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The Analysis of Different Factors Affecting the Red Wines Antioxidant Content

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Abstract

The benefits of moderate consumption of wines consist in the protective effects against cardiovascular diseases and anticarcinogenic is associated with their antioxidants content. There are plenty of analytical methods for assessing the antioxidant content of wines but unfortunately, there is not a standardized method. The antioxidant content of 'Cabernet Sauvignon', 'Merlot' and 'Pinot Noir' wines obtained from cultivated grapes varieties from Recas and Minis Romanian vineyard from different harvesting years were investigated. The electron paramagnetic resonance spectroscopy, using the 2,2,6,6-tetramethyl-4-hydroxypiperidine-N-oxyl (Tempol) free stable radical, was used to determine the antioxidant content of wines. Measurements were done in order to analyze the effects of the grape varieties, the harvesting year and the vineyard on the antioxidant content of wines. The obtained results revealed that the antioxidant content of studied red wines depends on the harvesting year of grape, the grape variety and on the vineyard. Using the observed values of the antioxidant content, a relationship was identified between the antioxidant content of wines and the harvesting year of grapes. Furthermore, the designed generalized nonlinear model revealed that the antioxidant content of wine depends on the combined effect of the grape variety and the vineyard, besides the year, the grape variety and the vineyard.

Keywords: antioxidant content, electron paramagnetic resonance (EPR) spectroscopy, harvesting year, red wines, relationship, variety, vineyard

Introduction

The increased number of patients diagnosed with cancer, degenerative, cardiovascular and other diseases years has been correlated with inadequate nutrition (Ferguson, 2010; Gibson *et al.*, 2010), stress (Guiraud *et al.*, 2010; Cano and Iovanna, 2010) and exposure to various environmental factors (Mathers *et al.*, 2010; Blonski *et al.*, 2010). The mortality rate due to cardiovascular diseases proved to be smaller in populations with diets that include a moderate consumption of wine, when populations with similar risk factors (the consumption of unsaturated fats, the high cholesterol level, obesity, smoke, etc.) were compared (Teissedre, 2000). Moreover, the content of wine phenolics proved to have anticarcinogenic and protective effects against cardiovascular diseases. (Lamuela-Raventós and de la Torre-Boronat, 1999). The protective effects were explained by the high level of antioxidant compounds of wines "French Paradox" (Renaud *et al.*, 1992), "Mediterranean diet" (Trichopoulou *et al.*, 2003)).

Antioxidants belong to different chemical classes of compounds: flavonols, catechins, anthocyanins, etc. (Dreosti, 2000, Vaya *et al.*, 2001). The antioxidants have the main effect on the neutralization of the free radicals (Halliwell, 1995). The antioxidants analysis is necessary to determine their structures, their quantity, and/ or to investigate their ability to react with free radical (the antioxidant activity), in order to estimate their *in vivo* and *in vitro* effects. Two classes of methods are used for antioxidant analysis: chromatographic methods such as high performance liquid chromatography (Fang *et al., 2007*), and thin layer chromatography (Cserháti *et al.*, 1998, Cimpoiu, 2006) and spectral methods such as spectrophotometry (UV-Vis) (Lachman *et al.*, 2007), and electron paramagnetic resonance (EPR) spectroscopy (Staško *et al.*, 2002)). Both classes of methods could be joined in different circumstances, depending on the objectives of the analysis. The second class of methods are generally used to measure the antioxidant content of pure compounds or of different samples of food (including wines) and body fluids based on their reaction with different free radicals. The frequently applied spectral method is UV-Vis spectrometry.

The antioxidant content of wine samples proved to be influenced by the variety of grape (Tsanova-Savova *et al.*, 2002), the climatic conditions (Pena-Neira *et al.*, 2000), the material used for sample preparation (e.g. seeds, skin), the conditions under samples are obtained (Fernandez-Pachon *et al.*, 2004; Halpern, 2008, Hosu *et al.*, 2010) and so on.

The aim of the research was to investigate the influence of three factors (grape variety, year of harvest and vineyard) on the antioxidant content of wines.

Materials and methods

Materials

Wines from three different varieties of *Vitis vinifera* sp.: 'Cabernet Sauvignon'-commercial name 'Cabernet Sauvignon' (abbreviated as CS), 'Pinot Noir'-commercial name 'Burgund' (abbreviated as PN) and 'Merlot'-commercial name 'Merlot' (abbreviated as TM), obtained from different harvest years from Recas (I) and Minis (II) vineyard (Romania) (Tab. 1) were studied in terms of their antioxidant capacity. Samples of wine, purchased from the market, were analyzed directly, without any preparation step.

The solution of 2,2,6,6-tetramethyl-4-hydroxypiperidine-N-oxyl (Tempol) (Fluka, Germany) free radicals (0.01%) was prepared in methanol (Chimopar, Bucharest, Romania). The number of free radical molecules decreases in time, with different rates, depending on the concentration of antioxidants, when the samples of wine containing antioxidant compounds react with Tempol molecules.

Tab. 1. The analyzed wines

Commercial name	Vineyard	Year	Alcohol content (%V)
CS	I	2002	13.5
CS	I	2003	13.5
CS	I	2005	13.0
CS	II	1995	12.5
CS	II	2000	13.5
TM	I	2003	13.5
TM	I	2005	13.0
PN	I	2005	12.5

CS= 'Cabernet Sauvignon', TM= 'Merlot', PN= 'Burgund'
Vineyards: I=Recas, II=Minis

EPR measurement of antioxidant activity

Five samples of 20 µl of red wine were injected using a Hamilton microsyringe into a quartz capillary (10 cm length and interior diameter 1mm) and 5 µl of methanolic solution of Tempol were rapidly added. Samples were monitored using an EPR Spectrometer (ADANI portable EPR Spectrometer PS8400", Resonance Instruments Instruments Inc., Germany), operating in X-band (9.1 GHz÷9.6 GHz) equipped with a computer acquisition system. The spectrometer parameters were: modulation frequency 100 KHz, sweep width 100 G, sweep time 30 s, receiver gain 2×10^1. The EPR spectra were recorded from 2 to 2 minutes, for 20 minutes. The antioxidant capacity of extracts was characterized by the decrease in time of the relative concentration of the paramagnetic species obtained by double integration of EPR signals (Petrişor *et al.*, 2008).

Determination and analysis of antioxidant content

The values obtained by double integration of the initial EPR signal of the free radicals (S_0), and the values determined after 20 minutes following adding the extracts of wines (S_{20}) were used in order to determine the antioxidant content (Hosu *et al.*, 2011). The following formula was used:

$$\text{Antioxidant content (\%)} = [(S_0-S_{20})/S_0]\cdot100$$

The normal distribution of the experimental data was tested by EasyFit (v.5.2) and Statistica (v.8.0) software. The following statistical tests were used in assessment of experimental data normality: Kolmogorov-Smirnov (K-S) (Kolmogorov, 1941; Smirnov, 1948), Wilk-Shapiro (W-S) (Shapiro, 1968), Anderson-Darling (A-D) (Anderson and Darling, 1952) and Jarque-Bera (J-B) (Jarque and Bera, 1980; Jarque and Bera, 1981). The analysis of variances was conducted using Statistica (v.8.0) software. The summary of antioxidant content was expressed as mean, associated 95% confidence interval, standard deviation, and coefficient of variation. The 95% confidence intervals associated with proportions were calculated as described by Fieller (1940). The Chi-Square test (Fisher, 1923) was applied to test the independence between pairs of factors (variety and harvesting year) that are able to influence the antioxidant content of the studied wines.

Results and discussion

The antioxidant content (expressed as %) calculated for each sample is presented in Tab. 2.

The results of normality analysis of experimental data are presented in Tab. 3.

Tab. 2. The antioxidant content of studied wines

AC%	Samples				
	1	2	3	4	5
CSI 2002	49.87	50.04	50.21	49.98	49.91
CSI 2003	27.91	28.02	27.99	28.00	27.97
CSI 2005	18.85	18.79	18.83	18.98	18.87
CSII 1995	69.98	69.98	69.96	70.03	70.12
CSII 2000	69.49	69.53	69.51	69.6	69.56
TMI 2003	56.45	56.72	56.64	56.38	56.62
TMI 2005	35.68	35.93	35.79	35.92	35.7
PNI 2005	28.13	28.42	28.40	29.09	28.42

AC% = Antioxidant content (%)
CS = 'Cabernet Sauvignon', TM = 'Merlot', PN = 'Burgund'

Tab. 3. The analysis of normality of antioxidant content

Wine	K-S	p_{K-S}	W-S	p_{W-S}	A-D	p_{A-D}	J-B	p_{J-B}
CSI 2002	0.188	0.980	0.932	0.609	0.253	0.733	1.088	0.581
CSI 2003	0.225	0.914	0.912	0.479	0.312	0.683	2.095	0.351
CSI 2005	0.266	0.790	0.909	0.464	0.330	0.669	2.461	0.292
CSII 1995	0.301	0.662	0.839	0.161	0.466	0.570	2.524	0.283
CSII 2000	0.173	0.992	0.970	0.875	0.179	0.800	0.353	0.838
TMI 2003	0.259	0.815	0.923	0.548	0.287	0.704	0.971	0.615
TMI 2005	0.237	0.882	0.863	0.238	0.362	0.644	1.807	0.405
PNI 2005	0.380	0.366	0.814	0.106	0.592	0.491	4.160	0.125

CS= 'Cabernet Sauvignon', TM= 'Merlot', PN= 'Burgund'
pX=probability from the test X, where X: K-S=Kolmogorov-Smirnov, W-S=Wilk-Shapiro, A-D=Anderson-Darling, J-B=Jarque-Bera

The results presented in Tab. 3 showed that the normal distribution of all observed data cannot be rejected at 5% significance level. Additional, for only three of cases a greater departure from normality could be observed ranging from 10% to 20% (p_{W-S} and p_{J-B} for PNI 2005; p_{W-S} for CSII 1995). All other cases proved to be in a greater agreement with the theoretical normal distribution. Therefore, the statistical analysis under proved normal distribution was further conducted, since the results showed that the calculated probability of all statistical tests and all observables considered, was higher than 0.05. The results of analysis of the variance conducted in order to estimate the values of the antioxidant content of wines are presented in Tab. 4.

Tab. 4. Antioxidant content: descriptive statistics

Wine	Mean	StD	CV%	CI95%
CSI 2002	50.00	0.13	0.27	50.00±0.17
CSI 2003	27.98	0.04	0.15	27.98±0.05
CSI 2005	18.86	0.07	0.38	18.86±0.09
CSII 1995	70.01	0.06	0.09	70.01±0.08
CSII 2000	69.54	0.04	0.06	69.54±0.05
TMI 2003	56.56	0.14	0.25	56.56±0.18
TMI 2005	35.80	0.12	0.33	35.80±0.15
PNI 2005	28.49	0.36	1.25	28.49±0.44

Wine: grape variety, vineyard and harvesting year; Mean = arithmetic mean; StD = Standard deviation; CV% = coefficient of variation; CI95% = half of the confidence interval width of mean

Tab. 5. Antioxidant content ratios between wines (relative to the highest value)

Wine	Value	Proportion
CSII 1995	70.01±0.08	1.000±0.001
CSII 2000	69.54±0.05	0.993±0.001
TMI 2003	56.56±0.18	0.808±0.002
CSI 2002	50.00±0.17	0.714±0.002
TMI 2005	35.80±0.15	0.511±0.001
PNI 2005	28.49±0.44	0.407±0.005
CSI 2003	27.98±0.05	0.400±0.001
CSI 2005	18.86±0.09	0.269±0.001

Wine: grape variety, vineyard and harvesting year; Value: result from Tab. 4 Proportion (from highest) and associated 95%CI

Tab. 4 provided along with the mean value of the antioxidant content, its confidence interval calculated at a 5% significance level (Value column in Tab. 4). The coefficients of variation (CV %) proved to be less than 1% for most of the wine samples; these values showed a low variability of the observations from repeated measurements, and in the same time indicate a good agreement between them.

The analysis from Tab. 4 revealed the following statements:

÷ The antioxidant content of wines vary from 18.86±0.09 (for CSI 2005) to 70.01±0.08 (for CSII 1995) all of them being statistically different by each other (Tab. 5);

÷ The 95% confidence intervals of PNI 2005 and CSI 2003 are near to be overlapped; when ratio between them are calculated (Tab. 5), the confidence of the ratio for 95% confidence vary from 1.005 to 1.032; thus the statistically significant difference between them are assured at 5% significance level (both limits are greater than 1). It should be noted that same result can be obtaining by applying student t-test to compare two means.

Tab. 5 presented the relative antioxidant content of wines reported to the highest value (CSII 1995). As Tab. 5 revealed, all antioxidant contents significantly varied (at a significance level of 5%) among the samples analyzed. The ratio of antioxidant content varied from 1 (when are compared the CSII 1995 to itself) to 0.269 (for CSI 2005). In view of that, the range of antioxidant content of the investigated wines varied significantly (the lowest antioxidant content is less than one third of the highest content).

An assumption can be verified for the data presented in Tab. 4: if there is (or not) a relationship between the observed mean (of antioxidants content) and the year (of harvesting) and variety (of cultivated grapes). The results of the Chi-Square test for the four entries presented in Tab. 4 (CSI 2003, CSI 2005, TMI 2003, and TMI 2005) are presented in Tab. 6.

The analysis of results presented in Tab. 6 clearly indicates that there is a relationship between antioxidant content and year and variety. By using the same data presented in Tab. 4 a relationship between the year of harvesting (y_h in Eq.1) and the antioxidant content (AC(%) in Eq.1) was identified:

$$AC(\%) = 9490(\pm 6654) - 4.72(\pm 3.32)\cdot y_h \quad (1)$$

$n = 8$; $r = 0.82$; $r^2_{adj} = 0.61$; $SE = 12.26$; $F_{(1)} = 12$; $p_F = 1.3\%$;

$t(9490) = 3.49$; $p_t(3.49) = 1.3\%$; $t(4.72) = 3.47$; $p_t(3.47) = 1.3\%$;

The hypothesis verified in Tab. 6 that there is no reason to reject the hypothesis that the wine antioxidant content is a observable dependent on the harvesting year is further confirmed by the statistical analysis resulted and presented in eq. (1). Eq. 1 provides an estimation of the true relationship between the populations of all wine sorts harvested in different years (did not take into account the sort-variety or vineyard, but estimates the average of them). Fig. 1 presents the relationship given in Eq. (1).

Tab. 6. Is the antioxidant content dependent by year and variety? Results

Variety\ Year	2003	2005	
CSI	27.98 (28.45)	18.86 (18.39)	
TMI	56.56 (56.09)	35.8 (36.27)	X^2=0.029; p(0.029,1)≈0.86

χ^2 test on contingency between variety (on rows) and years (on cols) for antioxidant content of wines; cells: observed averages (expected averages)

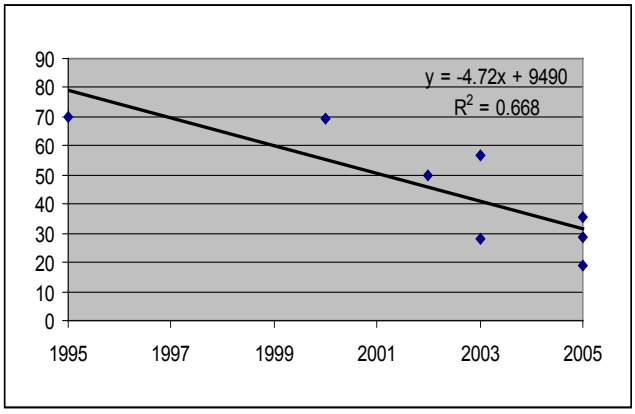

Fig. 1. Antioxidant contents of wines depending on harvesting year

Over 61% of the variance in the data could be explained by the linear relationship (1) between antioxidant content and harvesting year. A general view of wines population reveals the evolution in time of their antioxidant content; the population constituted from the three wines from 2005 year and the three characteristic points corresponding to the antioxidant content of different variety of wines could represent an estimator of mean antioxidant content of wines population from that year. Consequently, using the identified equation, the maturation time needed to reach a given antioxidant content could be probably estimated.

A generalized nonlinear model (GLZ) was designed in order to deeply explain the variance of antioxidant content using year (continuous variable), variety and vineyard (categorical variables) as predictors: $\hat{Y} = \Sigma a_i X_i + \Sigma b_{ij} X_i X_j$. Only first 7 entries from Tab. 4 were included into the analysis (the last entry in the table provides no information regarding the association, being the only one containing PN variety). Four significant components were identified in the model: year, variety, vineyard, and combined effect of variety and vineyard (Tab. 7).

The statistical analysis presented in Tab. 7 revealed that all effects are statistically significant (with p values less than

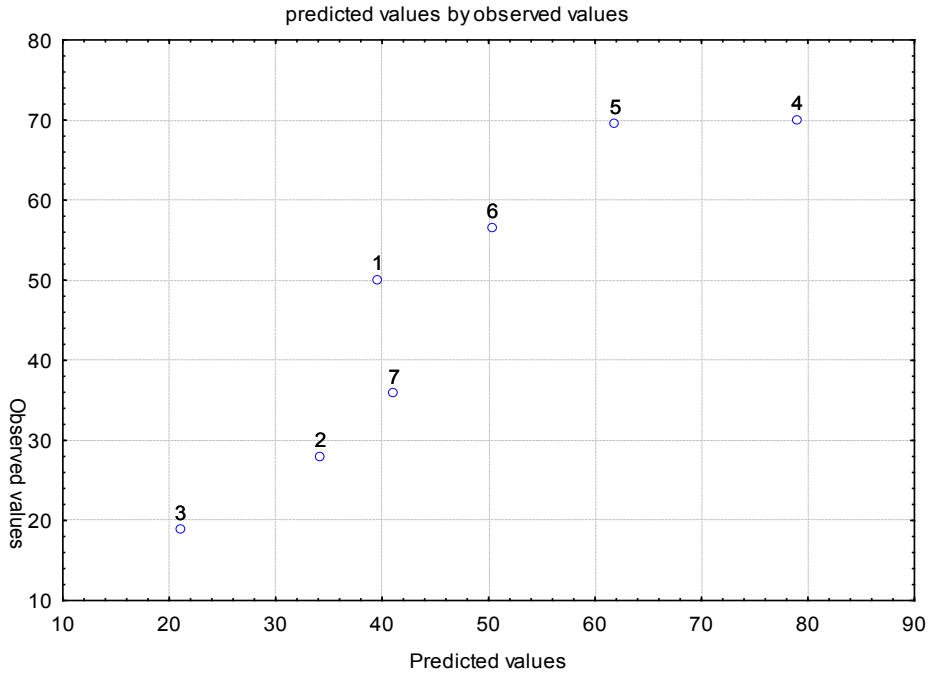

Fig. 2. GLZ estimated vs. observed antioxidant content (n = 7; r = 0.92; r^2_{adj} = 0.77)

Tab. 7. Analysis with GLZ Model (assumptions: NORMAL Distribution, POWER Link function)

Effects	Column	Estimate	95%CI	SE	Wald*	p
"Var3"	year	-114	[-196; -32]	42	7.4922	0.006197**
"Var1"	variety	228942	[65603; 392280]	83337	7.5469	0.006011**
"Var2"	vineyard	228948	[65356; 392540]	83467	7.5239	0.006088**
"Var1"*"Var2"	-	-229130	[-392482; -65779]	83344	7.5582	0.005974**
Scale		7		1.91	14.000	0.000183**

* Wald statistic; ** Statistically significant to the model

1%). Using the model, a good agreement was obtained between observed antioxidant content and estimated antioxidant content (Fig. 2).

The obtained GLZ model, through considering the categorical variables, was to improve the observed determination of the relationship relating antioxidant content and the production year (about 77% of the total variance are explained by the GLZ, and only 61% by the eq. 1) as the analysis with chi square test suggested (that antioxidants depends on both year and variety)

Conclusions

The presented study was conducted on the wines from three varieties of red grapes ('Cabernet Sauvignon', 'Pinot Noir' and 'Merlot'), produced in different years and obtained from different vineyards. The antioxidant content was assessed in terms of harvesting year of grape, the grape variety and vineyard. The obtained results revealed that the antioxidant content of studied red wines depends on the harvesting year of grape, the grape variety and on the vineyard. Using the observed values of the antioxidant content, a relationship was identified between the antioxidant content of wines and the harvesting year of grapes. Furthermore, the designed generalized nonlinear model revealed that the antioxidant content of wine depends on the combined effect of variety and vineyard, besides the year, the variety and the vineyard.

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References

Anderson TW, Darling DA (1952). Asymptotic theory of certain "goodness-of-fit" criteria based on stochastic processes. Ann Math Stat 23(2):193-212.

Blonski W, Kotlyar DS, Forde KA (2010). Non-viral causes of hepatocellular carcinoma. World J Gastroenterol 16(29):3603-3615.

Cano CE, Iovanna JL (2010). Stress proteins and pancreatic cancer metastasis. Scientific World Journal 10:1958-66.

Cimpoiu C (2006). Analysis of some natural antioxidants by thin-layer chromatography and high performance thin-layer chromatography. J Liq Chromatogr Rel Tech 29:1125-1142.

Cserháti T, Forgées E, Kosa Á, Csiktusnadi-Kiss G, Candais M (1998). Use of multistep gradient elution TLC to gradient separation in HPLC. J Planar Chromatog Mod TLC 11(1):34-37.

Dreosti IE (2000). Antioxidant polyphenols in tea, cocoa, and wine. Nutrition 16:692-694.

Fang F, Li MJ, Pan QH, Huang DW (2007). Determination of red wines flavonoids by HPLC and effect of aging. Food Chem 101:428-433.

Ferguson LR (2010). Dietary influences on mutagenesis--where is this field going? Environ Mol Mutagen 51(8-9):909-18.

Fernandez-Pachon MS, Villano D, Garcia-Parrilla MC, Troncoso AM (2004). Antioxidant activity of wines and relation with their polyphenolic composition. Anal Chim Acta 513:113-118.

Fieller EC (1940). The biological standardization of insulin. J Roy Stat Soc 7(1):1-64.

Fisher RA (1923). Studies in crop variation. II. The manurial response of different potato varieties. J Agric Sci 13:311-320.

Gibson TM, Ferrucci LM, Tangrea JA, Schatzkin A (2010). Epidemiological and clinical studies of nutrition. Semin Oncol 37(3):282-96.

Guiraud V, Amor MB, Mas JL, Touzé E (2010). Triggers of ischemic stroke: a systematic review. Stroke 41(11):2669-2677.

Halliwell B (1995). Antioxidant characterization. Biochem Pharmacol 49:1341-1348.

Halpern GM (2008). A celebration of wine: wine is medicine. Inflammopharmacology 16:240-244.

Hosu A, Cimpoiu C, Miclăuş V, Damian G, Tarsiche I, Pop N (2010). Influence of intermittent heating during maceration on the antioxidant capacity of some grape seeds and skins. Not Bot Hort Agrobot Cluj 38(1):41-43.

Hosu A, Cimpoiu C, Miclăuş V, Jantschi L (2011). Antioxidant content of three different varieties of wine grapes. Biotech Biotechnol Equip 25:2217-2221.

Jarque CM, Bera AK (1980). Efficient tests for normality, homoscedasticity and serial independence of regression residuals. Econ Lett 6(3):255-259.

Jarque CM, Bera AK (1981). Efficient tests for normality, homoscedasticity and serial independence of regression residuals: Monte Carlo evidence. Econ Lett 7(4):313-318.

Kolmogorov A (1941). Confidence Limits for an Unknown Distribution Function. Ann Math Stat 12(4):461-463.

Lachman J, Šulc M, Schillla M (2007). Comparison of the total antioxidant status of Bohemian wines during the wine-making process. Food Chem 103:802-807.

Lamuela-Raventós RM, de la Torre-Boronat MC (1999). Beneficial effects of white wines. Drugs Exp Clin Res 25(2-3):121-124.

Mathers JC, Strathdee G, Relton CL (2010). Induction of epigenetic alterations by dietary and other environmental factors. Adv Genet 71:3-39.

Pena-Neira A, Hernández T, García-Vallejo C, Estrella I, Suarez JA (2000). A survey of phenolic compounds in Spanish wines of different geographical origin. Eur Food Res Technol 210:445-448.

Petrişor D, Damian G, Simon S, Hosu A, Miclăuş V (2008).

Antioxidant activity, of some types of white wines and juices investigated by EPR spectroscopy. Mod Phys Lett B 22:2689-2698.

Renaud S, de Lorgeril M (1992). Wine, alcohol, plateles, and the French paradox for coronary heart disease. Lancet 339:1523-1526.

Shapiro SS, Wilk MB, Chen HJ (1968). A comparative study of various tests of normality. J Am Stat Assoc 63:1343-1372.

Smirnov NV (1948). Table for estimating the goodness of fit of empirical distributions. Ann Math Stat 19(2):279-281.

Staško A, Liptáková M, Malík F, Mišík V (2002). Free radical scavenging activities of white and red wines: An EPR spin trapping study. Appl Magn Reson 22:101-113.

Teissedre PL, Landrault N (2000). Wine phenolics: contribution to dietary intake and bioavailability. Food Res Int 33:461-467.

Trichopoulou A, Costacou T, Bamia C, Trichopoulou D (2003). Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med 348:2599-2608.

Tsanova-Savova S, Ribarova F (2002). Free and conjugated myricetin, quercetin, and kaempferol in Bulgarian red wines. J Food Compos Anal 15:639-645.

Vaya J, Aviram M (2001). Nutritional antioxidants: mechanism of action, analyses of activities and medicinal applications. Curr Med Chem: Immunol, Endocr Metab Agents 1:99-117.