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Scavenging Capacities of Some Wines and Wine Phenolic Extracts

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Summary

The aim of this study was to assess the ability of different wines – a sweet red, a dry red, a sweet white, and a dry white – to scavenge the stable 1,1′-diphenyl-2-picryl-hydrazyl radical (DPPH$^{\bullet}$) and to determine their phenolic composition. Both red wines contained, apart from anthocyanins, also higher concentration of total phenolics, tartaric esters, and flavonols than the two white wines. All wines exhibited scavenging activity analogous to their total phenolic content. However, their phenolics differed in antiradical potency, which was visible in their EC_{50} values. The dry red wine, Xinomavro, had a lower EC_{50} value, indicating the higher antiradical potency of its phenolics. The scavenging capacities of phenolic extracts from Xinomavro red wine on hydroxyl radicals, superoxide radicals, and singlet oxygen were also assessed. Wine total extract was fractionated by extraction, and each of the three fractions was then subfractionated by column chromatography into two subfractions. Wine total extract, and its fractions and subfractions exhibited scavenging capacity on hydroxyl radicals, superoxide radicals, and singlet oxygen, indicating the activity of many wine phenolics. The most active wine extracts towards hydroxyl radicals were characterized by the high peaks of flavanols, anthocyanins and flavonols in their HPLC-DAD chromatograms. The most active extract towards superoxide radicals was rich in flavanols and anthocyanins. The characteristic phenolics of the most active wine extracts towards singlet oxygen were flavanols, flavonols and phenolic acids. The ability of all red wine phenolic extracts to scavenge singlet oxygen, along with hydroxyl and superoxide radicals, emphasizes its health functionality.

Key words: wine, phenolics, scavenging activity, antioxidant activity, hydroxyl radicals, superoxide radicals, singlet oxygen

Introduction

Free radicals are extremely harmful to living organisms in that they attack different constituents of the cell, thus accelerating its ageing and destruction. Reactive oxygen species (ROS), including hydroxyl radicals ($OH^{\bullet}$), superoxide radicals ($O_2^{\bullet -}$) and singlet oxygen ($^1O_2$), are generated as byproducts of normal human metabolism. However, increased levels of ROS create oxidative stress and a cumulative oxidative damage in various biological macromolecules. Thus, they are implicated in the pathogenesis of various human diseases and disorders (*1–3*).

The harmful action of free radicals can be blocked and protection against ROS is provided by an array of different compounds contained in the human diet. Among them, polyphenols hold an important role since they behave as potent free radical and ROS scavengers.

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They represent a large group of antioxidant compounds widely distributed in fruits, vegetables and beverages (*4–6*).

Wine phenolics originate from grapes, particularly the skin, and they vary notably according to the grape variety (*7,8*). Red and white wines differ in their phenolic composition due to differences in phenolic composition of red and white grapes and in winemaking procedures. Red winemaking includes the procedure of maceration, while the skins are removed during the vinification of white wine. Moreover, red wines are aged in barrels while most white wines are not. Red wines contain much more polyphenols than white ones (*7*).

The effort of present work was to determine the scavenging activity and phenolic composition of different Greek wines. Moreover, the scavenging capacity of phenolic extracts of a red wine on reactive oxygen species was also evaluated.

Materials and Methods

Wines

Four Greek wines were used. They were Mavrodaphne of Patras, a sweet red wine; Xinomavro of Naoussa, a dry red wine; Muscat of Samos, a sweet white wine; and, Moschofilero of Mantinia, a dry white wine. All wines used are protected by Appellation of Origin.

Wine phenolic extracts

Total wine extract was dealcoholized wine concentrated by rotary evaporation at 25 °C and 80 mbar. Wine added to an equal volume of distilled water was concentrated to the original volume (25 °C, 80 mbar) in order to remove the alcohol without destroying the phenolic compounds (*9*). Liquid/liquid extractions of dealcoholized wine were performed to obtain three extracts containing different classes of polyphenol compounds (*10*). The dealcoholized wine (pH=2.0) was first extracted with ethyl acetate to obtain an aqueous phase containing mainly anthocyanins and an organic phase containing mainly flavanols, phenolic acids and flavonols. This aqueous phase was the first fraction (X1). The organic phase after evaporation was redissolved in water at pH=7.0, and extracted again with ethyl acetate to obtain an organic phase containing mainly flavanols and flavonols and an aqueous phase containing mainly phenolic acids and flavonols. This organic phase was the second fraction (X2). The aqueous phase was adjusted to pH=2.0 and extracted again with ethyl acetate to collect its phenolic compounds. This organic phase was the third fraction (X3). Each of the three fractions was subfractionated into non-polymeric (monomeric and dimeric) and polymeric polyphenols using a Sephadex LH-20 column (*11*). Non-polymeric polyphenols (subfraction a, X1a/X2a/X3a) were desorbed by methanol/acetic acid from the gel, and polymeric polyphenols (subfraction b, X1b/X2b/X3b) by acetone/acetic acid. In phenolic analysis samples in 10 % ethanol were used, whereas in scavenging assays samples in distilled water were used.

Analysis of phenolics

Total phenolic content of the samples was determined by the Folin-Ciocalteau method (*12*). Results were expressed as gallic acid equivalent (GAE).

The absorbance at 280, 320, 360 and 520 nm was also used to estimate total phenolic content, tartaric esters, flavonols and anthocyanins, respectively (*13*). The method consisted of placing 0.25 mL of sample in a test tube and adding 0.25 mL of 0.1 % HCl in 95 % ethanol and 4.55 mL of 2 % HCl. The solution was mixed and allowed to sit for approximately 15 min before reading the absorbance at 280, 320, 360 and 520 nm. As standard were used: gallic acid in 10 % ethanol for total phenolics, caffeic acid in 10 % ethanol for tartatic esters, quercetin in 95 % ethanol for flavonols, and malvidin-3-glucoside in 10 % ethanol for anthocyanins. The wines were analysed by high performance liquid chromatography and diode array detector (HPLC-DAD) for individual phenolic compounds. Samples were filtered using syringe filter (PTFE 0.45, Alltech) prior to the injection.

Waters 600E system with a 996-photodiode array detector and a 600E pump was used. Chromatograms were treated using the Millenium 32 program. The column used was a C18 reversed phase Spherisorb (4.0 × 250 mm) with 5-μm packing. The mobile phases were: A, water/glacial acetic acid (98:2); B, methanol/water/glacial acetic acid (60:38:2) and C, methanol/glacial acetic acid (98:2). The gradient used was 0–30 min, 100 % A at 0.20 mL/min; 30–40 min from 58.3 % A to 41.7 % B at 0.60 mL/min; 40–120 min, from 41.7 % A to 58.3 % B at 0.20 mL/min; 120–155 min, from 25 % A to 75 % B at 0.30 mL/min; 155–165 min, 100 % C at 0.60 mL/min; and 165–180 min, 100 % C at 0.90 mL/min.

Peak identification and classification were carried out as described previously (*14*). Some peaks were identified on the basis of the retention time and the UV-Vis spectra of several standards used. All peaks were classified using absorbance characteristics of the phenolic classes derived from the literature (*15,16*) and from our observations using several standards. The absorbance wave lengths of phenolic classes were as follows: benzoic acids at 250–280 nm; cinnamic acids at 305–330 nm, and several of them also at 290–300 nm; anthocyanins at 450–560 and 240–280 nm, and some of them at 315–325 nm; flavanols at 270–280 and around 230 nm; flavonols at 350–380 and 250–270 nm, and some of them at around 300 nm; flavones and isoflavones at 300–350 nm and at 245–270 nm; flavanones at 270–295 nm, and some of them at 300–320 nm. Unclassified peaks which exhibited maximum absorbance at 280–305 nm were expressed as unclassified 280 nm. Unclassified peaks that exhibited maximum absorbance at around 230 nm, and also absorbed at around 280 nm, were expressed as unclassified 230 nm. Subsequently, all peaks were classified into nine groups. As main phenolic peaks were taken those exhibiting high area at 280, 255, 320, 360 or 520 nm.

Radical scavenging

The ability of wines to scavenge the 1,1'-diphenyl-2-picryl-hydrazyl radical ($DPPH^{\bullet}$) was evaluated as described previously (*17*). In test tubes, 0.1 mL of sample

(wine or dilutions in 10 % ethanol) or 0.1 mL of 10 % ethanol (control) was added to 3.9 mL of DPPH$^{\bullet}$ solution ($6 \cdot 10^{-5}$ mol/L in methanol), and the mixture was mixed well. The absorbance at 515 nm was measured at t=0, 5, 10, 20, 30, 40, 50 and 60 min. A blank was prepared for each sample using methanol instead of the DPPH$^{\bullet}$ solution. For each wine concentration tested, the reaction kinetics was plotted. Moreover, the EC_{50} values of each wine, *i.e.* the concentration of total phenolics (in mg/L) required to lower the initial DPPH$^{\bullet}$ concentration by 50 %, were also calculated. The exact DPPH$^{\bullet}$ concentration was calculated from a calibration curve with the equation $C=9.5 \cdot A$, where C is the concentration of DPPH$^{\bullet}$ (in mg/L) and A is the absorbance at 515 nm.

Hydroxyl radicals were produced in a Fenton reaction by incubating for 60 min at 37 °C 20 μM $FeCl_3$, 1.4 mM H_2O_2, 2.8 mM deoxyribose, 100 μM EDTA, 100 μM ascorbate, 10 mM KH_2PO_4/KOH buffer, pH=7.4, and 100 μL of sample in a final volume of 1 mL. After incubation, 1 mL of 1 % thiobarbituric acid in 50 mM NaOH and 1 mL of 2.8 % trichloroacetic acid were added. The mixture was incubated in boiling water bath for 30 min, cooled and the absorbance at 532 nm was measured (*18,19*). DMSO (200 mM) was used as positive and distilled water as negative control. A blank was prepared for each sample using distilled water instead of ascorbate.

The percentage of scavenging capacity was estimated by the relation $[(A_{H_2O}-A_{sample})/(A_{H_2O}-A_{DMSO})] \cdot 100$, where A is the absorbance using H_2O, sample or DMSO.

Superoxide radicals were generated by enzymatic oxidation of hypoxanthine with xanthine oxidase. In 2.2 mL of 50 mM Tris-HCL buffer, pH=7.4, 100 μL of 1 mM nitroblue tetrazolium, 500 μL of 5 mM hypoxanthine, 100 μL of sample, and 100 μL of the solution of xanthine oxidase (grade I) were added. The nitroblue tetrazolium reduction was followed every 2.5 min for up to 30 min by measuring the absorbance at 560 nm. Influence on xanthine oxidase was tested by measuring uric acid formation under the conditions given above but with xanthine as substrate and the absorbance measured at 295 nm. Pyrogallol (100 ppm) was used as positive and distilled water as negative control (*20,21*). The remaining superoxide radicals were estimated by the relation $[(S_{sample}-S_{pyrogallol})/(S_{H_2O}-S_{pyrogallol})] \cdot 100$, where S is the initial (0–15 min) slope of the absorbance-time curve.

The singlet oxygen was produced by the reaction of hypochloride with H_2O_2. *N,N*-dimethyl-*p*-nitroanilide was used as a selective scavenger and histidine as a selective acceptor of singlet oxygen (*22*).

In 400 μL of sample, 400 μL of 10 mM histidine, 400 μL of 100 mM K_2HPO_4/KH_2PO_4 buffer, pH =7.1, 400 μL of 10 mM H_2O_2, 400 μL of 10 mM NaOCl, and 400 μL of 50 mM *N,N*-dimethyl-*p*-nitroanilide were added. The mixture was incubated at 37 °C for 40 min, and the absorbance at 440 nm was measured. A blank was prepared for each sample using distilled water instead of histidine. NaN_3 (20 mM) was used as positive and distilled water as negative control. The EC_{50} value of each sample, *i.e.* the concentration of total phenolics (γ, in mg/L of gallic acid equivalent) required to scavenge 50 % of each ROS, was estimated.

All analyses were run in triplicates, and results reported here are the means of the three runs. The one way analysis of variance (ANOVA), using the Duncan test at a level of significance $P<0.05$, was used for the statistical analysis (SPSS 11.5).

Results and Discussion

Scavenging activities and phenolic composition of different wines

Total phenolics, tartaric esters, flavonols and anthocyanins of Mavrodaphne, Xinomavro, Muscat and Moschofilero wines are presented in Table 1. Both red wines (Mavrodaphne and Xinomavro) exhibited higher total phenolics as well as tartaric esters, flavonols and anthocyanins than the two white wines (Muscat and Moschofilero). Xinomavro wine exhibited higher total phenolics and anthocyanins and lower tartaric esters and flavonols than Mavrodaphne wine. Muscat wine exhibited higher total phenolics, tartaric esters, and flavonols than Moschofilero wine. Both white wines did not contain anthocyanins as expected. It was noticed that total phenolics estimated by the Folin-Ciocalteau assay as well as by measuring the absorbance at 280 nm were analogous, but the results obtained by the former assay were much higher.

Wines were analysed by HPLC-DAD for their phenolic composition. Peaks of benzoic acids, cinnamic acids, flavonols, flavanols, flavones, unclassified compounds with maximum absorbance at 280 nm, and unclassified compounds with maximum absorbance at

Table 1. Total phenolics (Folin and A_{280nm}), tartaric esters, flavonols and anthocyanins of Mavrodaphne, Xinomavro, Muscat and Moschofilero wines

Wine	Total phenolics by Folin γ(gallic acid)	Total phenolics at 280 nm γ(gallic acid)	Tartaric esters at 320 nm γ(caffeic acid)	Flavonols at 360 nm γ(quercetin)	Anthocyanins at 520 nm γ(malvidin-3-glucoside)
	mg/L	mg/L	mg/L	mg/L	mg/L
Mavrodaphne	1710^{b*}	1225^{b}	210^{a}	139^{a}	66^{a}
Xinomavro	2825^{a}	1353^{a}	196^{b}	119^{b}	130^{b}
Muscat	450^{c}	309^{c}	99^{c}	59^{c}	4^{c}
Moschofilero	267^{d}	172^{d}	65^{d}	29^{d}	0^{c}

*Means in every column with different superscript differ significantly at $P<0.05$

230 nm were determined in all wines. In Mavrodaphne and Xinomavro red wines, peaks of anthocyanins were also measured. Xinomavro wine exhibited several main peaks of all phenolic classes. The major peaks of Mavrodaphne wine were of benzoic and cinnamic acids, while Moschofilero wine exhibited a major peak of cinnamic acid. HPLC-DAD chromatogram of Muscat wine (Fig. 1) revealed that it contained mainly benzoic and cinnamic acids, unclassified compounds and also flavonols (Fig. 1).

The kinetics of DPPH$^{\bullet}$ scavenging by Mavrodaphne, Xinomavro, Muscat and Moschofilero wines are presented in Fig. 2. The order of scavenging activities was Xinomavro > Mavrodaphne > Muscat > Moschofilero, indicating that their scavenging activity depends on their total phenolic content. Similarly, a correlation between antiradical efficiency and total phenolic content of red and white wines has been observed by others (*23–27*). A higher antiradical efficiency of red over white wines has also been reported by several other researchers (*23–28*). The antiradical potency of wines tested was dose dependent, since the scavenging activities of each wine sample decreased by decreasing their total phenolic content with dilution.

The EC_{50} values of four wines for the scavenging of DPPH$^{\bullet}$ are presented in Table 2. EC_{50} values at 5 min

Table 2. EC_{50} values of Mavrodaphne, Xinomavro, Muscat and Moschofilero wines for the scavenging of DPPH$^{\bullet}$ radical

Wine	γ/(mg/L)	
	EC_{50} at 5 min	EC_{50} at 60 min
Mavrodaphne	255^{b*}	$155^{a,b}$
Xinomavro	200^{c}	92^{c}
Muscat	314^{a}	134^{b}
Moschofilero	$282^{a,b}$	177^{a}

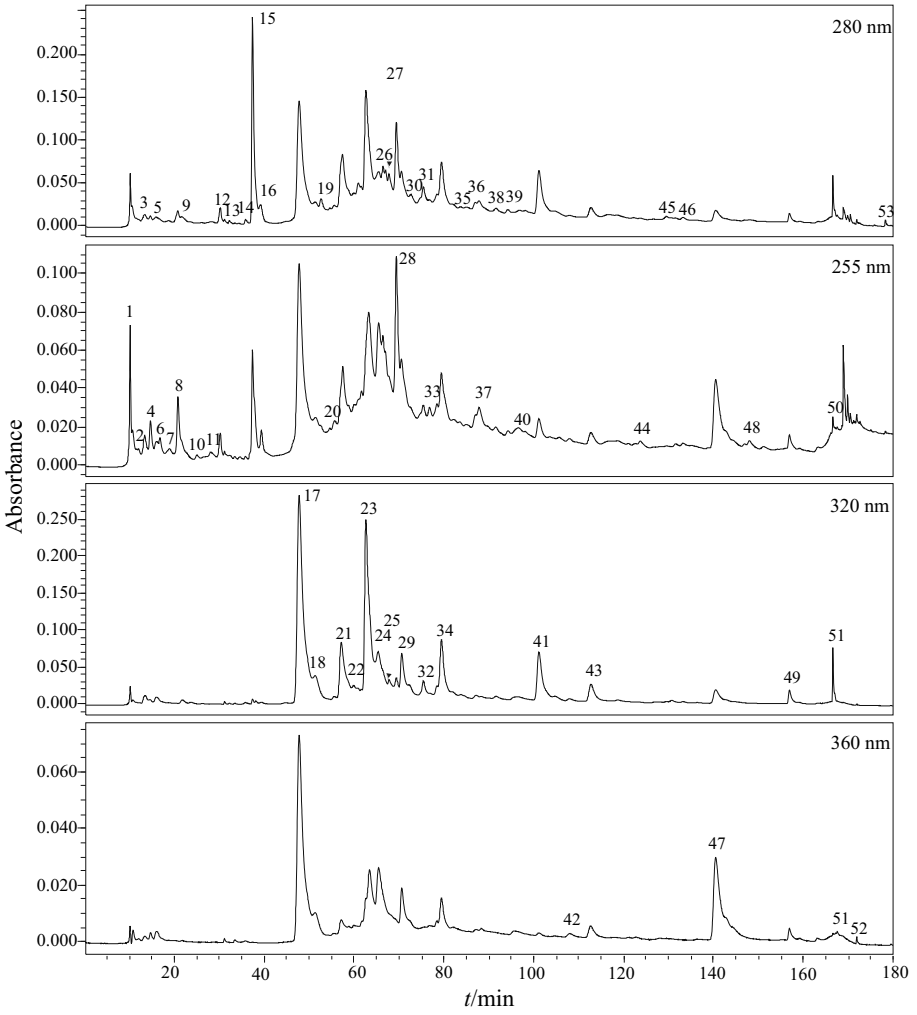

Fig. 1. HPLC chromatograms of Muscat wine at 280, 255, 320, and 360 nm
Peaks: 1-2, unclassified 230 nm; 3, unclassified 280 nm; 4, benzoic acid; 5, unclassified 280 nm; 6–8, benzoic acids; 9, unclassified 280 nm; 10–12, benzoic acids; 13, unclassified 280 nm; 14, flavanol; 15–16, unclassified 280 nm; 17–18, cinnamic acids; 19, flavone; 20, benzoic acid; 21–25, cinnamic acids; 26–27, flavanols; 28, benzoic acid; 29, cinnamic acid; 30–31, flavanols; 32, cinnamic acid; 33, benzoic acid; 34, cinnamic acid; 35, flavanol; 36–37, benzoic acids; 38–39, flavanols; 40, benzoic acid; 41, cinnamic acid; 42, flavonol; 43, cinnamic acid; 44, benzoic acid; 45, flavanone; 46, unclassified 280 nm; 47, flavonol; 48, benzoic acid; 49, cinnamic acid; 50, benzoic acid; 51–52, flavonols; 53, benzoic acid

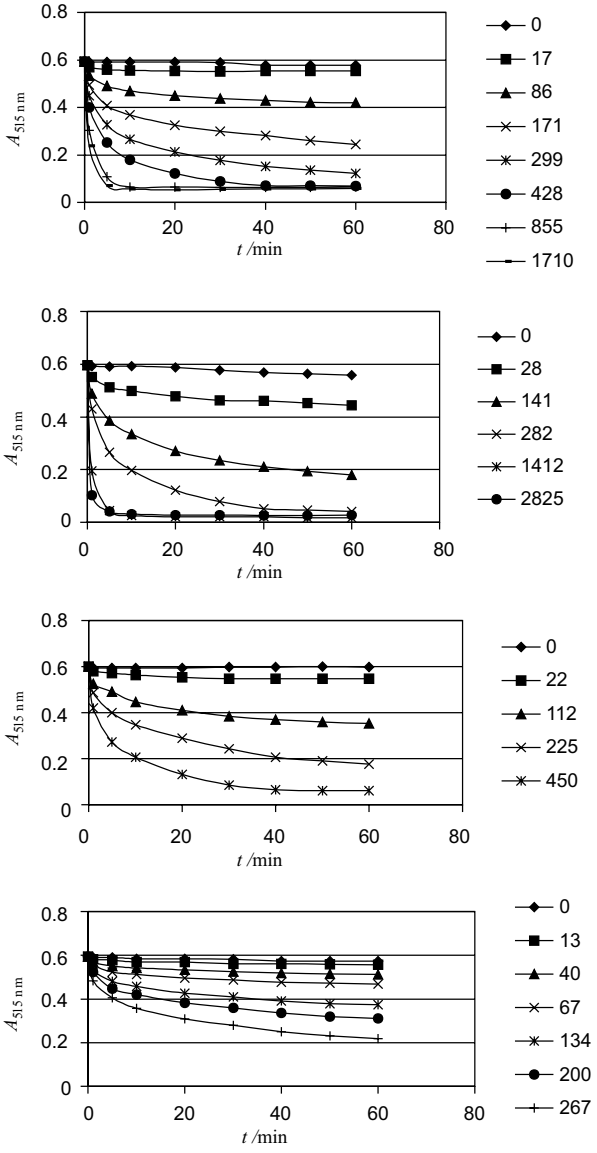

Fig. 2. Kinetics of DPPH• scavenging by Mavrodaphne, Xinomavro, Muscat and Moschofilero wines. The symbols indicate the total phenolic content (in mg/L) of wine dilutions used

(initial rate of scavenging activity) and at 60 min (total scavenging activity) were estimated. Xinomavro wine exhibited lower EC_{50} values than the others, indicating the higher potency of its phenolic compounds. The other red wine, Mavrodaphne, did not exhibit lower EC_{50} values than the two white wines. Between white wines, Muscat exhibited lower EC_{50} value at 60 min than Moschofilero wine.

Scavenging of reactive oxygen species by wine phenolic extracts

Xinomavro red wine total extract was tested for its scavenging capacities on hydroxyl radicals, superoxide radicals and singlet oxygen. Moreover, it was fractionated into three fractions, each fraction was then subfractionated into two subfractions. Fractions and subfractions were also tested. The main phenolics of all wine extracts (total extract, fractions, subfractions), as determined by HPLC-DAD, are shown in Table 3.

The EC_{50} values of all red wine extracts in the scavenging of hydroxyl radicals are given in Table 4. Wine total extract exhibited some scavenging capacity, which has also been reported by others (*29*). Among the phenolic fractions, fractions 1 and 2 showed higher scavenging capacity than fraction 3. Similarly, the subfractions of fractions 1 and 2 (1a, 1b, 2a and 2b) had higher scavenging capacity than the subfractions of fraction 3 (3a and 3b). HPLC-DAD chromatograms of fractions 1 and 2 and their subfractions were characterized by the high peaks of flavanols and anthocyanins (1, 1a and 1b), and of flavanols and flavonols (2, 2a and 2b). It has been reported that among wine phenolics, higher correlation of scavenging of hydroxyl radicals appeared to be with total anthocyanins (*29*). It is noticed that no differences were observed between subfractions a (1a, 2a and 3a) and the respective subfractions b (1b, 2b and 3b), indicating that monomeric-dimeric and polymeric wine phenolics do not differ in hydroxyl radical scavenging capacity. One wine fraction (fraction 3) appeared to be less active than the total extract, and subfractions 3a and 3b were less active than their original fraction. The above

Table 3. Main phenolics of Xinomavro red wine total extract and its fractions and subfractions

Sample	Main phenolics
Total extract	Cinnamic acids, benzoic acid, flavanols, flavones, anthocyanins, unclassified 280 nm, flavanone, flavonols, unclassified 230 nm
Fraction 1	Flavanols, anthocyanins, unclassified 280 nm, unclassified 230 nm, cinnamic acids, benzoic acids, flavonols
Fraction 2	Benzoic acids, flavanols, flavonols, unclassified 280 nm, flavanones, tyrosol
Fraction 3	Cinnamic acids, benzoic acids, flavonols, unclassified 230 nm
Subfraction 1a	Flavanols, anthocyanins, unclassified 230 nm, unclassified 280 nm, flavones, cinnamic acids, benzoic acids, flavonols
Subfraction 1b	Flavanols, anthocyanin
Subfraction 2a	Benzoic acids, flavanols, flavonols, flavanones, tyrosol, unclassified 280 nm
Subfraction 2b	Flavanols, flavonols, *trans*-resveratrol
Subfraction 3a	Cinnamic acids, benzoic acids, unclassified 230 nm, flavonols, unclassified 280 nm
Subfraction 3b	Cinnamic acids, flavanols, flavonols, flavones

indicates that wine phenolics may have a synergistic action in scavenging of hydroxyl radicals.

The EC_{50} values of all red wine extracts in the scavenging of superoxide radicals are also given in Table 4. Wine total extract exhibited some scavenging capacity, which has also been reported by others (*30*). Among the phenolic fractions, fraction 1 appeared to be the most active, followed by fraction 3. The higher peaks of fraction 1 were of flavanols and anthocyanins, while those of fraction 3 were of phenolic acids and flavonols. The most active subfraction, 1a, was rich in flavanols and anthocyanins. It has been reported that wine fractions containing anthocyanins or oligomeric procyanidins were efficient scavengers of superoxide radicals (*21*,*31*). In accordance with that subfraction 1a exhibited higher scavenging capacity than subfraction 1b, indicating that monomeric-dimeric flavanols and anthocyanins may be more active than polymeric ones.

Two fractions appeared to be less active than the total extract, and several subfractions were less active than their origin fractions. The above indicates that wine phenolics may have a synergistic action in scavenging of superoxide radicals. On the other hand, the subfractions 2a and 2b appeared to be more active than their original fraction.

The EC_{50} values of all red wine extracts in the scavenging of singlet oxygen are given in Table 4. Wine total extract exhibited some scavenging capacity. Among the phenolic fractions, fractions 2 and 3 exhibited higher scavenging capacity than fraction 1. Similarly, the subfractions of fractions 2 and 3 (2a, 2b, 3a and 3b) exhibited higher scavenging capacity than the subfractions of fraction 1. HPLC-DAD chromatograms of fractions 2 and 3 and their subfractions were characterized by the high peaks of flavanols and flavonols (2, 2a and 2b), and of phenolic acids and flavonols (3, 3a and 3b).

It was noticed that no differences were observed between subfractions a (1a, 2a and 3a) and the respective subfractions b (1b, 2b and 3b), indicating that monomeric-dimeric and polymeric wine phenolics do not differ in singlet oxygen scavenging capacity. One wine fraction (fraction 1) appeared to be less active than the total extract, and subfractions 1a and 1b were less active than their original fraction. The above indicates that wine phenolics may have a synergistic action in scavenging of singlet oxygen. There is no evidence concerning scavenging capacity of wine on singlet oxygen. However, the red wine constituent resveratrol has an inhibitory effect on the production of singlet oxygen and also of superoxide radicals in platelets (*32*). Resveratrol was also found to be an effective scavenger of hydroxyl and superoxide radicals (*33*).

Table 4. Phenolic concentration (in mg/L) of Xinomavro wine phenolic extracts causing a 50 % decrease of hydroxyl radicals, superoxide radicals and singlet oxygen (EC_{50} values)

Sample	Hydroxyl radicals, EC_{50}	Superoxide radicals, EC_{50}	Singlet oxygen, EC_{50}
Total extract	5.5^{a}*	1.4^{a}	3.4^{a}
Fraction 1	5.5^{a}	1.5^{ab}	5.9^{b}
Fraction 2	6.2^{ab}	2.9^{ef}	2.9^{a}
Fraction 3	7.1^{b}	2.0^{c}	3.4^{a}
Subfraction 1a	6.4^{ab}	1.8^{bc}	6.7^{c}
Subfraction 1b	6.6^{ab}	2.4^{d}	6.8^{c}
Subfraction 2a	5.7^{a}	2.4^{d}	2.0^{a}
Subfraction 2b	6.0^{ab}	2.3^{d}	3.1^{a}
Subfraction 3a	10.6^{d}	3.2^{f}	1.6^{a}
Subfraction 3b	8.3^{c}	2.6^{de}	3.6^{ab}

*Means in each column with different superscript differ significantly at $P<0.05$

Conclusions

In the present work, the scavenging activities of four different wines were examined. All wines exhibited scavenging activity on DPPH radical analogous to their total phenolic content. However, their phenolics differed in antiradical potency, exhibiting differences in their EC_{50} values.

The scavenging capacities of several red wine phenolic extracts on reactive oxygen species were also assessed. All wine extracts exhibited scavenging capacity on hydroxyl radicals, superoxide radicals and singlet oxygen, indicating that many wine phenolics may be active. However, they differ in their potency towards the three ROS tested. The ability of all red wine phenolic extracts to scavenge singlet oxygen, along with hydroxyl and superoxide radicals, emphasizes its health functionality.

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References

1. W.A. Pryor: *Free Radicals in Biology, Vols. 1–5*, Academic Press, New York (1976–1982).
2. B.N. Ames, Dietary carcinogens and anticarcinogens: Oxygen radicals and degenerative diseases, *Science, 221* (1983) 1256–1264.
3. B. Halliwell: The Biological Toxicity of Free Radicals and Other Reactive Oxygen Species. In: *Free Radicals and Food Additives*, O.I. Aruoma, B. Halliwell (Eds.), Taylor & Francis, PA (1991).
4. C.A. Rice-Evans, N.J. Miller, Antioxidant activities of flavonoids as bioactive components of foods, *Biochem. Soc. Trans. 24* (1996) 790–795.
5. J.H. Weisburger, Mechanisms of action of antioxidants as exemplified in vegetables, tomatoes and tea, *Food Chem. Toxicol. 37* (1999) 943–948.
6. R. Rodrigo, G. Rivera, Renal damage mediated by oxidative stress: A hypothesis of protective effects of red wine, *Free Radic. Biol. Med. 33* (2002) 409–422.
7. S.R. Jackson: *Wine Science. Principles and Applications*, Academic Press, San Diego (1994).
8. G. Mazza, Anthocyanins in grapes and grape products, *Crit. Rev. Food Sci. Nutr. 35* (1995) 341–371.
9. M.H. Salagoity-August, A.J. Bertrand, Wine phenolics analysis of low molecular weight components by high performance liquid chromatography, *J. Sci. Food Agric. 35* (1984) 1241–1247.

10. A. Ghiselli, M. Nardini, A. Baldi, C. Scaccini, Antioxidant activity of different phenolic fractions separated from an Italian red wine, *J. Agric. Food Chem. 46* (1998) 361–367.
11. K. Kantz, V.L. Singleton, Isolation and determination of polymeric polyphenols using Sephadex LH-20 and analysis of grape tissue extracts, *Am. J. Enol Vitic. 41* (1990) 223–228.
12. V.L. Singleton, J.A. Rossi, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, *Am. J. Enol. Vitic. 16* (1965) 144–158.
13. G. Mazza, L. Fukumoto, P. Delaquis, B. Girard, B. Ewert, Anthocyanins, phenolics, and color of Cabernet Franc, Merlot, and Pinot Noir wines from British Columbia, *J. Agric. Food Chem. 47* (1999) 4009–4017.
14. I. Roussou, I. Lambropoulos, G.N. Pagoulatos, T. Fotsis, I.G. Roussis, Decrease of heat shock protein levels and cell populations by wine phenolic extracts, *J. Agric. Food Chem. 52* (2004) 1017–1024.
15. K. Robards, P.D. Prenzler, G. Tucker, P. Swatsitang, W. Glaver, Phenolic compounds and their role in oxidative processes in fruits, *Food Chem. 66* (1999) 401–436.
16. H.S. Lee, B.W. Widmer: Phenolic Compounds. In: *Handbook of Food Analysis*, *Vol. 1*, L.M. Nollet (Ed.), Dekker, New York (1996).
17. J.A. Larrauri, C. Sanchez-Moreno, F. Saura-Calixto, Effect of temperature on the free radical scavenging capacity of extracts from red and white grape pomace peels, *J. Agric. Food Chem. 46* (1998) 2694–2697.
18. O.I. Aruoma, Deoxyribose assay for detecting hydroxyl radicals, *Methods Enzymol. 233* (1994) 57–66.
19. G.R. Schinella, H.A. Tournier, J.M. Prieto, P. Mordujovich de Buschiazzo, J.L. Rios, Antioxidant activity of anti-inflammatory plant extracts, *Life Sci. 70* (2002) 1023–1033.
20. M. Paya, B. Halliwell, J.R.S. Hoult, Interactions of a series of coumarins with reactive oxygen species. Scavenging of superoxide, hypochlorous acid and hydroxyl radicals, *Biochem. Pharmacol. 44* (1992) 205–214.
21. N. Saint-Cricq de Gaulejac, Y. Glories, N. Vivas, Free radical scavenging effect of anthocyanins in red wines, *Food Res. Int. 32* (1999) 327–333.
22. S.Y. Wang, H. Jiao, Scavenging capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen, *J. Agric. Food Chem. 48* (2000) 5677–5684.
23. C. Sanchez-Moreno, J.A. Larrauri, F. Saura-Calixto, Free radical scavenging capacity of selected red, rose and white wines, *J. Agric. Food Chem. 79* (1999) 1301–1304.
24. D. De Beer, E. Joubert, W.C.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.
25. R.C. Minussi, M. Rossi, L. Bologna, L. Cordi, D. Rotilio, G.M. Pastore, N. Duran, Phenolic compounds and total antioxidant potential of commercial wines, *Food Chem. 82* (2003) 409–416.
26. M.S. Fernandez-Pachon, D. Villano, M.C. Garcia-Parrilla, A.M. Troncoso, Antioxidant activity of wines and relation with their polyphenolic composition, *Anal. Chim. Acta, 513* (2004) 113–118.
27. V. Katalinic, M. Milos, D. Modum, I. Music, M. Bodan, Antioxidant effectiveness of selected wines in comparison with (+)-catechin, *Food Chem. 86* (2004) 593–600.
28. C. Sanchez-Moreno, G. Cao, B. Ou, R.L. Prior, Anthocyanin and proanthocyanidin content in selected white and red wines. Oxygen radical absorbance capacity comparison with nontraditional wine obtained from highbush blueberry, *J. Agric. Food Chem. 51* (2003) 4889–4896.
29. A. Arnous, D.P. Makris, P. Kefalas, Correlation of pigment and flavanol content with antioxidant properties in selected aged regional wines from Greece, *J. Food Compos. Anal. 15* (2002) 655–665.
30. M. Sato, N. Ramarathnam, Y. Suzuki, T. Ohkubo, M. Takeuchi, H. Ochi, Varietal differences in the phenolic content and superoxide radical scavenging potential of wines from different sources, *J. Agric. Food Chem. 44* (1996) 37–41.
31. N. Saint-Cricq de Gaulejac, C. Provost, N. Vivas, Comparative study of polyphenol scavenging activities assessed by different methods, *J. Agric. Food Chem. 47* (1999) 425–431.
32. B. Olas, B. Wachowicz, J. Saluk-Juszczak, T. Zielinski, W. Kaca, A. Buczynski, Antioxidant activity of resveratrol in endotoxin-stimulated blood platelets, *Cell Biol. Toxicol. 17* (2001) 117–125.
33. S.S. Leonard, C. Xia, B.H. Jiang, B. Stinefelt, H. Klandorf, G.K. Harris, X.L. Shi, Resveratrol scavenges reactive oxygen species and effects radical-induced cellular responses, *Biochem. Biophys. Res. Commun. 309* (2003) 1017–1026.

Učinkovitost vina i njegovih fenolnih ekstrakata pri uklanjaju reaktivnih kisikovih spojeva

Sažetak

Svrha je rada bila utvrditi sposobnost različitih vina, slatko crveno, suho crveno, slatko bijelo i suho bijelo, da uklone stabilni 1,1'-difenil-2-pikril-hidrazil radikal (DPPH$^{\bullet}$). Osim toga određen je i njihov fenolni sastav. Oba crvena vina sadržavala su više ukupnih fenolnih spojeva, estera tartarata i flavonola od bijelih vina. Sva su vina imala sposobnost uklanjanja radikala proporcionalno udjelu ukupnih fenola, a njihovi su fenoli pokazivali različitu sposobnost, što se vidi prema razlici njihovih EC_{50} vrijednosti. Suho crveno vino (Xinomavro) imalo je nižu EC_{50} vrijednost, što je upućivalo na veći antiradikalni učinak njegovih fenola. Također je utvrđena djelotvornost fenolnih ekstrakata vina Xinomavro na uklanjanje hidroksilnih i superoksidnih radikala te singletnoga kisika. Ukupni vinski ekstrakti frakcionirani su ekstrakcijom, a svaka od triju frakcija bila je naknadno frakcionirana kromatografijom na koloni u dvije podfrakcije. Ukupni vinski ekstrakt, njegove frakcije

i podfrakcije pokazivali su sposobnost uklanjanja hidroksilnih i superoksidnih radikala te singletnoga kisika, što je značilo da su mnogi fenoli u vinu aktivni. Najaktivniji vinski ekstrakti prema hidroksilnim radikalima karakterizirani su visokim pikovima flavanola, antocijanina i flavonola u njihovim HPLC-DAD kromatogramima. Najaktivniji ekstrakt prema superoksid radikalima bio je bogat flavanolima i antocijaninima. Karakteristični fenoli u najaktivnijim vinskim ekstraktima prema singletnom kisiku bili su flavanoli, flavonoli i fenolne kiseline. Sposobnost ekstrakata svih crvenih vina da uklanjaju singletni kisik zajedno s hidroksilnim i superoksidnim radikalima ističe njihovu zdravstvenu vrijednost.