trans- AND *cis*-RESVERATROL IN RED WINES PRODUCED FROM AUTOCHTHONE GRAPE VARIETIES

Ariola Morina[1], Renata Kongoli[1]
[1]Faculty of Biotechnology and Food, Agriculture University of Tirana, Kodër Kamëz, Tirane, Albania
ariola_lika@hotmail.com, renatakongoli@aol.com

Abstract

Over the last few years is shown a great interest in autochthonous ('native') wine grape varieties amongst the research and wine communities. This has been due, in part, to their generally greater resistance against fungal and virus attacks and, particularly in synthesis of resveratrol. Chemically, resveratrol is a substance of a polyphenolic character from the group of phytoalexins-3, 5, 4'-trihydroxystilbene and exist in *cis* and *trans* isomer forms. Grapes of *Vitis vinifera* and especially red wine represent its main source in human diet. The average concentration in red wines of world provenience fluctuates between 1.0 and 3.0 mg/l. *trans*-, *cis*- and total resveratrol content, quantified as aglycone of resveratrol, has been determined in 25 mono varietal red wines from autochthonous grape varieties (*Shesh i zi, Serin i zi, Debinë e zeze, Kallmet* and *Vlosh*) and international grape varieties (*Merlot* and *Cabernet Sauvignon*). Resveratrol was determined by HPLC method with UV-Vis detection after direct injection of wine samples. As expected, red wines from autochthonous grape varieties appeared to contain relatively high levels of resveratrol (from 0.3 to 6.8 mg/l) and *trans*/*cis* ratio ranged from 0.04 to 4.6. The highest concentration is presented by *Serin i zi* followed by *Shesh i zi* red wines. Variation of *trans*-, *cis*- and total resveratrol concentrations between the same grape variety red wines could be due to the different year of production. The highest concentration of total resveratrol was typical for red wine growing in southeast region of Albania, where vineyards are exposed to higher environmental stress as low temperature and UV radiation.

Keywords: *red wine, autochthonous, trans-resveratrol, HPLC.*

Introduction

In developed countries one in every four person, in average, is likely to suffer from cancer, while scientist continuous their research to find cure for this mortal illness. From the chemical point of view some molecules have been identified because of their roles as protective agents. Special attention has been given to phytoalexins. Phytoalexins are groups of low molecular mass compounds existing in grape vines and a large number of plants in response to stressful situations, like microbial infections and UV irradiation (Dixon, 2001).

Resveratrol (*trans* – 3,4',5 – trihydroxystilbene) which is a phytoalexin belonging to stilbenes, has attracted great interests in recent years, stimulated by research concerning the "French paradox", a phenomenon known as a low incidence of cardiovascular diseases coexisting with

the intake of a high fat diet in France(Reanaud S., 1992; Ferre-Filmon C., 2000). Resveratrol can inhibit cellular events associated with tumor initiation, promotion, and progression (Fontecave M., 1998; Jang, 1997; Mgbonyebi, 1998), reduce cell death from oxidative stress (Chantavitayapongs, 1997), inhibit the oxidation of human low-density lipoprotein (LDL) (Frankel E. N., 1993; Frankel E.N., 1995; Me´rillon J. M., 1996; Vinson J. A., 1995), inhibit platelet aggregation and eicosanoid synthesis (Chung M.I., 1992; Kimura Y., 1995; Pace-Asciak C.R., 1995), and prevent anti-inflammatory activity (Ferrero M.E., 1997) and it is an agonist for the estrogen receptor (Gehm B.D., 1997).

Resveratrol is the parent compound of a family of molecules, including glycosides and polymers, existing in *ci*s and *trans* configurations (Fig.1) in a narrow range of spermatophytes of which vines, peanuts and pines are the prime representatives. Its synthesis from *p*-coumaryl CoA and malonyl CoA is induced by stress, such as injury, infection or UV-irradiation, and it is classified as a phytoalexin, anti-fungicide conferring disease resistance in the plant kingdom (Stervbo U., 2007; L., 1976; Landcake P., 1976.). Resveratrol has been identified from a number of dietary sources including red wines and berry fruits. It is also consumed in the forms of botanical dietary supplements and herbal formulations used in traditional Chinese medicine and Indian Ayurvedic medicine where it is commonly used as an active ingredient(Paul B., 1999).

In addition to the trans-isomer, first detected, other forms of this trihydroxystilbene have been found only in wine. The next to be described was cis-resveratrol(Goldberg D.M., 1995a; Jeandet, 1993; Soleas G.J., 1995), which in some wines is in higher concentration that in the trans-isomer (Jeandet P., 1995; Soleas G.J., 1995). Since cis-resveratrol has not been detected in grape skins or juices, it appears to be formed from the isomerization of trans-resveratrol or the breakdown of resveratrol polymers during skin fermentation (Roggero, 1996; Soleas G.J., 1995). Resveratrol contents in red wines are higher than those present in rose wines: likewise rose wines present a higher resveratrol level than white wines (Mattivi, 1993b). This difference in concentrations is linked to the winemaking process, especially to the contact of wine with the solid parts of the grape, since resveratrol is found in the skin but not in the flesh (Creasy, 1988).

Many studies have been carried out on the contents of *trans*-resveratrol in red wines. Among them, the following may be indicated: Jeandet (Jeandet, 1993) obtained an interval of resveratrol content ranging from 0.4 to 2.0 mg/L in French wines; in Italian wines, Mattivi (Mattivi, 1993b) measured concentrations up to 7.17 mg/L in wines from Trentino and Dell'Oro, Cravero, and Moraglio (1997) found a mean resveratrol concentration of 2.09 mg/L in wines from Piedmont. McMurtrey et al. (McMurtrey, 1994) studied California wines, obtaining a mean content of 0.99 mg/L; Lamuela-Raventos et al. (Lamuela-Raventos, 1995) obtained results ranging from 0.60 to 8.00 mg/L in Spanish wines, and Goldberg et al. (Goldberg, 1995b.) found mean concentrations of 3.16 mg/L for Canadian wines, 1.47 mg/L for Californian and Australian wines, 1.21 mg/L in South America wines and 1.76 mg/L for Italian wines. Japanese wines appeared to have a very low content of *trans*-resveratrol 0.08 – 0.244 mg/L, (Okuda, 1996.). Greek wines were also studied (Kallithraka, 2001), the levels being between 0.550 and 2.534 mg/L, similar to those of Spanish, Italian, Portuguese, French and Californian wines.

For the *cis*-isomer, fewer reports have emerged. Jeandet et al. (Jeandet, 1993) reported range of 0.19-1.30 mg/L in Burgundy wines; Lamuela-Raventos et al., (Lamuela-Raventos, 1995) found a range of 0.11-2.48 mg/L for Spanish red wines and Gonzalo et al. (Gonzalo, 1997) obtained an average of 1.33 mg/L in Cataluna red wines.

Not only can environmental factors affect resveratrol contents, but also the concentration of resveratrol in wine varies considerably and appears to depend on the grape variety. Lamuela-

Raventos et al., (Lamuela-Raventos, 1995) found, in Spanish red wines made from Pinot noir grapes, a high level of trans-resveratrol, averaging 5.13 mg/L compared to 2.43 mg/L for Garnacha, 1.42 mg/L for Cabernet sauvignon and 1.33 mg/L for Tempranillo. The cis-resveratrol levels were higher for Pinot noir (1.12 mg/L), followed by Garnacha (0.43 mg/L), Cabernet sauvignon (0.29 mg/L) and Tempranillo (0.28 mg/L).
At the present time there are no reports in the literature about trans and cis-resveratrol content in red wines from Albania. Our work corresponds to study the potential of our autochthones grape variety, the aim of which was to determine the concentration of *cis*- and *trans*-isomer resveratrol in red wines produced from these grape varieties, compared with Cabernet sauvignon and Merlot red wines grown in Albanian territory. We carried out a discriminated study on young and aged mono varietal red wines in order to find whether there were significant differences among the wines.

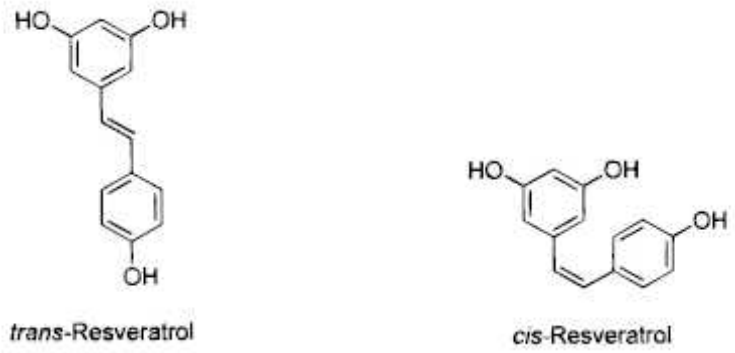

Figure 1. Chemical structure of resveratrol isomers.

MATERIAL & METHODS

Red wine samples

For this paper were taken in study red wines produced from Albanian grape varieties *Serin i zi* (cultivated in Korca and Përmet regions), *Debin e zezë* (Përmet), *Shesh i zi* (from Vlora and Tirana), *Kallmet* (from Shkodra), *Vlosh* (from Vlora), and international grape varieties *Merlot* and *Cabernet Sauvignon* (from Berat). The grapes were harvest at their mature state, brought at the canteen of the Faculty of Biotechnology and Food where was produced red wine using the traditional method of vinification. The fermentation process was controlled daily and fermentation temperature fluctuated from 25-27 ^{0}C. At the end of fermentation, red wines were not filtrated. Before bottling SO_2 level were regulate to 30mg/L, and then were stored in cellar of the Faculty of Biotechnology and Food. Young, one and two years aged red wines were analyzed from the above varieties.

Standard solutions

The *trans*-resveratrol standard (99% HPLC) was purchased from Merc. Methanol, acetonitril and glacial acetic acid (100% p. a) were HPLC grade.
A stock solution of 20 mg/L of *trans*-resveratrol was prepared in a 12% alcoholic (aqueous) solution. From the stock solution were prepared a set of standards with 0.2, 0.3, 0.5, 1.0, 2.0 dhe 3.0 mg/L, diluted in a 12% alcoholic (aqueous) solutions. The *cis*-resveratrol was prepared by UV irradiation at 254 nm of alcoholic solutions of *trans*-resveratrol according to Romero-Perez (Romero-Perez, 1996).

HPLC analysis

The concentrations of free *trans*- and *cis*-resveratrol monomers in selected wines were determined using a direct injection isocratic UV-HPLC method (Ratola, 2004). The HPLC system used was composed with a Varian UV-VIS 330 detector. Each standard and sample was injected in triplicates. For the analysis was used a column from Varian C18 (250 x 4.6mm, with 5µm particles). Mobile phase consisted of a water / acetonitrile / acetic acid mixture (volume ratio 70 / 29.9 / 0.1) with a flow rate of 1.0 mL/min. Injection volume was 20 µL. Detection was performed at a 306 nm wavelength. Run time was 20 min. Wine samples were filtered in 0.45 µm membrane and directly injected through a manual fixed loop. *Trans*-resveratrol and *cis*-resveratrol peaks were identified by their retention times using external standart method (Ratola, 2004).

Variety	***Trans*-resveratrol Means ±SD**	***Cis*-resveratrol Means ±SD**	**Total resveratrol Means ±SD**
Kallmet	0.296 ± 0.39	1.084± 0.44	1.17 ± 0.09
Debin e zeze	0.281 ± 0.16	0.162 ± 0.02	0.443 ± 0.15
Serin i zi	1.862 ± 0.98	1.547 ± 1.62	3.409 ± 2.15
Shesh i zi	1.056 ± 0.34	1.096 ± 0.89	2.152 ± 1.07
Vlosh	0.038 ± 0.21	0.699 ± 0.04	0.729 ± 1.06
Merlot	0.919 ± 0.42	1.333 ± 0.72	1.808 ± 1.29
Cabe. Sauvi.	0.522 ± 0.32	0.595 ± 0.74	1.116 ± 1.31

Table 1. Concentrations of resveratrol in 25 red wines (mg/L) based on their varieties.

RESULTS AND DISCUSSION

We have grouped the wines, depending on their grape variety and the results of *trans*-, *cis*- and total resveratrol contents are presented in Table 1. Means and standard deviations of the concentrations are expressed in mg/L. For most red wines, these results are similar to those found by different authors like Goldberg et al. (Goldberg, 1995b.) and also Jeandet et al. (Jeandet, 1993). The concentration of resveratrol in wine varies considerably and appears to depend on the grape variety. In this work the average concentration of resveratrol wines from Albanian grape varieties range from 0.038 to 1.862 mg/L *trans*-resveratrol, from 0.162 to 1.547 mg/L *cis*-resveratrol and 0.443 to 3.409 mg/L for total resveratrol. The highest level of *trans*-resveratrol was found on *Serin i zi* followed by *Shesh i zi* red wine, both of these are autochthonous grape variety. The lowest level was presented by *Vlosh* red wine which had a quantity of 0.038 mg/L. International grape varieties *Merlot* and *Cabernet sauvignon* had 0.919 and 0.522 mg/L *trans*-resveratrol respectively, which positions them in the between.

If we take in consideration the quantity of *cis*-isomer we find the highest level again on *Serin i zi* 1.547 mg/L followed by *Merlot* with 1.333 mg/L and then is *Shesh i zi* with 1.096 mg/L *cis*-resveratrol.

On the other hand quantity of *trans*-, *cis*-, total resveratrol concentrations and ratios between *trans* - and *cis*-isomers for the 25 red wines analyzed for this study are presented in table 2. The different concentrations of *trans* -, *cis*- and total resveratrol between young and aged red wines could be attributed to the different climatic conditions of each vintage.
The higher content of resveratrol in wines could be due to a more extreme environmental stress of the vineyards, because *Serin i zi* is located at a height of about 800 m, while the height of the other varieties is lower and their climatic conditions are warmer.
From the chart1we find that the excess concentration of *cis*-isomer is found in aged red wine *Serin i zi* from Permeti and Korca area, followed by *Shesh i zi* from Vlora area. We see a variation of *trans*-, *cis*- and total resveratrol concentrations between the same grape variety red wine and this could be due to the different year of production.

Variety	***trans*-resveratrol**	***cis*-resveratrol**	**Total resveratrol**	**Ratio t/c**
Ka '09	0.046	1.234	1.28	0.037
Ka '11	0.093	1.012	1.105	0.092
Ka '12	0.75	0.375	1.125	2.00
DZ'09	0.45	0.145	0.595	3.11
DZ'11	0.139	0.162	0.301	0.86
DZ'12	0.254	0.178	0.432	1.43
SP'09	2.62	4.16	6.78	0.63
SP'11	0.646	0.684	1.33	0.94
SP'12	3.47	0.75	4.22	4.63
SK'09	0.877	0.384	1.261	2.28
SK'11	1.699	2.68	4.379	0.63
SK'12	1.86	0.625	2.485	2.98
ShT'09	1.64	1.36	3	1.21
ShT'11	0.684	0.637	1.321	1.07
ShT'12	1.612	0.775	2.387	2.08
ShV'09	0.945	0.391	1.336	2.42
ShV'11	0.783	0.522	1.305	1.51
ShV'12	0.67	2.89	3.56	0.23
V'09	0.03	0.699	0.729	0.05
M'09	0.689	n.d.	0.689	0.00
M'11	1.408	1.845	3.253	0.76
M'12	0.661	0.82	1.481	0.81
Cs'09	0.875	1.44	2.315	0.61
Cs'11	0.238	0.075	0.313	3.17
Cs'12	0.452	0.269	0.721	1.68

Table 2. Concentration of *trans*-, *cis*-, total resveratrol and the ratio between *trans*- and *cis*-isomers.
(Ka – Kallmet from Shkodra; DZ – Debin e zeze from Përmeti; SP – Serin i zi from Përmeti; SK – Serin i zi from Korca; ShT – Shesh i zi from Tirana; ShV – Shesh i zi from Vlora; V- Vlosh from Vlora; M – Merlot from Berat; Cs – Cabernet sauvignon from Berat. Number '09; '11; '12 – year of production of red wines)

Based on these results the direct injection procedure was effective despite the potential increase of interference in the initial stages of each chromatogram. Whenever doubts in the identification of the peak for isomers rose in consequence of interfering peak in its vicinity, the technique of standard addition to the sample (at different concentration) was employed to confirm isomers peaks identity.

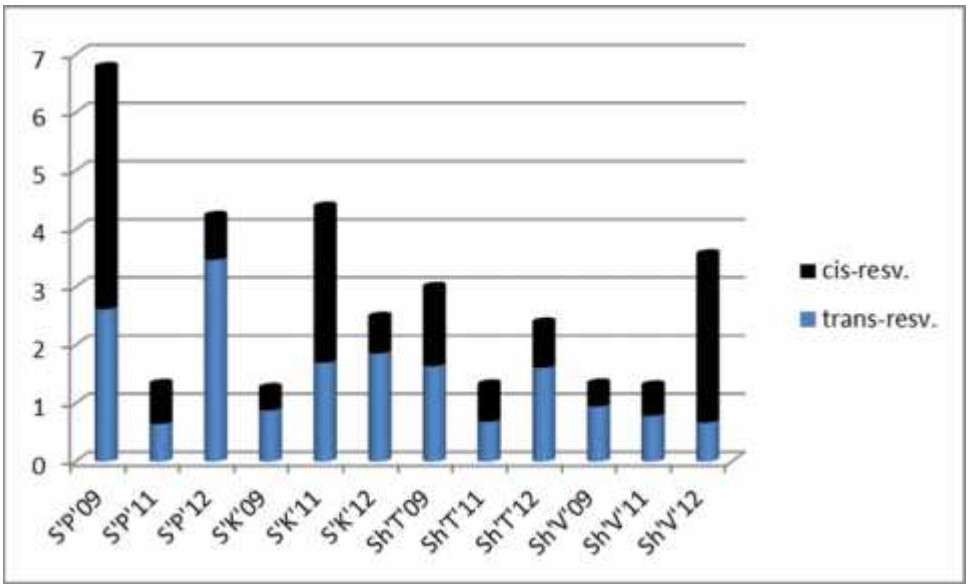

Chart 1. Concentration of *trans*- and *cis*-resveratrol for *Serin i zi* (from Korca and Përmeti area) and *Shesh i zi* (from Tirana and Vlora area) red wines.

CONCLUSION

This work enabled the implementation of an easy and fast methodology for analysis of *trans*- and *cis*-resveratrol in red wines. The use of direct injection in HPLC with UV detection allows the screening of a large number of samples in a short time.

For Albanian red wines the highest concentration of resveratrol was found in autochthonous red wine as expected. According to the obtained results, Albanian autochthonous red wines *Serin i zi* and *Shesh i zi* could be a good dietary source of resveratrol. Because resveratrol contents in *Serin i zi* red wines appeared to be highly variable, it is possible that resveratrol contents of these wines are principally influenced by the climatic conditions of each vintage. In these areas certain producers have managed to preserve free resveratrol monomer in the amount sufficient to make them interesting nutritional products. The values of total resveratrol contents found here can be taken as positive characteristic of the red wines from autochthonous grape varieties. The producers should take in consideration that resveratrol content is a function of many variables, grape variety, local and environmental factors in the vineyard, and the processing techniques.

REFERENCES

Chantavitayapongs, S. D.-L. (1997). Amelioration of oxidative stress by antioxidants and resveratrol in PC12 cells. . *Neuroreprot, 8* , 1499-1502.

Chung M.I., T. C. (1992). An antiplatelet principle of Veratrum formosanum. *Planta Med* , 274-276.

Creasy, L. C. (1988). Phytoalexin production potential of grape berries. *Journal of American Society of Horticulture Science, 113* , 230-234.

Dixon, R. (2001). Natural products and plant disease resistance. *Nature* , 843-847.

Ferre-Filmon C., D. L. (2000). Catalytic methods for synthesis of stilbenes with anemphasis on their phytoalexins. *Coord. Chem Rev.* , 323-2336.

Ferrero M.E., C. M. (1997). Activity of resveratrol on endothelial adhesion molecules. *Int. J. Tissue React.19* , 121-124.

Fontecave M., L. M. (1998). Resvertrol, a remarkable inhibitor of ribonucleotide reductase. *FEBS Lett.* , 277-279.

Frankel E. N., W. A. (1993). Inhibition of human LDL oxidation by resveratrol. *Lancet 341* , 1103-1104.

Frankel E.N., W. A. (1995). Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *J. Agric. Food Chem. 43* , 890-894.

Gehm B.D., M. J. (1997). Resveratrol, a polyphenolic compound found in grapes and wine, is an agonist for the estrogen receptor . *Proc. Natl.Acad. Sci. U.S.A. 94* , 14138-14143.

Goldberg D.M., Y. J. (1995a). A gas chromatographic mass spectrometric method to assay cis-resveratrol in wines: preliminary survey of its concentration in commercial wines. *Journal of Agricultural and Food Chemistry, 43* , 1245-1250.

Goldberg, D. Y. (1995b.). A global survey of trans-resveratrol concentrations in commercial wines. . *American Journal of Enology and Viticulture, 46* , 159-165.

Gonzalo, A. V. (1997). Contenido de resveratrol en vinos de Cataluna. *Sevi, 2.638* , 684-687.

Jang, M. C. (1997). Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science 275* , 218-220.

Jeandet P., B. R. (1995). Resveratrol content of wines of different ages: relationship with fungal disease pressure in the vineyard. *American Journal of Enology and Viticulture, 46* , 1-4.

Jeandet, P. B. (1993). Analysis of resveratrol in Burgundy wines. *Journal of Wine Research, 4* , 79-85.

Kallithraka, S. A.-Z. (2001). The application of an improved method for trans-resveratrol to determine the origin of Greek red wines. *Food chemistry. 75* , 355-363.

Kimura Y., O. H. (1995). Effects of stilbenes isolated from medicinal plants on arachidonate metabolism and degranulation in human polymorphonuclear leukocytes. *J. Ethnopharmacol. 45* , 131-139.

L., I. (1976). 3,5,4'-Trihydroxystilbene as a phytoalexyn from ground nuts (A.hypoggaea). *Phytochem 15* , 1791–1793.

Lamuela-Raventos, R. R.-P.-B. (1995). Direct HPLC analysis of cis and trans resveratrol and piceid isomers in Spanish red Vitis vinifera wines. *Journal of Agricultural and Food Chemistry, 43* , 281-283.

Landcake P., P. R. (1976.). Production of resveratrol by Vitis vinifera and other members of the Vitaceae as a response to infection or injury. *Ohisiology and Plant Path 9* , 77-86.

Mattivi, F. (1993b). Resveratrol content in red and rose wines produced in Trentino ((Italy) and currently available on the market. *Rivista di Viticultura e di Enologia, 1* , 37-45.

McMurtrey, K. M. (1994). Analysis of wines for resveratrol using direct injection high-pressure liquid chromatography with electrochemical detection. *Journal of Agricultural and Food Chemistry, 42* , 2077-2080.

Me´rillon J. M., F. B. (1996). Antioxidant activity of wine phenolic compounds in inhibiting oxidation of human low-density lipoproteins. *18th International Conference on Polyphenols.* Bordeaux (France): Polyphenols Communications 96.

Mgbonyebi, O. R. (1998). Antiproliferative effect of synthetic resveratrol on human breast epithelial cells. . *Int. J. Oncol.* , 865-869.

Okuda, T. Y. (1996.). Trans-resveratrol concentrations in berry skins and wines from grapes grown in Japan. *American Journal of Enology and Viticulture, 47(1)* , 93-99.

Pace-Asciak C.R., H. S. (1995). The red wine phenolics trans-resveratrol and quercetin block human platelet aggregation and eicosanoid synthesis: Implications for protection against coronary heart disease. *Clin. Chim. Acta* , 207-219.

Paul B., M. I. (1999). Occurrence of resveratrol and pteriostilbene in age-old Darkchasava, an Ayurvwedic medicine from India. *J Ethnopharmacol 68* , 71–76.

Ratola, N. F. (2004). t-Resveratrol in wines from Alentejo Region. *Food Technol. Biotechnol. 42 (2)* , 125-130.

Reanaud S., D. L. (1992). Wine, alcohol, platelets and the French paradox for coronary heart disease. *Lancet* , 1523-1526.

Reaunaud S. (n.d.).

Roggero, J. (1996). Changes in resveratrol and piceid contents in wines during fermentation or aging. Comparison of grenache and mourvedre varieties. *Sci. Aliment, 16* , 631-642.

Romero-Perez, A. L.-R.-B. (1996). Levels of cis- and trans-resveratrol and their glucosides in white and rose vitis vinifera wines from Spain. *J Agric Food Chem 44* , 2124–2128.

Soleas G.J., G. D. (1995). Influence of viticulture and oenological factors on changes in cis and trans-resveratrol in commercial wines. *Journale of Wine Research, 6(2)* , 107-121.

Stervbo U., V. O. (2007). A review of the content of the putative phytoalexin resveratrol in red wine. *Food Chem 101* , 440–457.

Vinson J. A., J. J. (1995). Plant polyphenols exhibit lipoprotein-bound antioxidant activity using an in vitro oxidation model for heart disease. *J. Agric. Food Chem* , 2798-2799.