ISSN 1330-9862 *original scientific paper*
(FTB-1453)

A Comparison of Methods Used to Define the Phenolic Content and Antioxidant Activity of Croatian Wines

Jasenka Piljac[1*], *Sanja Martinez*[2], *Lidija Valek*[2], *Tamara Stipčević*[3] *and Karin Kovačević Ganić*[4]

[1]Department of Molecular Biology, Ruđer Bošković Institute, Bijenička c. 54, PO Box 180, HR-10000, Zagreb, Croatia

[2]Department of Electrochemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, Savska c. 16/I, PO Box 177, HR-10000, Zagreb, Croatia

[3]Department of Molecular Medicine, Ruđer Bošković Institute, Bijenička c. 54, PO Box 180, HR-10000, Zagreb, Croatia

[4]Department of Food Technology, Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, HR-10000, Zagreb, Croatia

Received: November 17, 2004
Revised version: April 4, 2005
Accepted: June 28, 2005

Summary

Concentrations of phenolic antioxidants and antioxidant activities were determined for three different vintages of red varietal Plavac mali wines (Grgich), white varietal Pošip wines (Grgich) and white varietal Žlahtina wines (Gršković). All three mentioned cultivars (*Vitis vinifera* L.) are well exploited in vineyards along the Croatian coast. Two different tests, the spectrophotometric Folin-Ciocalteau test and redox derivative potentiometric titration with electrogenerated chlorine, were used to quantify phenolic antioxidants and express them in gallic acid equivalents. The sequence of wines obtained by the two methods, ranked according to the increasing phenolic content, was comparable. Among all the tested wines, Plavac mali of the vintage 2003 showed the highest phenol content of ~5 g/L. As expected, due to the lack of anthocyanins and other pigments present in red wines, all six white wines showed approximately ten times lower phenolic levels in comparison with red wines, averaging between 190–380 mg/L. This study demonstrates the utilization of quick and reliable analytical techniques, spectrophotometry and derivative potentiometric titration, in quantification of wine phenolics. The change in free radical scavenging ability of the same set of wines was evaluated according to the Brand-Williams assay. The results show, on average, eight times higher free radical scavenging ability of red wines. Also, a slight decrease in the free radical scavenging ability of the older vintage white wines was observed, while the antioxidant activities of the older vintage red wines (Plavac mali) were slightly higher, due to formation of condensed tannins with time.

Key words: wine antioxidants, polyphenols, antioxidant activity, potentiometric titration, Folin-Ciocalteau test

*Corresponding author; Phone: ++385 (1) 45 60 987; Fax: ++385 (1) 45 61 177; E-mail: jpiljac@irb.hr

Introduction

Croatia has a very long tradition of grape cultivation and winemaking that dates back to the ancient Greek and Roman times (*1*). Old records point to several hundred native varieties existing in this region in recent past (*2,3*). Today, with more than 80 native cultivars registered in the official cultivar list (and additional 50 rare genotypes) (*4*), and more than 1000 different wine types produced (*5*), Croatia significantly contributes to the diversity of grape and wine products on the Adriatic coast. Although there are indications that over 10 % of the Croatian population uses wine products to earn a living (*6*), very few published studies have been focused on the analysis of wines made from native Croatian grape varieties. One recent study has pointed to a significantly larger total phenol content of a top quality Croatian red wine, Zlatan Plavac, in comparison with Cabernet Sauvignon and Merlot produced in New Zealand (*7*). This difference in the concentration of total antioxidants was attributed to traditional processing of grapes in Croatia, with prolonged maceration times, but may also be due to the inherent natural richness in polyphenols, typical for certain Croatian varieties such as Plavac mali.

The aim of the research presented in this paper was to investigate the polyphenolic content and antioxidant activity of three consecutive vintages of varietal Croatian wines Plavac mali (Grgich), Pošip (Grgich), and Žlahtina (Gršković). For this purpose the 2001, 2002 and 2003 vintages of each wine were tested using the standard Folin-Ciocalteau and Brand-Williams tests and the more recently adapted potentiometric titration method. In order to exclude the influence of maceration and vinification parameters, which have been proven to significantly affect the total phenol content (*8*), and maximally focus on the change of the concentration of phenolic antioxidants in each consecutive vintage, the grapes were subjected to identical processing procedures and each vintage was stored in oak barrels (except in the case of Žlahtina, where only one of the two 2002 vintages is a barrique). For quantification of phenolic antioxidants, the well-established spectrophotometric (Folin-Ciocalteau test) and the recently adapted potentiometric titration methods were utilized and the results are expressed in gallic acid equivalents (GAE). The Brand-Williams (*9*) assay was employed to determine the free radical scavenging ability of each sample.

Material and Methods

Wines

Three red varietal Plavac mali wines and three white varietal Pošip wines produced by the Grgich winery in Trstenik on the Pelješac peninsula, as well as three varietal Žlahtina white wines produced by the Gršković family from the island of Krk, Croatia, were used for the study. These three grape varieties belong to *Vitis vinifera* L. and are considered to be autochthonous Croatian cultivars suitable for the production of highest quality wines. Since this study was undertaken with the aim of investigating the change in total phenols and antioxidant activity of three different vintages, each set of three wines consisted of a 2001, 2002 and 2003 vintage and the grapes of each vintage came from the same vineyards and were processed in the same manner. All nine tested wines are high-quality products with low SO_2 content (<10 mg/L). Such low concentrations of SO_2 are below the detection limit of potentiometric titration (with currently chosen current density of chlorine generation) and do not represent interference in the Folin-Ciocalteau (FC) test. In the case of Žlahtina wines, because the vintage 2001 was not available, a comparison between the two 2002 vintages was made, with the first one aged in inox and the 2002 Žlahtina barrique aged in oak barrels.

Spectrophotometry

Total phenol content of wines was determined using the Folin-Ciocalteau micro method adapted by Andrew Waterhouse and readily available on the internet (http://waterhouse.ucdavis.edu/phenol/folinmicro.htm). Absorbance of each wine solution (white wines undiluted, red wines diluted 10×), as well as the absorbance of gallic acid standard in concentrations of 0, 50, 100, 150, 250, and 500 mg/L, were determined at 765 nm against the blank (the »0 mL« solution). Four absorbance readings for each sample were averaged and taken as the final result. A calibration curve absorbance *vs*. *c*(gallic acid) was used to derive the GAE concentrations for wines.

Potentiometric titration

Measurements were carried out in a 0.2 M KCl solution buffered to the pH=2. The working electrode with a platinum spiral and the indicator electrode with a platinum plate were immersed in the anodic compartment of the electrochemical cell along with a saturated calomel reference electrode (SCE), and separated by fritted glass from the cathodic compartment containing the platinum counter electrode. The electrochemical cell was connected to a PAR model 273 potentiostat/galvanostat and data acquisition was performed with the help of a PC. Solutions of the common phenolic standard, gallic acid (9.94 mg in 100 mL of redistilled water + 10 % ethanol mixture), were introduced into 60 mL of the reaction solution in an electrolysis cell. After starting a magnetic stirrer, a stabilized current of 20 mA was passed through the solution in order to generate chlorine at the working electrode. The same procedure was applied to 1-mL samples of undiluted white wines and 1-mL samples of red wines diluted 20 times, in order for the results to fall within the calibration range.

The titration curves were recorded by measuring the potential between a platinum indicator electrode and the SCE as a function of time. The end-point (inflection point) calculations were performed in Mathematica®, including the fitting of the measured S-shaped potentiometric curves by the natural cubic spline method.

Antioxidant activity (AA)

The samples were analyzed according to the technique reported by Brand-Williams *et al*. (*9*). Briefly, a volume of sample (5 μL of red wine (volume ratio *V*(methanol):*V*(wine)=1:1) and 20 μL of white wine) was added to a volume of 2,2-diphenyl-1-picrylhydrazyl (DPPH)

0.094 mM in methanol up to 1 mL. The free radical scavenging activity using the free radical DPPH reaction was evaluated by measuring the absorbance at 515 nm after 60 min of reaction at 20 °C in a spectrophotometer. The reaction was carried out in closed Eppendorf tubes shaken at 20 °C. The results were expressed as mmol/L Trolox equivalents (TE), a vitamin E analogue.

pH determination

Iskra digital pH meter MA 5722 (Iskra Elektrozveze, Ljubljana, Slovenia) was used to determine the pH values of wines.

Results and Discussion

In a previous potentiometric titration study applied to wine (*10*), both mimosa tannin and a set of investigated wines at pH=2 showed a high degree of linearity of the curve titration time *vs*. volume of added sample. Also, at pH=2 the observed titration curves were steep, producing well-defined peaks upon derivation. Therefore, pH=2 (stomach acid pH) was chosen as the optimal experimental pH for determination of the level of phenolic antioxidants present in nine Croatian wines.

Potentiometric titration curves for the gallic acid standard obtained for different test volumes (ranging from 0.5–5 mL) at pH=2 are shown in Fig. 1. The behaviour observed for gallic acid confirms the reliability of potentiometric titration and justifies its use in other studies aimed at quantification of wine polyphenols (*11*). The equivalent values for wines have been interpolated from the calibration curves for titration times of 1 mL of test solutions. The results of potentiometric titration as well as the FC micromethod, expressed as GAE, are shown in Table 1 along with the wine pH. The good correlation between these two methods (r^2=0.967) is indicated by the plot in Fig. 2.

Assuming 100 % electrochemical production of chlorine, it may be calculated that each molecule of gallic acid reacts with approximately 3 molecules of Cl_2 giving up 6 electrons, which is about three times more than required for oxidation of a catechol or pyrogallol group.

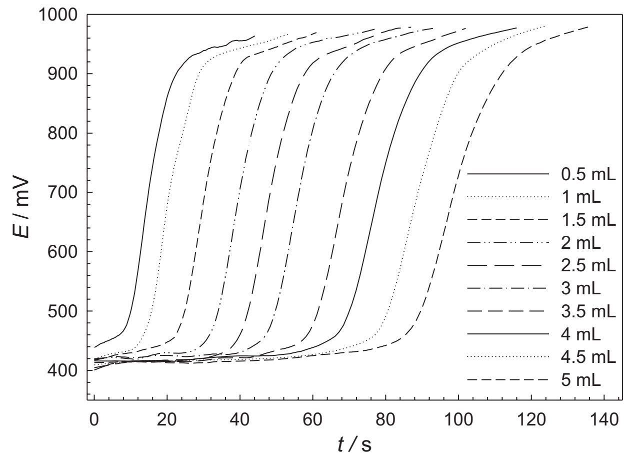

Fig. 1. Potentiometric titration curves for the gallic acid standard recorded at pH=2. The curves from left to right were obtained for test volumes ranging from 0.5 to 5 mL in 0.5-mL increments

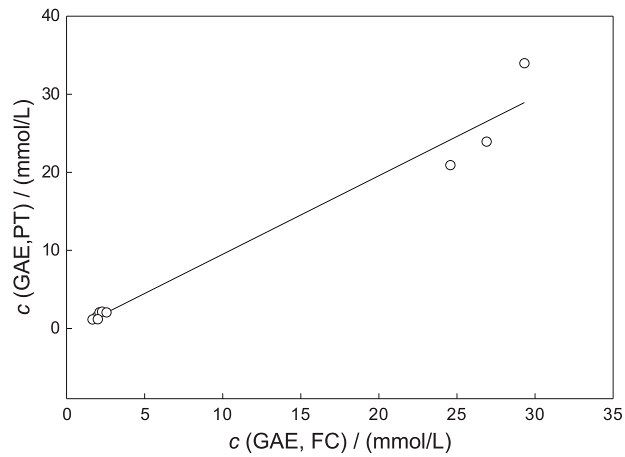

Fig. 2. Correlation between the results of PT and FC methods expressed in GAE (r^2=0.967)

The high number of electrons consumed by gallic acid may be explained either by the occurrence of side reactions at an electrode, which lower the efficiency of chlo-

Table 1. Basic wine parameters and antioxidant levels expressed in gallic acid and catechin equivalents, for a set of nine Croatian varietal wines – three red and six white ones

Wine	pH	FC γ(GAE)/(mg/L)	PT γ(GAE)/(mg/L)	AA *c*(TE)/(mmol/L)
Grgich Plavac mali 2003	3.83	4989±43	5778±14	3.960±0.051
Grgich Plavac mali 2002	3.82	4181±32	3558±11	3.760±0.072
Grgich Plavac mali 2001	3.75	4576±34	4070±10	4.128±0.094
Grgich Pošip 2003	3.22	328±13	254±6	0.534±0.011
Grgich Pošip 2002	3.21	358±11	350±4	0.482±0.006
Grgich Pošip 2001	3.24	387±10	364±8	0.459±0.008
Gršković Žlahtina 2003	3.75	436±19	347±7	0.478±0.005
Gršković Žlahtina 2002[b]	3.54	283±14	196±3	0.500±0.009
Gršković Žlahtina 2002	3.56	280±9	190±3	0.446±0.005

Abbreviations: FC (Folin-Ciocalteu test); PT (potentiometric titration); GAE (gallic acid equivalents); AA (antioxidant activity), TE (Trolox equivalents); b – barrique. All measurements were performed in triplicate rine production, or by oxidation reactions, other than removal of electron and/or hydrogen from the substrate. Recent results of Westerhoff *et al.* (*12*) show that chlorine consumption by phenolic compounds proceeds in two stages, the rapid initial consumption (1–5 min) followed by the slower consumption. The initial chlorine demand is most likely associated with highly reactive organic matter sites, *i.e.* the electron donating functional groups such as hydroxyl groups. High molar halogen consumption per mole of phenolic compound also correlates with the number of -OH functional groups in the molecule. A rapid decrease in the oxidation power of the gallic acid solution (in concentrations comparable to those of ref. *12*) and wine samples after the titration with chlorine indicates oxidation of the fast reacting moieties of phenolic molecules, most probably the *o*-diphenol groups, without the formation of the compounds of intermediate antioxidant activity.

The trend of wines obtained by the FC test, in terms of increasing content of phenolic antioxidants expressed in GAE, coincides with that obtained by potentiometric titration (Figs. 3 and 4), except in the case of 2002 and 2003 Pošip wines, which swapped places. The reason behind this observation is probably that the overall content of phenolic antioxidants present in both wines is too small for the difference between them to be pronounced. Among the tested Plavac mali, Pošip and Žlahtina wines, the 2003 vintage of Plavac mali exhibited the highest phenolic content as determined by both the FC assay and potentiometric titration. This result is indeed plausible, because the 2003 vintage of red grapes in Croatia was severely affected by a dry spell experienced in the late summer and early autumn. As a consequence, the grapes were already slightly dried out at harvest time and the phenols were more concentrated in the juice.

If the total phenol content observed for all three vintages of Grgich Plavac mali is compared to the results observed for Cabernet Sauvignon, Merlot and Pinot noir published by Kilmartin *et al.* (*13*), it becomes clear that Plavac mali stands out as a variety inherently rich in antioxidants. This finding was previously assumed by the authors in a cyclic voltammetry study of the phenolic content of several Croatian wines (*7*). Even in comparison with another high quality Croatian red wine, Babić produced in the Primošten winegrowing region (*8*), Plavac mali wine made from grapes grown on the slopes of the Pelješac peninsula, at appellations exposed to direct sunlight and sunlight reflected off the sea surface, exhibits the total phenol concentration greater by about 2 g/L. As a consequence, Plavac mali wines are famous for their bitter taste and astringency, organoleptic sensations that are in red wines mostly attributed to oligomeric and polymeric pronathocyanidins, respectively, consisting of flavan-3-ol units such as (+)-catechin and (–)-epicatechin (*14*).

As expected (*15*), the total phenol content of white wines, Pošip and Žlahtina, is approximately ten times smaller than that observed for red wines. The reason for this is the higher content of anthocyanin and condensed tannins in red wines as a consequence of better extraction of phenolic compounds from grape pomace during fermentation of the juice on the skins and seeds (*16*,*17*). The results of both the spectrophotometric FC test and potentiometric titration exhibited the same trend and pointed to the highest content of antioxidants in the young wine, the 2003 Žlahtina. Similar values observed for the total phenol content of the two 2002 Žlahtina wines (inox and barrique), indicated that the ageing process equally affected both 2002 vintages of Žlahtina. Also, the barrels used for storage of the 2002 Žlahtina barrique had already been reused on several occasions, thus, heartwood phenolics did not significantly contribute to the total phenol content. In general, wines undergo more drastic changes during the first months of ageing and the variability in the polyphenolic content as well as colour parameters tend to level out with time (*18*). In old barrels, the kinetics of phenol transport from wood to wine is damped by the resistance to mass transfer on the wood side (*19*). In comparing the phenolic content of three vintages of Pošip and Žlahtina wines, a drop from 436 to 280 mg/L was observed in Žlahtina between the 2003 and 2002 vintages. The differences found in the concentration of polyphenols between wines are expected to result from the vintage year, in addition to the phenomena that occur during ageing. In order to draw conclusions about the effect of ageing on degradation of polyphenols in these wines, an

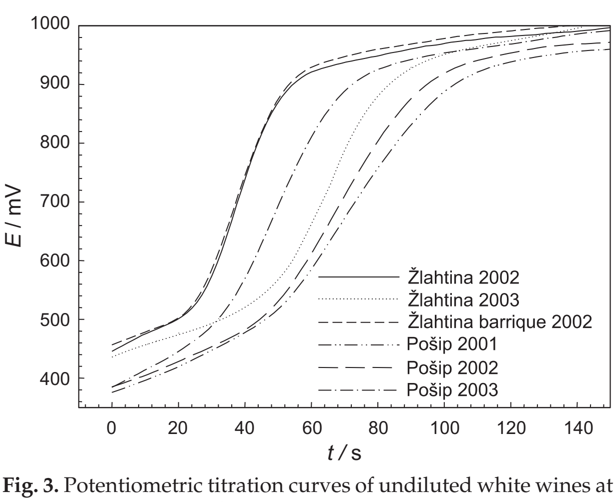

Fig. 3. Potentiometric titration curves of undiluted white wines at pH=2. Tested volume for each wine was 1 mL

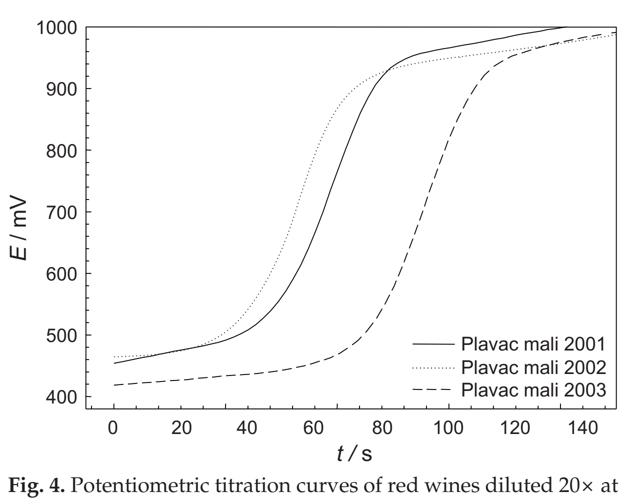

Fig. 4. Potentiometric titration curves of red wines diluted 20× at pH=2. Tested volume for each wine was 1 mL

experiment should be set up where the same wines are followed for several years.

All three Pošip wines maintained a relatively large concentration of phenolics in all three vintages, with the 2001 vintage exhibiting the highest value. The Pošip wines, in comparison with the New Zealand Chardonnay and Riesling wines studied by Kilmartin *et al.* (*13*), show comparable FC values. The slightly higher concentration of phenolics exhibited by the 2001 Pošip may be attributed to ageing in new oak barrels and slow extraction of heartwood tannins soluble in wine. However, precise HPLC determination of the polyphenolic profile of each Pošip wine aged in identical barrels should be performed to prove this claim, since the extraction of tannins from wooden barrels depends on a variety of factors (*20*), including the ethanol content of wine, the age of the wood, storage temperature, *etc.*

When the antioxidant activities for all nine wines, determined according to the Brand-Williams assay, are compared (Table 1), it becomes clear that red wines are, on average, 8× more potent free radical scavengers than white wines (c(TE)=~4 mM as opposed to c(TE)=~0.5 mM). This finding is in accordance with previous studies (*15*) and may be explained by significantly larger total phenol content of red wines in comparison with white wines.

In the case of three Plavac mali wines, the oldest 2001 vintage exhibited the highest antioxidant activity followed by the 2003 and 2002 vintage. The differences in the concentration of polyphenols observed between different vintages may also explain the differences found in the antioxidant activity of Plavac mali wines.

All six white wines showed a decreasing trend in antioxidant activities in the order 2003>2002>2001. The only exception is the 2002 Žlahtina barrique, which maintained an antioxidant activity of 0.5 mM TE, due to extraction of heartwood tannins during ageing in oak barrels. Considering the fact that favonols are much less concentrated in white wines since they are extracted from the skins (*21*), this decrease in the antioxidant activities of white wines may be expected.

In drawing any final conclusions regarding the antioxidant activities of wines, the other bioactive compounds (vitamins and minerals) present in wine and their potential synergistic effect should also be taken into consideration. Also, HPLC profiles of wines that would enable the quantification of individual phenolic antioxidants, those more and less active, could be helpful in explaining the correlation between the total phenol content and antioxidant activity and will be the subject of our next publication. A mathematical model published by Soleas *et al.* (*22*) has thus far been most successful in prediction of the total antioxidant status of wines based on the content of vanillic acid, *trans*-polydatin, catechin, *m*-coumaric acid, epicatechin, quercetin, *cis*-polydatin, and *trans*-resveratrol.

Conclusions

In general, this paper demonstrates the usefulness of spectrophotometric and potentiometric titration analyses that yield comparative results and, in combination with the Brand-Williams assay, enable us to draw conclusions regarding the antioxidative property of wines. In comparing the different vintages produced using the same technology, it may be concluded that the ageing of three monovarietal wine types, Pošip, Žlahtina and Plavac, exerts a more pronounced effect on the total phenol content as opposed to the antioxidant activity, which is in accordance with previous findings of Zafrilla *et al.* (*21*). In fact, due to polymerization reactions that lead to formation of condensed tannins during ageing of red wines, the antioxidant activity of the oldest wine, the 2001 Plavac mali, was the highest (c(TE)=4.128 mM).

In addition, the results of our study point to a high phenolic content of Plavac mali wines. This observation, along with the fact that white wines show 10× lower levels of antioxidants in comparison with red wines, points to the potential use of Plavac mali, and other red Croatian varieties, as rich sources of natural antioxidants.

Since the skin fermentation time has the largest influence on the total phenol content of wine, especially on the concentration of anthocyanins and proanthocyanidins including catechin (*23,24*), wines from Plavac mali grapes rich with antioxidants will soon be produced by prolonging the skin/juice contact time. This incentive has been launched at the Faculty of Agriculture in Zagreb in collaboration with the Medical School in Split and will shed new light on the potential of the already well-exploited Plavac mali grapes in prevention of coronary heart disease.

Acknowledgements

We thank the Ministry of Science of the Republic of Croatia for financial support. We also thank the Grgich and Gršković families for their wine donations.

References

1 N. Fazinić: *Vineyards and Wines of Croatia*, Association for Development of Winegrowing, Zagreb (1981).

2. S. Bulić: *Dalmatinska ampelografija*, Poljoprivredni nakladni zavod, Zagreb (1949).

3. M. Jelaska, K. Briza: *Ampelografija.* In: *Poljoprivredna enciklopedija*, Jugoslavenski leksikografski zavod, Zagreb (1967).

4. I. Pejić, E. Maletić, J. Karoglan-Kontić, B. Kozina, N. Mirošević, Diversity of autochthonous grapevine genotypes in Croatia, *Acta Hortic. 528* (2000) 73–89.

5. S. Herjavec, N. Mirošević, M. Fazinić, J. Karoglan-Kontić, Đ. Peršurić, V. Milat, Lj. Gašparec-Skočić, M. Ričković, J. Bolić: *Hrvatska vina i vinari – Croatian Wines and Vintners*, AGMAR press, Zagreb (2002).

6. D. Bulum: Establishing Wine Land Register in Croatia. In: *Proceedings of the 1st International Conference – Prospects for Viticulture and Enology*, Zagreb (2000) p. 22.

7. J. Piljac, S. Martinez, T. Stipčević, Ž. Petrović, M. Metikoš-Huković, A cyclic voltammetry investigation of the phenolic content of Croatian wines, *Am. J. Enol. Vitic. 55* (2004) 417–422.

8. I. Budić-Leto, T. Lovrić, U. Vrhovšek, Influence of different maceration techniques and ageing on proanthocyanidins and anthocyanins of red wine cv. Babić (*Vitis vinifera* L.), *Food Technol. Biotechnol. 41* (2003) 299–303.

9. W. Brand-Williams, M.E. Cuvelier, C. Berset, Use of a free radical method to evaluate antioxidant activity, *Lebensm. Wiss. Technol. 28* (1995) 25–30.

10. S. Martinez, L. Valek, J. Piljac, M. Metikoš-Huković, Determination of wine antioxidant capacity by derivative potentiometric titration with electrogenerated chlorine, *Eur. Food Res. Technol. 220* (2004) 658–661.

11. V. Castaignede, H. Durliat, M. Comtat, Amperometric and potentiometric determination of catechin as model of polyphenols in wines, *Anal. Lett. 36* (2003) 1707–1720.

12. P. Westerhoff, P. Chao, H. Mash, Reactivity of natural organic matter with aqueous chlorine and bromine, *Water Res. 38* (2004) 1502–1513.

13. P.A. Kilmartin, H. Zou, A.L. Waterhouse, Correlation of wine phenolic composition versus cyclic voltammetry response, *Am. J. Enol. Vitic. 53* (2002) 294–302.

14. R. Gawel, Red wine astringency: A review, *Aust. J. Grape Wine Res. 4* (1998) 74–95.

15. D. De Beer, E. Joubert, C.A. Wentzel, A. Gelderblom, M. Manley, Antioxidant activity of South African red and white cultivar wines: Free radical scavenging, *J. Agric. Food Chem. 51* (2003) 902–909.

16. J.-J. Macheix, A. Fleuriet, J. Billot: *Fruit Phenolics*, CRC Press, Boca Raton (1990).

17. J. Oszmianski, F.M. Romeyer, J.C Sapis, J.-J. Macheix, Grape seed phenolics: Extraction as affected by some conditions occurring during wine processing, *Am. J. Enol. Vitic. 37* (1986) 7–12.

18. M.D. Sanza, J.L.B. Yague, C.G. De La Vega, The influence of the type of barrels and oak variety on the ageing of a Ribera del Duero red wine, *Food Sci. Technol. Int. 6* (2000) 483–493.

19. D. Kadim, C.H. Mannheim, Kinetics of phenolic extraction during ageing of model wine solution and white wine in oak barrels, *Am. J. Enol. Vitic. 50* (1999) 33–39.

20. J.L. Puech, F. Feuillat, J.R. Mosedale, The tannins of heartwood: Structure, properties, and their influence on wine flavor, *Am. J. Enol. Vitic. 50* (1999) 469–478.

21. P. Zafrilla, J. Morillas, J. Mulero, J.M. Cayuela, A. Martinez-Cacha, F. Pardo, J.M.L. Nicolas, Changes during storage in conventional and ecological wine: Phenolic content and antioxidant activity, *J. Agric. Food Chem. 51* (2003) 4694–4700.

22. G.J. Soleas, G. Tomlinson, E.P. Diamandis, D.M. Goldberg, Relative contributions of polyphenolic constituents to the antioxidant status of wines: Development of a predictive model, *J. Agric. Food Chem. 45* (1997) 3995–4003.

23. V. Kovac, E. Alonso, M. Bourzeix, E. Revilla, Effect of several enological practices on the content of catechins and proanthocyanidins of red wines, *J. Agric. Food Chem. 40* (1992) 1953–1957.

24. R. J.M. Da Silva, V. Cheynier, A. Samsom, M. Bourzeix, Effect of pomace contact, carbonic maceration, and hyperoxidation on the procyanidin composition of Grenache-blanc wines, *Am. J. Enol. Vitic. 44* (1993) 168–172.

Usporedba metoda za određivanje udjela fenola i antioksidacijske aktivnosti hrvatskih vina

Sažetak

Određene su koncentracije fenolnih antioksidansa, te antioksidacijske aktivnosti triju različitih berba crnih vina Plavac mali (Grgić) i bijelih vina Pošip (Grgić) te Žlahtina (Gršković). Sva tri kultivara (*Vitis vinifera* L.) trenutačno se primjenjuju u vinogradima duž dalmatinske obale. Dva različita testa, spektrofotometrijski Folin-Ciocalteauov test te derivacijska potenciometrijska redoks-titracija s elektrogeneriranim klorom, upotrijebljena su za kvantitativno određivanje fenolnih antioksidansa, a vrijednosti izražene u ekvivalentima galne kiseline. Sljedovi vina poredanih prema sve većoj koncentraciji fenola, određeni ovim dvjema metodama, usporedivi su. Od svih ispitanih vina, Plavac mali berbe 2003. imao je najveću koncentraciju fenola (~5 g/L). Kao što se i očekivalo, zbog manjka antocijanina i drugih pigmenata prisutnih u crnim vinima, svih šest bijelih vina imalo je otprilike deset puta manju koncentraciju fenola (prosječno između 190–380 mg/L) od crnih vina. Ovo istraživanje pokazuje učinkovitost brzih i pouzdanih analitičkih metoda, kao što su spektrofotometrija i derivacijska potenciometrijska redoks-titracija, u kvantitativnom određivanju fenola u vinu. Različite mogućnosti uklanjanja slobodnih radikala za isti set vina određene su prema Brand-Williamsovu testu. Rezultati su pokazali prosječno osam puta veću učinkovitost crnih vina pri uklanjanju slobodnih radikala od bijelih vina. Isto tako, manja učinkovitost primijećena je u sortama bijelih vina starijih berba (2001. i 2002), dok je antioksidacijska aktivnost starijega crnog vina (Plavac mali 2001) nešto veća zbog nastajanja kondenziranih tanina.