BOTTLE STORAGE AND AIR EXPOSED RED WINES: INVESTIGATION OF THEIR ANTIOXIDANT ACTIVITY

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Abstract

The antioxidant activity of bottle storaged ani air exposed Greek red wines was investigated. For this purpose, 9 high quality, single-variety wines, namely Cabernet-Merlot, Cabernet-N.Drys, Metohi, Chromitsa, Syrah, Megas Oinos, Amyntaio, Nemea and Kaba of the vintages of 2001, 2002, 2003, 2004 and 2005 were selected. Reactivity of wine samples against 1,1- diphenyl-2-dipicrylhydrazyl (DPPH) free radical showed a decrease in antioxidant activity caused by storage in bottles, which was confirmed by cyclic voltammetry analysis. This phenomenon may be connected with decrease in anthocyanines content as well as to reactions converting the low molecular to high molecular-weight phenolics. The antioxidant profile of air exposed wine samples was also studied. It was found that air exposure of wine samples leads to a substantial decrease of their antioxidant properties, eliminating the 50% of their antiradical scavenging in 8-12 days at 25^{o}C or in 11-16 days at 4^{o}C.

Keywords: *Red wine; antioxidant activity; bottle storage; air exposed wine samples; cyclic voltammetry; DPPH.*

Introduction

The beneficial effect of moderate wine consumption to human health has been widely accepted [Trichopoulou and Lagiou 1997; Renaud et al. 1999]. Indeed, epidemiological studies showed a decrease in susceptibility of Low-Density Lipoprotein (LDL) to oxidation, which implies the contribution of wine consumption to the prevention of arteriosclerosis development and to the increase of serum antioxidant capacity [Cooper et al. 2004]. Furthermore, wine consumption has been associated with decrease in chronic inflammatory diseases incidence, provoked by oxygen free radicals and, even, cancer [Scalbert et al. 2005]. This action can be explained on the basis of antioxidant activity of wine, attributed to many polyphenolic antioxidants, including flavonoids, such as flavonol and anthocyanins and non-flavonoid antoxidant compounds, such as gallic and caffeic acid [Monagas et al. 2005]. However, the polyphenolic and, hence the antioxidant profile of wines differs essentially between red and white wines and it is also greatly affected by the vinification technology used [Alen-Ruiz et al. 2009].
Further parameters influencing the antioxidant activity of wines, include among others the bottle storage and its disposal conditions after bottle opening. The effect of aging to antioxidant capacity of wines has been a controversial topic in the literature. Thus, some authors stated decrease in antioxidant capacity of wines by aging [Giovanell and Brenna 2007; Alen-Ruiz et al. 2009; Mulero at el. 2009], while others found no correlation between aging and antioxidant activity [Zafrilla et al. 2003]. Other studies found an increase in antioxidant activity of wines as a result of aging [Burns et al.

2001; Echeverry et al. 2005]. On the other hand, no references exist in the literature referring to the influence of wines' disposal conditions and, particularly, the antioxidant profile of air exposed wines.
In the present study, we found it interesting to investigate the effect of bottle storage to the antioxidant content of nine popular Greek wine varieties from South and North Greece. Furthermore, antioxidant profiles of air exposed wine samples were studied at two different conditions (room temperature / fridge temperature). The latter study provides some interesting results regarding to the deterioration of the nutritional benefits consumers, to wine consumer, if the wine has been air exposed for a few days.

Materials and Methods

Instrumentation

Spectrophotometric measurements were carried out by a Hitachi U-2000 UV-Vis spectrophotometer (Varian). All samples were analyzed in 10 mm quartz cells at room temperature. Cyclic voltammetric study of the investigated red wines was performed using the polarograph 747 VA Stand (Metrohm) connected with the 746 VA Trace Analyzer (Metrohm) microprocessor. The working electrode was a glassy carbon electrode, the reference a Ag/AgCl one, filled with 3 M KCl (≥99.5% p.a., Merck) in High Purity Water (HPW) supplied by an EASYpure II (Model D 7381, Barnsted International) water purification system, and the auxiliary a Pt electrode. The glassy carbon working electrode was polished at the beginning of each measurement with alumina powder (0.3 μm) for 3 min, using a polishing cloth and it was rinsed with deionized water. Furthermore, in the end of each working day, it was sonicated for 5 min firstly in distilled water and, secondly, in acetone. The state of the electrode was checked using a 0.1 M $Fe(CN)_6^{4-}$ solution.

Reagents

All reagents were of analytical grade and they were purchased by Chemilab (Athens, Greece). 1,1- diphenyl-2-dipicrylhydrazyl (DPPH) (≥ 95%, Fluka Biochemica) was used for the evaluation of the antioxidant activity of wines, while Trolox (97%, Merck) was employed for as the reference antioxidant material. Methanol (Merck) was used as solvent for the dilution of wines samples and the preparation of DPPH solutions and $LiClO_4$ (≥99.0%, p.a., Fluka Biochemica) was employed in a concentration of 0.1 M as electrolyte for cyclic voltammetry.

Wine Samples

Nine commercial wines of the Greek vineyard, produced according to standard procedures, were investigated. All studied samples are dry red wines of regional origin and they were made from the following grape varieties: (1) Cabernet-Merlot; (2) Cabernet- N. Drys; (3) Metochi; (4) Chromitsa; (5) Syrah; (6) Megas Oinos; (7) Amyntaio; (8) Nemea; (9) Caba. The geographical origin of these wines is depicted in Figure 1 and their oenological parameters are presented in Table 1. It should be noted that the investigated samples represent some of the most popular wine varieties of Peloponnesus and North Greece. These wine varieties are available in the Greek market, and widely consumed. All samples were stored at 8^o C in obscurity.

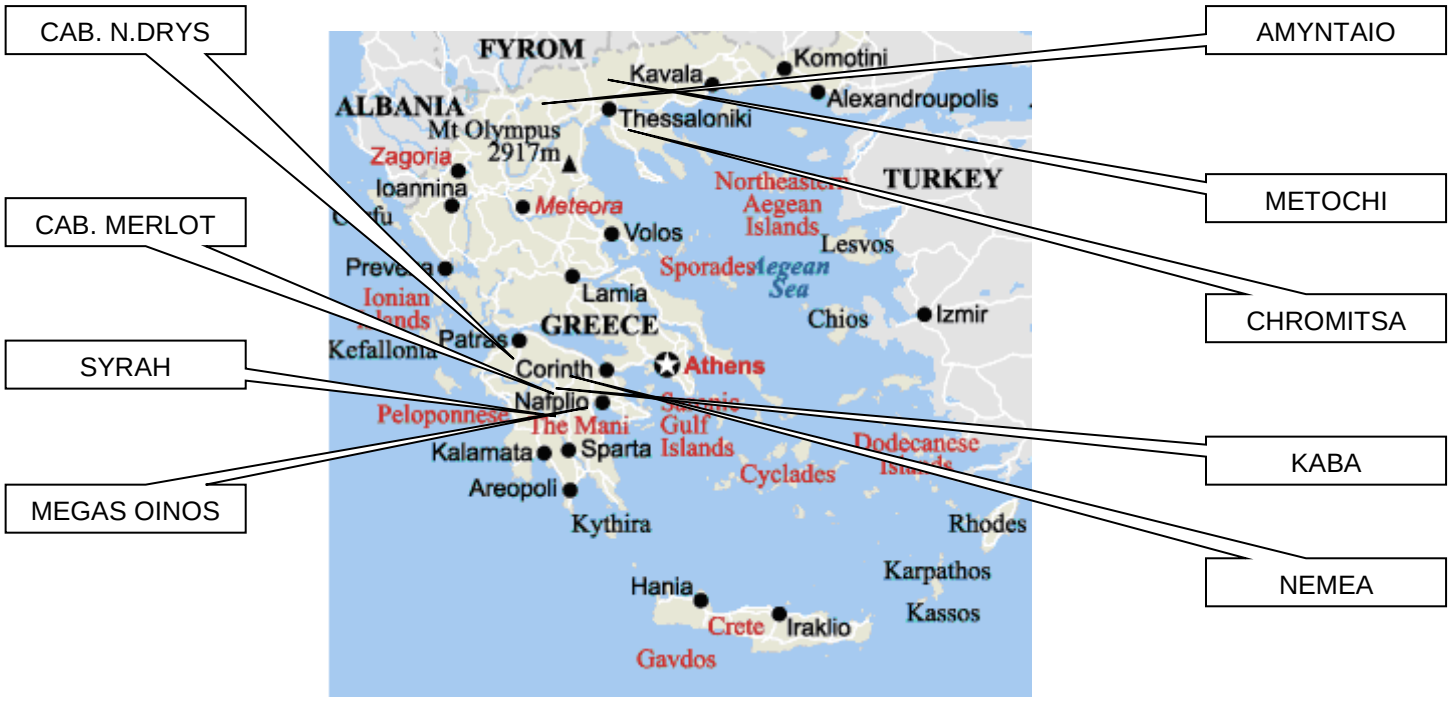


Figure 1: Map of Greece and geographical origination of the investigated Greek red wines

Table 1: Origin, varietal composition and characteristic oenological parameters of the tested wines. [a]Letters N and S denote Northern and Southern Greece respectively

Appellation	**Cultivar(s)**	**Alcoholic Degree %**	**Varietal Composition**	**Location[a]**
Cabernet Merlot	Tselepos	13	Cabernet – Sauvignon Merlot	Peloponese (S)
Cabernet Nea Drys	Antonopoulos	14	Cabernet – Sauvignon Cabernet - Franc	Peloponese (S)
Syrah	Ktima Gerovasileiou	13.5	Syrah	Peloponese (S)
Megas Oinos	Skouras	13.5	Agiorgitiko Cabernet - Sauvignon	Peloponese (S)
Amyntaio	Svolos Selections	12.5	Xinomauro	Macedonia (N)
Nemea	Svolos Selections	13	Agiorgitiko	Peloponese (S)
Metochi	Tsantalis	13.5	Cabernet – Sauvignon Limnio	Macedonia (N)
Kaba	Boutari	12.5	Cabernet – Sauvignon	Peloponese (S)

			Agiorgitiko - Merlot	
Chromitsa	Tsantalis	13	Cabernet – Sauvignon Limnio	Macedonia (N)

Analytical Procedure

a) Effect of aging in bottles to antioxidant profile of wine samples

All studies were carried out in room temperature (22 ± 1°C). For each investigated wine, 6 measurements of antioxidant activity were carried out in order to obtain means and standard deviations.
Antioxidant activity was measured according to the protocol described elsewhere [Arnous et al. 2001]. Briefly, an aliquot of 25 μl of the appropriately diluted (1:10 in methanol immediately before the analysis) wine sample was added to 0.975 ml of 60 mM of DPPH solution in MeOH. Absorbance was read at 515 nm at t=0 and t=30 min after the addition of DPPH.

The antioxidant activity (A_{AR}) was measured according to the formula (1):

$$A_{AR} = 0.015 \cdot \%\Delta A_{515} + 0.006 \quad (r^2 = 0.9947) \qquad (1)$$

as measured by linear regression after plotting $\%\Delta A_{515}$ of known solutions of Trolox against its concentration in the range of 0.4-1.5 mM and $\%\Delta A_{515}$ is calculated according to the equation (2) :

$$\%\,\Delta A_{515} = \frac{\left(A_{515(0)} - A_{515(30)}\right)}{A_{515(0)}} \cdot 100 \qquad (2)$$

Results were expressed as Trolox equivalents (mM TRE)

b) Antioxidant profile of air exposed wine samples

For the investigation of air exposure to antioxidant activity of wine samples, 10 ml of sample was transferred to an open flask and stored at room temperature (25°C) and in fridge (4°C). Antiradical activity measurements were carried out, according the above mentioned protocol, every 24 hours for 7 days.

c) Cyclic voltammetry

For cyclic voltammetric analysis, 1 mL of the investigated wine sample was transferred in a volumetric flask of 10 mL. Then, the appropriate amount of $LiClO_4$, corresponding to a final concentration of 0.1 M, was added and the aliquot was diluted with MeOH up to a final volume of 10 mL. As blank, 0.1 M $LiClO_4$ in 90% MeOH, 8.7 % HPW and 1.3 % EtOH was considered, taking into account that the mean value of alcoholic degree of the studied wine varieties is about 13%. Cyclic voltammograms were recorded between -100 and 1000 mV with a scanning rate of 20 mV s^{-1}.

Statistical Analysis

The statistical treatment of experimental data was carried out using the Statistica – Axa 7.0 software package (StatSoft, Tulsa, Oklahoma, USA).

Results and Discussion

Effect of bottle storage to the antioxidant activity of Greek red wines

In the first place, the effect of bottle storage to the antioxidant activity of Greek red wines was studied. For this purpose, samples of 5 different vintages (2001, 2002, 2003, 2004 and 2005) of the 9 investigated Greek red wine varieties (Cabernet-Merlot, Cabernet- N. Drys, Metochi and Chromitsa, Syrah, Megas Oinos, Amyntaio, Nemea, Kaba) were selected. The antioxidant activity of the studied varieties as a function of aging is depicted in Figure 2. As shown, a decrease in antioxidant capacity of all samples is observed by increasing the years of bottle storage. This decrease is not linear but it is followed in most cases by a plateau, where an almost constant antiradical activity was observed for a certain period of storage. For example, Cabernet- Merlot showed an almost constant antioxidant activity during the 6th year of its bottle storage, while for other wines' varieties the corresponding plateau was evident during the 7th (Cabernet- N.Drys) of the 8th year (Megas Oinos, Nemea, Syrah) of storage.

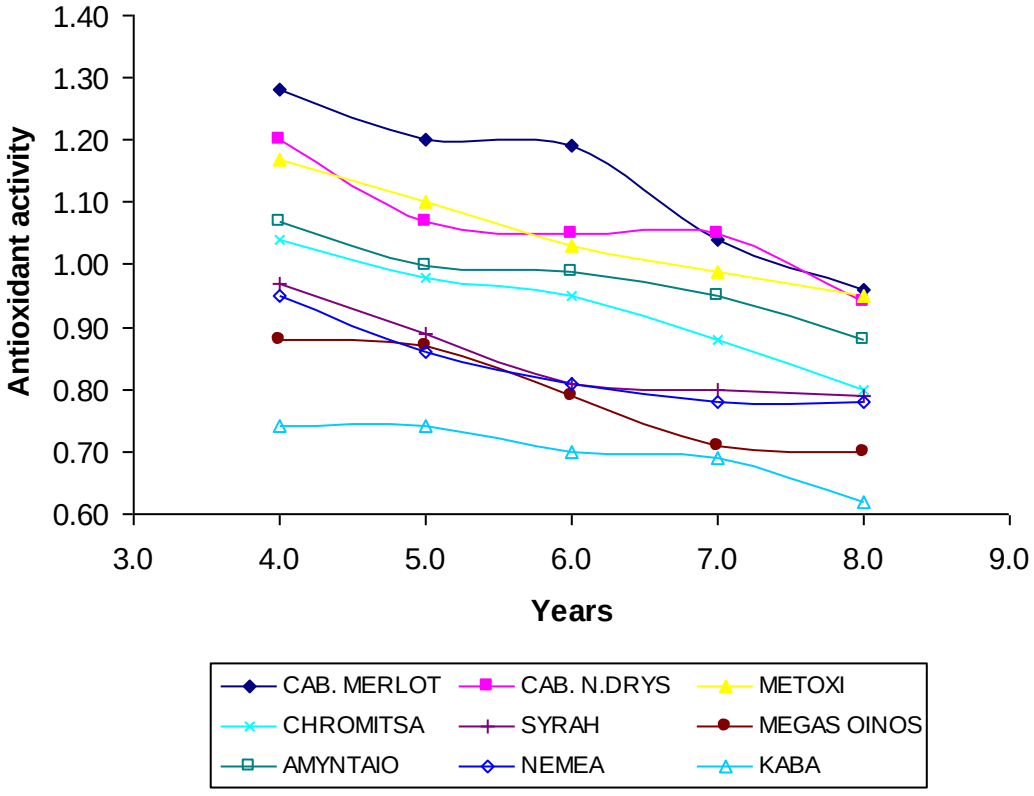
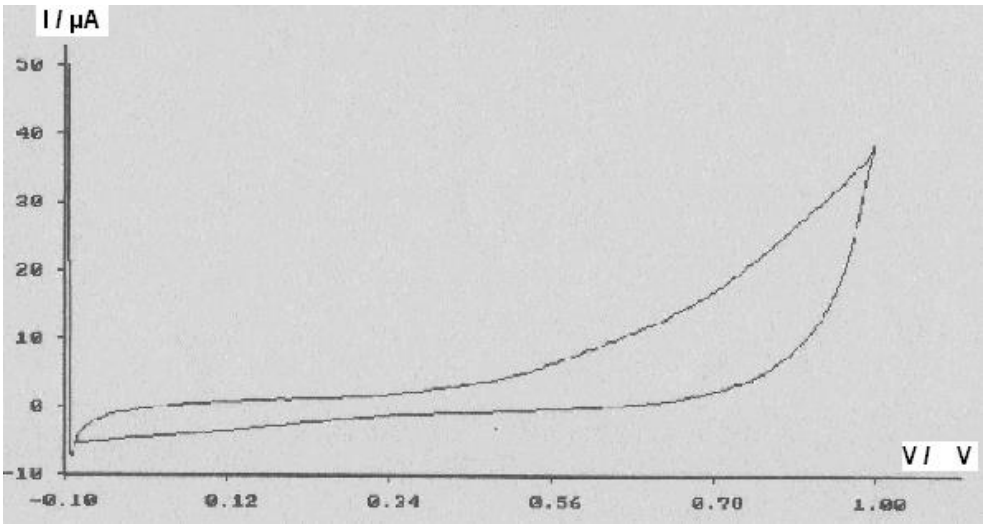


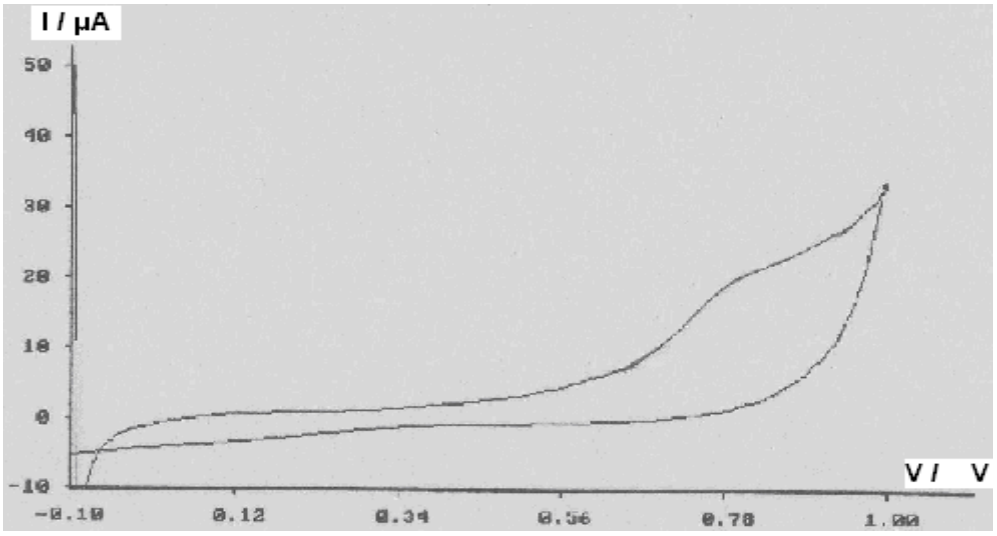
Figure 2: Effect of aging to the antiradical activity of the nine investigated wine samples

In order to confirm the decrease in antioxidant profile of wines as a result of bottle storage, the same wine samples were also studied by cyclic voltammetry. It should be noted that cyclic voltammetry has been widely employed for the evaluation of the antioxidant capacity of natural products and biological issues, such as human and

horse plasma, animal tissues, edible plants, wines and different types of tea and coffees [Martinez et al. 2006; Zielinska et al. 2007; Yakovleva et al. 2007]. 0.1 M $LiClO_4$ was selected as electrolyte, because it had been successfully used in the past for electrochemical studies in the organic phase, showing good compatibility with a wide range of organic solvents [Ochsenkühn et al. 2002]. The representative cyclic voltammograms of "Semeli" wine of the vintages of 2001 and 2005 are presented in Figure 3. As shown, a plateau at a voltage of +0.82±0.04 V is shown in the case of the vintage of 2005 (Figure 3(b)), implying weak antioxidant properties. The presence of the plateau instead of a distinct peak may be attributed to the possible presence of multiple overlapping peaks, which can be attributed to electrochemical oxidations involving more than one successive oxidation steps. However, the corresponding cyclic voltammogram in the same wine variety coming from the vintage of 2001 (Figure 3(a)) shows absence of such plateau, indicating deterioration of antioxidant activity. Analogous phenomenon was also observed for the other wine samples.



(a)



(b)

Figure 3: Cyclic voltammograms of "Semeli" wine samples (a) of the vintage of 2001 and (b) of the vintage of 2005.

The decrease in antioxidant activity of wine samples as a function of storage has also been described by other authors [Giovanell and Brenna 2007; Alen-Ruiz et al. 2009; Mulero at el. 2009]. One assumption for this decrease is the substantial decrease in anthocyanines content of wines in relation to age and to reactions that modify the structure of anthocyanin monomers [Burns et al. 2001], or even the decrease of their total phenolic content [Negi and Dey 2009]. These reactions are possibly complex and, according to our findings, do not uniformly take place. However, as it has been stated before, the influence of bottle storage to the antioxidant activity of wines has not been widely accepted, as other authors stated increase in antioxidant capacity of wines as a result of storage [Burns et al. 2001; Echeverry et al. 2005], while others found no correlation between storage and antioxidant activity [Zafrilla et al. 2003].

Antioxidant capacity of air exposed wines

The investigation of the antioxidant activity of the air exposed wines was set a major aim of the present study as no relevant studies existed in the literature. For this purpose, wine samples of the 9 wine varieties were investigated in open flasks in both room temperature (25^{o}C) and in the fridge (4^{o}C). These temperatures represent the typical air exposure conditions of wines after the opening of their bottles. It was found that antioxidant activity of wine samples decreased soon after the opening of their bottle. The reduction rate of antioxidant activity of all wine samples was close between the two investigated temperatures. The plots of antioxidant activity of the 9 investigated wines as a function of air exposure at fridge (4^{o}C) and room temperature (25^{o}C), in the case of samples coming from the vintage of 2005 are presented in Figures 4 and 5, respectively.

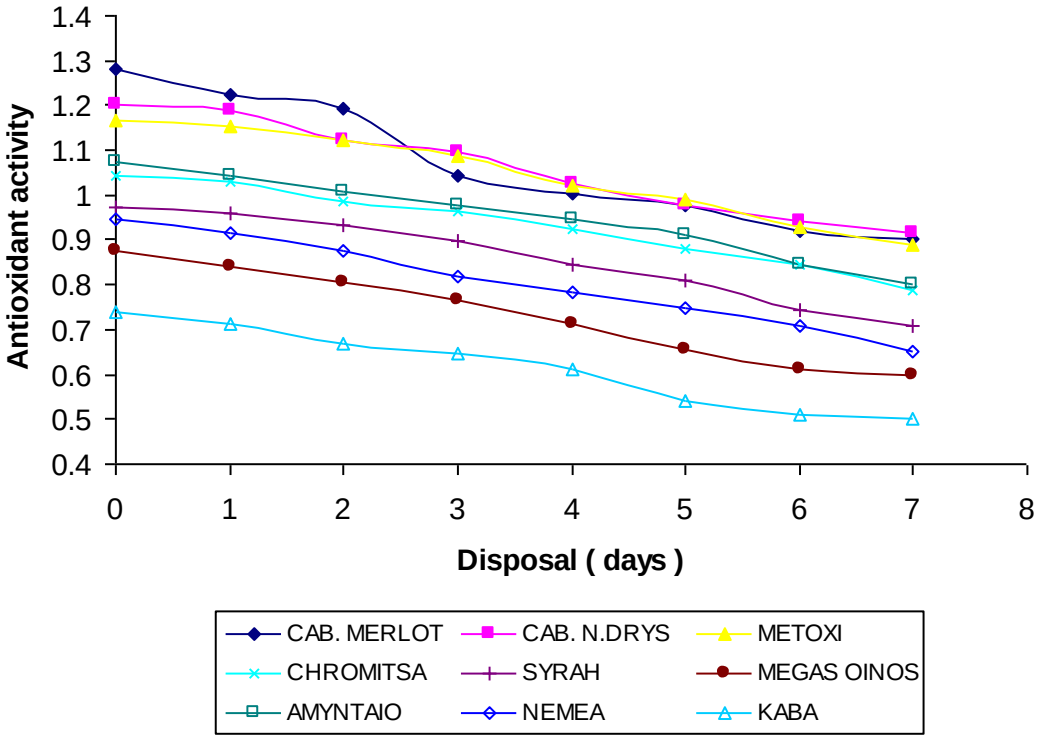


Figure 4: Effect of disposal time to the antiradical activity of the nine wine samples at 4^{o}C

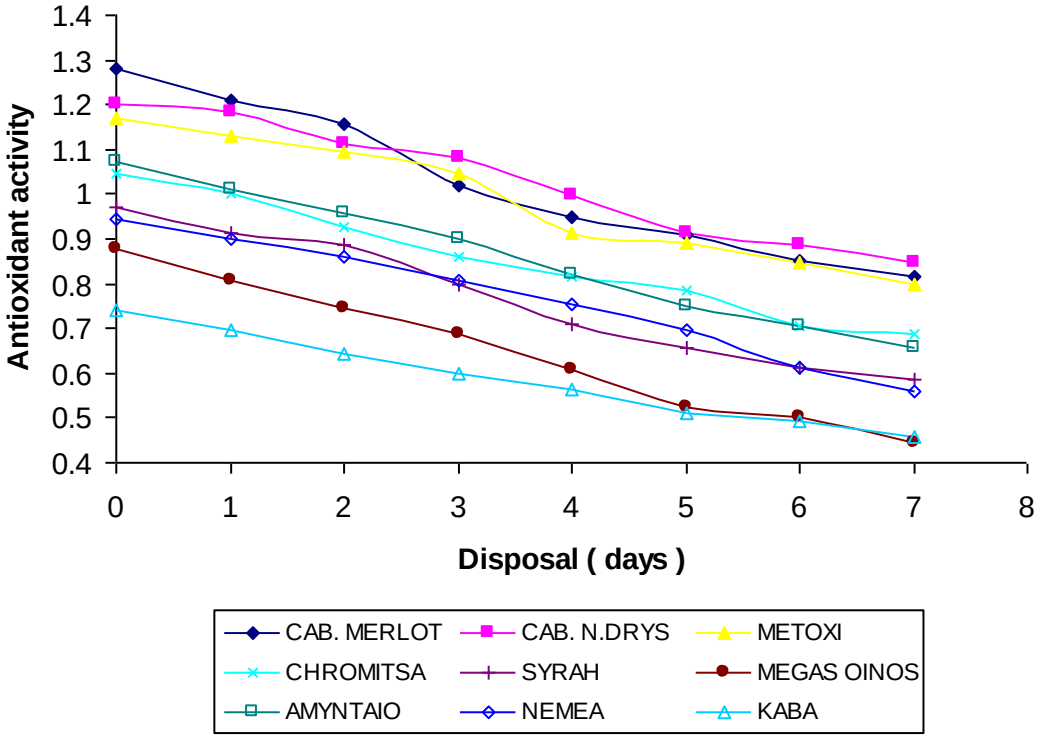


Figure 5: Effect of disposal time to the antiradical activity of the nine wine samples at 25^{o}C

As shown, a uniformly rapid decrease of antiradical capacity is observed for all samples after the beginning of their contact with air. In the case of room temperature, the decrease in antioxidant activity is slightly higher compared to the fridge temperature. Analogous decrease was observed in all wine samples independently of their vintage or the temperature. This observation is of great interest because in many cases wine bottles after their opening, remains open for a long time till the consumption of the whole quantity of wine. Therefore, the benefits to human health from the consumption of a wine stored in an open bottle can be much lower compared to a wine not exposed to air. This substantial decrease of antioxidant activity can be attributed to the reaction of atmospheric oxygen with wines' phenolic content [Fulcrand et al. 2006].

By the plot of antioxidant activity/air exposure time, the half-time of the decrease of the wines' antioxidant activity can be determined. These values correspond to the required time for the 50% decrease of the initial antiradical activity of each wine and they are presented in Table 2. As shown, the effect of temperature is not uniform for all investigated wine samples. A slight influence can be considered for wine varieties, such as Kaba, while a more considerable effect is observed in the case of Chromitsa, Nemea and Megas Oinos.

Table 2: Half-time (days) of the antioxidant activity reduction of investigated wine varieties during open disposal at 25° C and 4° C.

Wine varieties	**Half-time (days) for the reduction of antioxidant activity of wine samples during open disposal at different conditions**	
	Room Temperature (25°C)	**Fridge (4°C)**
Cabernet-Merlot	9.7	12.1
Cabernet- N. Drys	12	14.6
Metochi	11	14.6
Chromitsa	10.2	16.2
Syrah	8.8	12.1
Megas Oinos	7.3	11
Amyntaio	9	13.8
Nemea	8.6	14.3
Kaba	10.3	10.9

Conclusions

Bottle storage leads to a moderate decrease in antioxidant activity of the investigated Greek red wines, which can be attributed to the reduction of their anthocyanines content and to possible decrease in their total phenolic content. Air exposure of wine samples provokes a considerable decrease of their antioxidant properties, possibly due to reactions with atmospheric oxygen. An elimination of about 50% of the antiradical activity of the investigated wine samples is observed in 8-12 days at 25°C and in 11-16 days at 4°C.

References

1. Alen- Ruiz, F., Garcia- Falcon, M.S., Perez- Lamela, M.C., Martinez- Carballo, E., Simal-Gandara, J. (2009). Influence of major polyphenols on antioxidant activity in Mencia and Brancellao red wines. Food Chemistry 113: 53-60.
2. Arnous, A., Makris, D., Kefalas, P. (2001). Effect of principal polyphenolic components in relation to antioxidant characteristics of aged red wines. Journal of Agriculture and Food Chemistry 49: 5736-5742.
3. Burns, J., Gardner, P.T., Matthews, D., Duthie, G.G., Lean, M.E.J., Crozier, A. (2001). Extraction of phenolics and changes in antioxidant activity of red wines during vinification. Journal of Agriculture and Food Chemistry 49: 5797-5808.
4. Cooper, K.A., Chopra, M., Thurnham, D.I. (2004). Wine polyphenols and promotion of cardiac health: a review. Nutrition Research Reviews 17: 111-129.
5. Echeverry, C., Ferreira, M., Reyes- Parada, M., Abin- Carriquiry, J.A., Blasina, F., Gonzelez-Neves, G., Dajas, F. (2005). Changes in antioxidant capacity of Tannat red wines during early maturation. Journal of Food Engineering 69: 147-154.
6. Fulcrand, H., Duenas, M., Salas, E., Cheynier, V. (2006). Phenolic reactions during winemaking and aging. American Journal of Enology and Viticulture 57: 289-297.
7. Giovanelli, G., Brenna, O. (2007). Oxidative stability of red wine stored in packages with different oxygen permeability. European Food Research and Technology 226: 169-179.

8. Martinez, S., Valek, L., Resetic, J., Ferenec Ruzic, D. (2006). Cyclic voltammetry of plasma antioxidant capacity- Comparison with the DPPH and TAS spectrophotometric methods. Journal of Electroanalytical Chemistry 588: 68-73.

9. Monagas, M., Bartolome, B., Gomez- Cordoves, C. (2005). Updated knowledge about the presence of phenolic compounds in wine. Critical Reviews in Food Science and Nutrition 45: 85-118.

10. Mulero, J., Pardo, F., Zafrilla, P. (2009). Effect of principal polyphenolic components in relation to antioxidant activity in conventional and organic red wines during storage. European Food Research and Technology 229: 807-812.

11. Negi, B., Dey, G. (2009). Comparative analysis of total phenolic content in Sea Buckthorn wine and other selected fruit wines. World Academy of Science Engineering and Technology 54: 396-399.

12. Ochsenkühn-Petropoulou, M., Tsopelas, F. (2002). Speciation analysis of selenium using voltammetric techniques. Analytica Chimika Acta 467: 167-178.

13. Renaud, S.C., Gueguen, R., Siest, G., Salomon, R. (1999). Wine, Beer, and Mortality in Middle-aged Men from Eastern France. Archives of Internal Medicine 159: 1865-1870.

14. Scalbert A, manach C, Morand C, Remesy C, Jimenez L. (2005). Dietary polyphenols and the prevention of diseases. Critical Reviews in Food Science and Nutrition. 45: 287-306.

15. Trichopoulou, A., Lagiou P. (1997). Healthy traditional Mediterranean diet: an expression of culture, history, and lifestyle. Nutrition Reviews 55: 383-389.

16. Yakovleva, K.E., Kurzeev, S.A., Stepanova, E.V., Fedorova, T.V., Kuznetsov, B.A., Koroleva, O.V. (2007). Characterization of plant phenolic compounds by cyclic voltammetry. Applied Biochemistry and Microbiology. 43: 661-668.

17. Zafrilla, P., Morillas, J., Mulero, J., Cayuela, J.M., Martinez- Cacha, A., Pardo, F., Lopez, J.M. (2003). Changes during storage in conventional and ecological wine: Phenolic content and antioxidant activity**.** Journal of Agriculture and Food Chemistry 51: 4694-4700.

18. Zielinska, D., Szawara- Nowak, D., Zielinski, H. (2007). Comparison of spectrophotometric and electrochemical methods for the evaluation of antioxidant capacity of buchwheat products after hydrothermal treatment. Journal of Agriculture and Food Chemistry 55: 6124-6131.