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Antioxidant action of Greek red wines correlated to early maturation / storage

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Abstract:*In this article the antioxidant activity of selected Greek red wines was investigated and correlated to early maturation / storage. For this purpose 12 single-variety Greek red wines, of the vintages of 1999, 2000, 2002, 2003, 2004 and 2005 were selected. Reactivity of wine samples against 1,1- diphenyl-2-dipicrylhydrazyl (DPPH) free radical were performed which showed a decrease (in the majority of the samples) in antioxidant activity caused by storage in barrels / maturation in glass bottles. This phenomenon may be connected with decrease in anthocyanines content as well as to reactions converting the low molecular to high molecular-weight phenolics.*

Keywords:*Greek red wines, phenolic compounds, antioxidant activity, free radical, bottle storage, maturation, DPPH.*

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I. INTRODUCTION

Wines, especially red ones are a rich source of antioxidants due to its high content of phenolic compounds. The latter have attracted great interest in recent years due to their potential beneficial effects on human health. Researches have shown that red wine consumption leads to prevention of pathologies associated witho xidative stress (Renaud et al. 1999). 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). The powerful antioxidant capacity of phenolic compounds is involved in the total phenomenons. We must mention that moderate wine consumption is most common in Mediterranean countries (e.g. Greece, South France, Italy, Spain) where a majority of the population follows the principles of the mediterranean diet (Trichopoulou et al. 1997).

The mediterranean diet is defined as a traditional eating pattern found among populations living in the Mediterranean Basin (countries of South Europe and North Africa) mainly during the 50s and 60s of the 20th century. The main characteristics are a low consumption of meat and meat products, with very low consumption of red meat. Red wine must be consumed in moderate amounts. It contains several bioactive polyphenols (e.g. hydroxytyrosol and tyrosol, oleocanthal, and resveratrol) with postulated anti-inflammatory properties (Martínez-González et al. 2019). Moderate alcohol consumption is defined as having up to 1 drink (a glass, 150 mL 12% v/v alcohol wine) per day for women and up to 2 drinks (two glasses, 300 mL 12% v/v alcohol wine) per day for men(Cassino et al. 2016).

Besides that wine aging is one of the key practices for improving the overall quality (mainly the organoleptic characteristics) of wines in order to increase their commercial value. This procedure can be performed in bottles or in wooden barrels. Unfortunately antioxidant properties of wines can be greatly affected during storage. According to literature, earlier studies show that there is conflicting evidence about the effects of aging on the antiradical activity of wines. Some researches show no relation (Zafrilla et al. 2003), others observe increase or decrease in phenolic compounds (Echeverry et al. 2005).

In this context the aim of our work was to show how maturation effects antioxidant action of Greek wines. More specifically the antiradical activity (A_{AR}) was studied. According to previous studies one of the most popular techniques for the evaluation of antioxidant properties of wines is the reducing ability against the stable 1,1- diphenyl-2-dipicrylhydrazyl (DPPH) free radical, which is considered as a rapid and general measure of free radical scavenging ability.

II. EXPERIMENTAL PROCEDURE

1.1 Samles

At first different brands of red Greek wines were purchased. Our basic aim was to collect wines from consecutive years which were stored for a period of 15 years in glass bottle sealed with cork stopper. We must

notice that the varieties we used cover some of the most important viticulture areas in Greece, are available in Greek market and they can be considered as representative of the most popular consumed wines in Greece.

More specifically samples were produced using grapes grown in different areas of Greece (with a variety of soils and climatic characteristics) and from different cultivars. In this way, the set of samples guarantees adequate heterogeneity of the agronomic and viticulture characteristics.

The main characteristics (samplename, year of production, geographical origination) of wine samples are described in the following table (1).

Table 1. Characteristics of wine samples

Sample name	Year of production			Geographical origination
Merlot	2004	2005	--	Thiva
Cabernet	2003	2004	2005	Thiva
Metoxi	2002	2003	2004	Thessaloniki
Wine of Paros	1999	2000	--	Paros
Wine of Nemea	1999	2000	--	Nemea

1.2 Measurement of the Antiradical Activity (A_{AR})

First of all we must mention that all samples were initial stored at refrigerator (temperature approximately 4 ± 1°C). Studies were carried out in room temperature (approximately 25 ± 1°C). For each investigated wine sample, 4 measurements of antioxidant activity were carried out in order to obtain means and standard deviations.

During the experimental procedure the antiradical activity (A_{AR}) in 12 Greek varietal red wines was evaluated using the DPPH free radical method. The DPPH assay is one of the most commonly employed methods for measuring antioxidant activity (Carmona et al. 2014). The antioxidant activity of the samples was measured according to the protocols described in previous studies (Bassil, et all. 2005), (Arnous et al. 2001).

According to protocol an aliquot of 0.025 ml of wine sample (diluted 1/10 in methanol CH_3OH immediately before the analysis) was added to 0.975 ml of DPPH solution (concentration 60 μM in methanol CH_3OH). The mixture was vortered and the absorbance was read at 515 nm at t=0 and t=30 minutes using a U-2000 Hitachi Spectrophotometer after the addition of DPPH. During the experiments (total time 30 minutes) we can observe a change in the color of the sample we investigate from purple to gentle yellow as shown in the next picture (1).

Picture 1. Wine sample before (right) and after (left) the reaction with DPPH

1.3 Expression of results

Results were expressed using the equation (1):

$$A_{AR} = 0{,}018 \text{ x } \%\Delta A_{515} + 0{,}017$$

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) :

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

Final results were expressed as Trolox equivalents (mM TRE)

III. RESULTS AND DISCUSSIONS

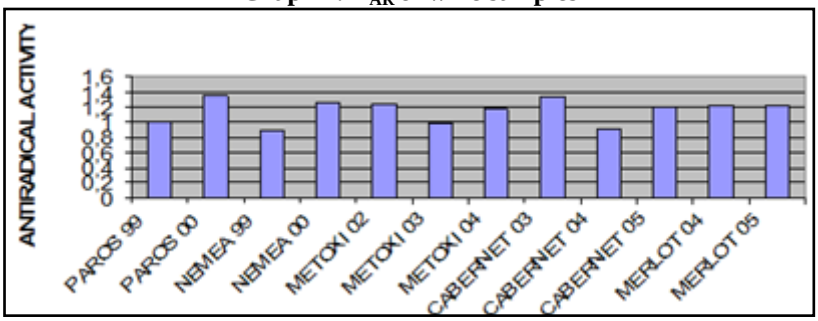
During the experiments changes in antiradical activity of wines during early maturation was measured (years 1999, 2000, 2002, 2003, 2004). Both a decrease and an increase in the antiradical activity (A_{AR}) of wines investigated were observed. We must mention that in the majority of thesampleswe observe reduction in antiradical action.

These changes probably could be correlated to variations in parameters of wine making techniques, different conditions during maturation in the barrel, different conditions during storage in bottles, or finally different conditions of fermentation of each wine sample. The results are shown in the following table (2) and graphs (1, 2).

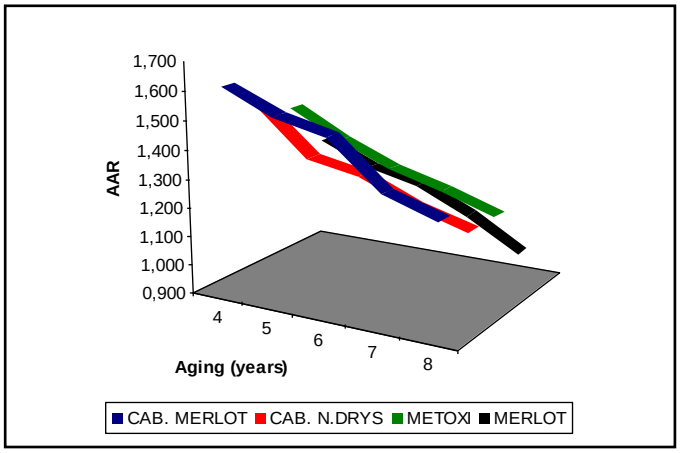
Table 2. Measurements of A_{AR} in wine samples

Sample name	Years of production	A_{AR}
MERLOT 04	2004	**1,228**
MERLOT 05	2005	**1,225**
CABERNET 03	2003	**1,330**
CABERNET 04	2004	**0,904**
CABERNET 05	2005	**1,208**
METOXI 02	2002	**1,242**
METOXI 03	2003	**0,994**
METOXI 04	2004	**1,181**
PAROS 99	1999	**1,011**
PAROS 00	2000	**1,344**
NEMEA 99	1999	**0,887**
NEMEA 00	2000	**1,248**

Graph 1. A_{AR} of wine samples



Graph 2. A_{AR} of wine samples in relation to time



IV. CONCLUSIONS

Aging leads to a moderate decrease (as observed in the majority of samples) in antioxidant activity of the investigated Greek red wines, which can be attributed to the reduction of their anthocyanines content and to possible reactions converting the low molecular to high molecular-weight phenolics. Indeed storage in glass bottles or wooden barrels improves the organoleptic characteristics of wines but also a decrease in their antioxidant action takes care also.

The decrease in antioxidant activity of wine samples as a function of storage has also been described in the literature (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 total structure of anthocyanin monomers (Burns et al. 2001), or even the decrease of their total phenolic content (Negi and Dey 2009). In addition substantial decrease of antioxidant activity can be attributed to the reaction of atmospheric oxygen, which remains in the bottle after it is sealed, with wines' phenolic content (Fulcrand et al. 2006).

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 while others found no correlation between storage and antioxidant activity.

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