportionality constant

Results were plotted with the volume of the standard added to each flask (V_S) as the independent variable and the resulting peak height in centimetres as the dependent variable. Equation 1 can be represented by a straight line where

$$\text{slope} = k.C_S/V_T \quad (2)$$

and

$$\text{intercept} = k.V_U.C_U/V_T \quad (3)$$

Rearranging Equations 2 and 3,

$$C_U = (\text{measured intercept}/\text{measured slope}) (C_S/V_U) \quad (4)$$

This standard addition method proved to be an acceptable procedure for analysis of resveratrol in wine. This procedure, used in combination with our selected HPLC procedure, was reproducible, and with no significant differences between calculated levels of resveratrol between replicate wine samples within any variety.

Grapes and wine

Noble and Cabernet Sauvignon grapes were obtained from Post Familie Vineyards, Altus, Arkansas; Cynthiana was obtained from the research vineyards of the Agricultural Experiment Station, University of Arkansas, Fayetteville. All grapes were hand-harvested and delivered to the University of Arkansas Food Science Department for processing that same day.

Each of these three species of grapes was destemmed, crushed, and placed in food-grade polyethylene fermentation containers. Samples were taken for titratable acidity, soluble solids (°Brix) and pH. Soluble solids were ameliorated to 21°Brix. The must was inoculated with 71B yeast (Lallemand, Inc., Montreal, Canada) and fermented at 21°C. Grapes were then fermented on skins to dryness (0°Brix). The must was pressed in a #25 Enrossi bladder type press (Enoagricol Rossi srl, Calzolaro, Italy). Resulting wine was collected in glass carboys. Fermenting wine was racked three times to remove spent yeast cells and to prevent production of off-flavours. Fermentation was completed to less than 0.5% residual sugar. After completion of fermentation, sulfur dioxide was added as potassium metabisulfite to each treatment at a rate of 175 mg/L.

Fining agents used for these studies were bentonite, carbon, egg white, gelatin + kieselsol, and PVPP at different levels. Bentonite was used as Vitiben™ (Holchem, Inc). The type of carbon used was activated deodourising carbon. Gelatin was used as Finacol 8000® (Cellulo Co) and counterfined with Kieselsol (Nalco® 1072; Nalco Chemical Co). PVPP was used as Polyclar® VT (GAF Chemicals Corporation). Bentonite, gelatin + kieselsol, and PVPP were prepared according to the manufacturer's recommendations. Egg white was prepared by using the procedure from Zoecklein et al. (1995).

Twenty-four hours after addition of fining agents, wines were pre-filtered and bottled into 114 mL glass bottles with screw caps and stored at 21°C until analysed. The standard addition method of analysis, as outlined above, was used to determine resveratrol levels in wine samples.

Study 1

Fining agents bentonite, egg white, gelatin + kieselsol, and PVPP were used at different levels in Cabernet Sauvignon wine. Recommended maximum amount of all fining agents was used for Level 3; Level 2 was half of Level 3 (half of the recommended maximum). Level 1 was half of Level 2, resulting in one-fourth of the recommended maximum amount. Level 0 served as a control and received no fining agents. These additions are summarized below:

<u>Fining agent</u>	<u>Level 0</u>	<u>Level 1</u>	<u>Level 2</u>	<u>Level 3</u>
PVPP (g/L)	0	0.18	0.36	0.72
Bentonite (g/L)	0	0.12	0.24	0.48
Egg white (mL/L)	0	0.57	1.14	2.29
Gelatin + kieselsol (mL/L)	0	2.0	4.0	8.0

Treatments representing each fining agent (bentonite, egg white, gelatin + kieselsol, and PVPP) were applied in a completely randomised design with 2 replications of each level (Level 0, Level 1, Level 2, and Level 3). Statistical analysis was based on the SAS PROC GLM procedure. Treatment means were regarded as significantly different when separated by the equivalent of a least significant difference (LSD). Polynomial models (linear, quadratic) were fitted in PROC REG for each fining agent to assess resveratrol response to fining level.

Study 2

Fining agents carbon, egg white, and gelatin + kieselsol, were added to the Cabernet Sauvignon, Cynthiana and Noble wines. The recommended maximum amount was used for Level 2 of all fining agents (Zoecklein et al. 1995). Level 1 was half of Level 2 resulting in half of the recommended maximum amount. Level 0 was the control which received no fining agents. These additions are summarised below:

<u>Fining agent</u>	<u>Level 0</u>	<u>Level 1</u>	<u>Level 2</u>
Carbon (g/L)	0	0.24	0.48
Egg white (mL/L)	0	1.28	2.56
Gelatin + kieselsol (mL/L)	0	4.0	8.0

Treatment design was once again a Completely Randomised Design, but with 3 replications (cf. 2 in Study 1). The treatment design contained 3 factors; viz. variety (Cabernet Sauvignon, Cynthiana and Noble), fining agent (carbon, egg white, and gelatin + kieselsol), and fining agent level (Level 0, 1 and 2). Statistical analysis was based on the SAS PROC GLM procedure. As in Study 1, treatment means separated by an LSD were regarded as significant. As undertaken in Study 1, polynomial models (linear, quadratic) were fitted in PROC REG for each variety x agent to define resveratrol response to each fining level.

Results and discussion

Fining agents such as bentonite, carbon, egg white, gelatin + kieselsol, and PVPP, are routinely added to wine in order to enhance appeal by altering colour and removing off-flavours. One possible trade-off is an accompanying loss of desirable constituents, including resveratrol. Analyses of experimental wines in these present studies have helped define the extent of such losses.

Study 1

Resveratrol levels in Cabernet Sauvignon wine (Table 1) were reduced by all fining agents added at concentrations that accorded with manufacturer's specifications. Wine fined with PVPP had the least amount of resveratrol compared to wine fined with bentonite, egg white or gelatin + kieselsol. Sieman and Creasy (1992), as well as Vrhovsek et al. (1997), also noted a significant removal of resveratrol from red wine by PVPP. Supporting data were also obtained by Goldberg et al. (1997) and Soleas et al. (1995) who found no major effect of Egg white or bentonite on resveratrol and only a slight removal by gelatin and silica.

Statistical analysis of addition level on resveratrol concentration for each type of fining agent, established some quantitative trends (Table 1). Addition of bentonite or gelatin + kieselsol resulted in linear trends. An increase of 1 g/L of bentonite yielded a decrease of 0.135 mg/L of resveratrol in the wine. An increase of 1 mL/L of gelatin + kieselsol yielded a decrease in resveratrol of 0.008 mg/L. PVPP addition resulted in a cubic trend, and egg white addition resulted in no trend.

In all cases, resveratrol concentrations corresponding to fining agents at Level 0 were significantly different to those at Level 3, but Level 0 was not significantly different from Level 1. In wine fined with egg white, gelatin + kieselsol, and PVPP; resveratrol levels were significantly different for Level 0 versus Level 2. Resveratrol levels were significantly different in egg white and PVPP fined wine samples for Level 1 versus Level 2 and Level 3. Previous research on fining agents has produced equivocal outcomes, but our data now show that addition of certain fining agents increases removal of resveratrol from Cabernet Sauvignon wine. Nevertheless, those same data imply that a low level of certain fining agents can be added without incurring significant loss of resveratrol.

Table 1. Effect of four different fining agents viz. bentonite, egg white, gelatin + kieselsol, and PVPP on the resveratrol level of wine made from Cabernet Sauvignon grapes.

Fining agent level	Bentonite	Egg white	Gelatin+ kieselsol	PVPP
0	0.2905 A*	0.3142 A	0.3165 A	0.2502 A
1	0.2660 AB	0.3570 A	0.2789 AB	0.2691 A
2	0.2680 AB	0.2609 B	0.2635 B	0.1975 B
3	0.2220 B	0.2465 B	0.2505 B	0.1911 B
Trend	Linear trend $y = 0.29 - 0.135$ (level) $R^2 \text{ Adj} = 0.7$	No trend ^b	Linear trend $y = 0.3 - 0.008$ (level) $R^2 \text{ Adj} = 0.54$	Cubic trend

* Means with the same letter within columns are not significantly different ($p \leq 0.05$)

^b No trend indicates that there was not a statistically significant linear, quadratic, or cubic model that could be fitted to the means.

Cabernet Sauvignon wine, carbon resulted in a linear trend, while fining with egg white and gelatin + kieselsol resulted in quadratic trends. Carbon fining level did not have a significant effect on resveratrol concentration in Cabernet Sauvignon wine, but where either egg white or gelatin + kieselsol were added, Level 0 was significantly higher than Level 2. By implication, addition of egg white at 1 mL/L would have yielded a resveratrol level of 0.2 mg/L in wine from Cabernet Sauvignon, while addition of gelatin + kieselsol at 1 mL/L would have yielded a resveratrol level of 0.23 mg/L.

In Cynthiana wine, carbon fining resulted in a linear trend while egg white and gelatin + kieselsol fining resulted in a trend that was linear with evidence of significance in the quadratic and cubic trends. Thus, neither egg white nor gelatin + kieselsol fining had a significant effect on resveratrol level of Cynthiana wine, but carbon fining did have a significant effect. Resveratrol concentration corresponding to Level 0 was significantly higher than Level 2 resveratrol in the carbon-fined sample. By implication, every 1 g/L increase in carbon would result in a decrease of 0.09 mg/L of resveratrol in Cynthiana wine.

In Noble wine, carbon fining resulted in a quadratic trend, egg white fining resulted in a trend that was linear with evidence of significance in the quadratic and cubic trends, while gelatin + kieselsol fining resulted in a linear trend. When Noble wine was fined with egg white, resveratrol levels trended downwards, but differences were not significant (Table 2). However, when Noble wine was fined with either carbon or gelatin + kieselsol, significant effects did emerge. The addition of carbon at 0.1 g/L would have yielded a resveratrol level of 0.36 mg/L, while every 1 mL/L increase in gelatin + kieselsol would have decreased resveratrol by 0.012 mg/L.

Study 2

All fining agents reduced resveratrol levels in Cabernet Sauvignon, Cynthiana, and Noble wine (Table 2), although the extent of that reduction differed strongly between variety (representing each of three species). In Cynthiana and Noble wine, resveratrol level was lowest in the carbon-fined sample. By contrast, Cabernet Sauvignon wine fined with carbon retained a higher mean concentration compared with wine fined by other agents. Since carbon binds non-specifically, the presence of other phenolic compounds may have influenced removal of resveratrol from Cabernet Sauvignon wine. In accordance with previous observations (Goldberg et al. 1997, Soleas et al. 1995) high levels of carbon removed significant amounts of resveratrol from Cynthiana and Noble wines (Table 2).

As undertaken for Study 1, the effect of fining agent level within fining agent was analysed and regression trends were established (Table 2). Increased addition of any fining agent decreased resveratrol level in all varieties of wine, but not always significantly. In

Table 2. Effect of three fining agents viz. carbon, egg white, and gelatin + kieselsol, on resveratrol concentration in wine from Cabernet Sauvignon, Cynthiana, and Noble grapes

Variety	Fining Level	Carbon	Egg White	Gelatin+ Kieselsol
Cabernet	0	0.2692 A ^a	0.2409 A	0.2492 A
	1	0.2552 A	0.2008 B	0.1924 B
	2	0.2438 A	0.2004 B	0.1829 B
Trend analysis	no trend ^b	quadratic y=0.24-0.05 (level) +0.012(level) ² R ² Adj=0.6	quadratic y=0.25-0.02 (level) +0.001 (level) ² R ² Adj=0.82	
Cynthiana	0	0.2000 A	0.2126 A	0.2210 A
	1	0.1873 AB	0.2090 A	0.1978 A
	2	0.1557 B	0.2026 A	0.1979 A
Trend analysis	linear y=0.2-0.09(level)	no trend	no trend	
Noble	0	0.4433 A	0.4446 A	0.4580 A
	1	0.2973 B	0.4296 A	0.4181 A
	2	0.2602 B	0.4095 A	0.3645 B
Trend analysis	quadratic y=0.44-0.84 (level) +0.95(level) ² R ² Adj=0.79	no trend	linear y=0.46-0.012 (level) R ² Adj=0.75	

^a Means with the same letter within variety and columns are not significantly different ($p \leq 0.05$)

^b No trend indicates that the linear trend was significant but there was not a statistically significant quadratic or cubic model that could be fitted to the means.

In conclusion, all fining agents, when added in accordance with maximum recommended levels, provided instances of reduced resveratrol in wines from all three varieties viz. Cabernet Sauvignon, Cynthiana, and Noble. Nonetheless, due to a complex relationship between wine variety and fining agents (in terms of both type and addition level) our results imply that low levels of fining agents can be used without significant removal of resveratrol. Further comparisons between these same three varieties from each of these same three species, but sourced from a wider range of vineyards than presently employed, would be required to confirm variety distinctiveness with respect to resveratrol losses during fining operations.

References

- Bisson, L.F., Butzke, C.E. and Ebeler, S.F. (1995) The role of moderate ethanol consumption in health and human nutrition. *American Journal of Enology and Viticulture*. 46, 449-462.
- Blond, J., Denis, M. and Bezar, J. (1995) Antioxidant action of resveratrol in lipid peroxidation. *Sciences des Aliments*. 15, 347-358.
- Demrow, H.S., Slane, P.R. and Folts, J.D. (1995) Administration of wine and grape juice inhibits in vivo platelet activity and thrombosis in stenosed canine coronary arteries. *Circulation*. 91, 1182-1188.
- Ebeler, S.E., Clifford, A.J., Ebeler, J.D., Bills, N.D. and Hinrichs, S.H. (1997) An In Vivo experimental protocol for identifying and evaluating dietary factors that delay tumor onset: Effect of red wine solids. In: *Wine Nutritional and Therapeutic Benefits*. ACS Symposium Series 661. (American Chemical Society, Washington, D.C.) pp. 215-229.
- Ector, B.J., Magee, J.B., Hegwood, C.P. and Coign, M.J. (1996) Resveratrol concentration in muscadine berries, juice pomace, purees, seeds, and wines. *American Journal of Enology and Viticulture*. 47, 57-62.
- Fitzpatrick, D.F., Coffey, R.J. and Jantzen, P.T. (1997) Endothelium-dependent vasorelaxing activity of wine, grapes, and other plant products. In: *Wine Nutritional and Therapeutic Benefits*. ACS Symposium Series 661. (American Chemical Society, Washington, D.C.) pp. 237-246.
- Fitzpatrick, D.F., Hirschfield, S.L. and Colley, R.G. (1993) Endothelium-dependent vasorelaxing activity of wine and other grape products. *American Journal of Physiology* 265, H774-H778.
- Frankel, E.N., Manner, J., German, J.B., Parks, E. and Kinsella, J.E. (1993) Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet* 341, 454-457.
- Frhrman, B., Lavy A. and Aviram, M. (1995) Consumption of red wine with meals reduces the susceptibility of human plasma and low-density lipoprotein peroxidation. *American Journal of Clinical Nutrition* 61, 549-554.

- Goldberg, D.M., Soleas, G.J., Hahn, S.E., Diamandis, E.P. and Karumanchiri, A. (1997) Identification and assay of trihydroxystilbenes in wine and their biological properties. In: Wine Nutritional and Therapeutic Benefits. ACS Symposium Series 661. (American Chemical Society Washington, D.C.) pp. 24-43.
- Jang, M., Cai, L., Udeani, G.O., Slowing, K.V, Thomas, C.F., Beecher, C.WW., Fong, H.H.S., Farnsworth, N.R., Kinghorn, A.D., Mehta, R.G., Moon, R.C. and Pezzuto, J.M. (1997) Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science* 275, 218-220.
- Moorehead, D. (1996) Wine fining for the finer wines. *American Wine Society Journal* 28, 7-9.
- Nielsen, S.S. (ed). (1994) 'Introduction to the Chemical Analysis of Foods' (Jones and Bartlett Publishers, Inc. Boston).
- Rimm, E.B., Giovannucci, E.L., Willett, W.C., Colditz, G.A., Ascherio, A., Rosner, B. and Stampfer, M.J. (1991) Prospective study of alcohol consumption and risk of coronary disease in men. *Lancet* 338, 464-468.
- Robinson, J. (ed.) (1994) 'The Oxford Companion to Wine'. (Oxford University Press: New York).
- Seigneur, M., Bonnet, J., Dorian, B., Benchimol, D., Drouillet, F., Gouverneur, G., Larrue, J., Crockett, R., Boisseau, M., RibereauGayon, P. and Bricaud, H. (1990) Effect of the consumption of alcohol, white wine and red wine on platelet function and serum lipids. *Journal of Applied Cardiology* 5, 215-222.
- Siemann, E.H. and Creasy, L.L. (1992) Concentration of the phytoalexin resveratrol in wine. *American Journal of Enology and Viticulture* 43, 49-52.
- Soleas, G.J., Goldberg, D.M., Karumanchiri, A., Diamandis, E.P. and Ng, E. (1995) Influence of viticulture and oenological factors on changes in *cis*- and *trans*-resveratrol in commercial wines. *Journal of Wine Research* 6, 107-121.
- Vrhovsek U., Wendelin, S. and Eder, R. (1997) Effects of various vinification techniques on the concentration of *cis*- and *trans*-resveratrol and resveratrol glucoside isomers in wine. *American Journal of Enology and Viticulture* 48, 214-219.
- Zoecklein, B.W, Fugelsang, K.C., Gump, B.H. and Nury F.S. (1995) 'Wine Analysis and Production' (Chapman and Hall: New York).