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ANTIOXIDANT PROPERTIES OF SOME BULGARIAN RED WINES

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Abstract

A study on antioxidant capacity and content of polyphenols in some Bulgarian wines is carried out. The wines are selected from one vintage, but from different regions and different grape cultivars. Methods for determination of polyphenols and antioxidant capacity are presented and discussed with regard of their easy practical applications. The most reliable of them are applied to determine the total content of polyphenols and antioxidant capacity of all tested wines. The wines are evaluated according to the data obtained depending on region of origin and cultivar. The results are useful for searching correlation between the total concentration of phenols and the wine antioxidant capacity.

Key words: *polyphenols, antioxidant capacity, red wine*

1. INTRODUCTION

As it is known, some biochemical reactions in human body produce free radicals, which are unhealthy because of their high oxidizing power [1-3]. They provoke premature aging, many heart deceases, atherosclerosis, diabetes, some cancer deceases, etc. Concerning foods, the oxidation of lipids is responsible for formation of unpleasant flavors and undesirable chemical compounds, which worsen food quality and may be detrimental to human health. In order to hamper these unwanted processes, synthetic or natural antioxidant additives are used. However, many studies have demonstrated that the synthetic nutrition additives as BHA (ButylHydroxyAnisol), BHT (ButylHydroxyToluene), TBHQ (Tertiary-ButylHydroQuinone) have negative effects on the human organism [4,5,6]. For this reason, nowadays a large interest is paid to the natural antioxidants, in particular the polyphenolic compounds present in many botanicals, especially fruits and vegetables.

Wine, particularly the red cultivars, is considered to be one of the reachest sources of natural antioxidants - polyphenols [7,8,9], namely flavonoids (flavanols, antocyanines), phenolic acids (gallic, protocanthinic, caffeic, cyanidinic, ferrulic, etc.), procyanidins (B1, B2), phenolic alcohols, stilbens (trans- and cis-resverathrol), etc. [10,11]. In parallel to taste qualities, polyphenol concentration and antioxidant capacity (AOC) of a wine are regarded as objective quantity parameters. Although the existing correlation between them, it is considered that polyphenol content and AOC have to be reported as separate values [12,13,14].

In this study, appropriate methods for determination of total polyphenols and AOC are described and applied for determination of these characteristics of some of Bulgarian red wines, distributed throughout the domestic market.

2. GENERAL

2.1. Determination of total polyphenolic content

The phenolic profile of wines is dominated by the phenolic acids, mainly gallic acid (which has the most powerful antioxidant activity per unit mass) and phenol-containing flavonoids – catehins and procyanidins B1 and B2. Identification and determination of the individual phenols is made by HPLC technique. It is to account that the specific role of each component is not fully elucidated, and their antioxidant capacity is rather different. The concentration of a particular component strongly depends on climate, soil, grape variety and maturity, production technology, conditions of storage etc. A common example is the degradation of high-molecular polyphenols in wines kept at higher temperature.

It is considered that the total polyphenolic content is representative for general estimation of the antioxidant activity of a product. Because of the unknown mean molecular weight of a polyphenols mixture, their global action is expressed as Gallic Acid Equivalent (TPC_{GA}) or as Ascorbic Acid Equivalent (TPC_{AA}) if the latter more popular substance, vitamin C, is considered.

Due to its simplicity and feasibility, the photometric method of Folin-Ciocalteau is most often used for determination of total polyphenolic content. It is based on a color reaction between the sample and Folin-Ciocalteau reagent. This method, selected also in our study, is described in details further in the paper.

2.2. Determination of antioxidant capacity (AOC)

Unlike the case of polyphenols, a number of methods are used for determination of AOC [8,15]. Usually they are named after the name of the substance used as source of free radicals, for example: FRAP (Ferric reducing antioxidant power), ORAC (oxygen radical absorbance capacity), ABTS or Trolox (2,2-azinobis 3-ethyl-benzothyazoline 6-sulphonate), $DPPH^+$ (2,2-diphenyl-1-picrylhydrazyl). It is to mention that different methods give rather different results and should be applied preferentially for comparison of similar products.

$DPPH^+$ method is largely used because of its simplicity and stable results [4,15,]. The measurement is based on the sample decolorization resulting from neutralization of the nitrogen electron by the hydroxyl group hydrogen atom of the antioxidant substance. A shortcoming of this method is its light sensibility. For this reason, the reaction is carried out at dark and in non-alkaline medium.

An important application aspect of $DPPH^+$ method is the difference in the reaction rates for the individual polyphenolic species. Consequently, the retention times before the photometric measurement should be different. To avoid this time effect, various authors recommend applying retention times, varying from 10 to 120 minutes [5,16,17,18,19]. In case of lack of information for the reaction kinetics of individual phenols, we recommend the maximal retention time value of 2 hours, which will cover the majority of cases. Although the long period applied, the reaction might not be fully completed. For this reason, the parameter IC_{50} is used for comparison at equivalent conditions. It represents the quantity of an antioxidant, which inhibits 50% of the source of free radicals. The method is described in details below.

3. MATERIALS AND METHODS

Ten bottled Bulgarian red wines from the shelves of a large supermarket were tested. They were from different grape varieties cultivated in different regions of the country, all 2006 vintage: foreign cultivars Merlot and Cabernet Sauvignon and local cultivars Mavrud, Melnik, and Rubin. A list of these wines is given in Table 1.

The wines were analyzed immediately after bottle opening, then they were conserved in the original dark colored bottles at temperature 4°C for consequent analyses.

Folin-Ciocalteau (FC) reagent (2N solution) is from SIGMA®.

Gallic acid (GA) solution: It was prepared in 100 ml volumetric flask by dissolving 0.5 g GA (p.a. SIGMA®) in 10 ml ethanol (96 % p.a. VALERUS®). The total volume was brought to 100 ml by adding distilled water.

Table 1. List of tested wines

Cultivar	Region	Sample code
Merlot	South-East Thracian Plane	Mer1
Merlot	North-East Danubean Plane	Mer2
Merlot	North-East Thracian Plane	Mer3
Merlot	West Thracian Plane	Mer4
Cabernet Sauvignon	South-East Thracian Plane	Cab1
Cabernet Sauvignon	North-East Danubean Plane	Cab2
Mavrud	East Rhodopean Region	Mav1
Mavrud	North-East Thracian Plane	Mav2
Melnik	South-West Bulgaria	Mel
Rubin	East Rhodopean Region	Rub

Na_2CO_3 solution: 200 g dehydrated Na_2CO_3 (p.a. VALERUS®) was added to 800 ml distilled water and then boiled. After cooling to room temperature, some crystals from the salt were added. 24 hours later the saturated solution was filtered and the total volume was brought to 1 l by adding distilled water.

The calibration curve (GA concentration - light absorption A) was obtained by measurement of samples with different concentration of GA in distilled water (0.1, 0.2, 0.5, 0.75, 1 g/l). 20□□l from each sample were diluted with 1.58 ml water. Then 100 □l FC reagent was added and shaken well. After some rest (from 30 seconds to 8 minutes) 300 □l Na_2CO_3 solution was added and shaken well. The samples were kept in dark for 2 hours at room temperature, and then they were analyzed with double-beam UV-VIS-spectrophotometer (UNICAM®-Helios β) in order to measure the light absorption at 765 nm. The reference cuvette contained all components except GA solution. So, the dependence A = f (GA concentration) was derived and used to determine GA concentration or total phenol concentration as TPC_{GA}) corresponding to a measured light absorption A.

Wine samples were prepared similarly as above. Wine was diluted 10 or 20 times with 13 % ethanol and 20 □l diluted wine sample was added to the cuvette instead of GA solution. Light absorption was measured at 765 nm and the total polyphenolic concentration was determined from the calibration line $C_{GA} = f(A)$.

The antioxidant capacity (AOC) of tested wines was determined photometrically using the $DPPH^+$ method. 1 ml diluted wine sample was added to 4 ml 0.004 M solution of $DPPH^+$ (SIGMA®) in

methanol (4.73 mg/100 ml). After 120 min solution retention in the dark, the light absorption was measured in 1 cm standard cuvette at 517 nm. The reference liquid was methanol. The free radical inhibition (scavenging) was calculated by the expression

$$IC\ [\%] = (A_o - A_s)/A_o \times 100,$$

where A_o is absorption of the reference liquid not containing wine, A_s is absorption of the wine containing sample.

As mentioned above, the IC_{50} is a parameter representing the wine concentration, able to inhibit 50 % of the used $DPPH^+$ amount. It was determined by drawing a graph with sample concentration C_w (l wine/l sample or ml/ml) on the abscissa and free radical inhibition capacity IC [%] as ordinate. A series of samples was prepared as already described. The initial wine sample was diluted in a manner to obtain a linear graph which lies in the zone of 0 to over 50 % radical scavenging capacity. The wine concentration which reduces 50% of free radicals can be calculated by using graph equation or simply determined from the graph. Each sample was analyzed at least three times, and a mean value was calculated.

This method can be applied for relative comparison of AOC of different wines only. In order to characterize more objectively the AOC, the same procedure was applied to a popular and stable antioxidant – ascorbic acid (AA) - vitamin C, and its IC_{50} value was determined. Comparing wine IC_{50} and the same parameter obtained at the same conditions, for the ascorbic acid, the quantity of this acid equivalent to one litter of wine or $AOCE_{AA}$ in g/l can be evaluated.

4. RESULTS AND DISCUSSION

Table 2 represents the total polyphenolic content, expressed as gallic acid equivalent (TPC_{GA}) and $AOCE_{AA}$ for all tested wines.

Table 2. Total polyphenolic content and antioxidant capacity of tested wines

Sample code	Region	TPC_{GA} [g/l]	$AOCE_{AA}$ [g/l]
Mer1	South-East Thracian Plane	2.45 ± 0.02	3.12
Mer2	North-East Danubean Plane	2.55 ± 0.01	2.89
Mer3	North-East Thracian Plane	2.28 ± 0.31	2,40
Mer4	West Thracian Plane	1.80 ± 0.01	2.34
Cab1	South-East Thracian Plane	2.66 ± 0.02	2.80
Cab2	North-East Danubean Plane	2.14 ± 0.06	2.65
Mav1	East Rhodopean Region	2.09 ± 0.06	1.55
Mav2	North-East Thracian Plane	2.12 ± 0.02	1.61
Mel	South-West Bulgaria	1.70 ± 0.11	2.00
Rub	East Rhodopean Region	2.16 ± 0.03	2.37

The data presented in Table 2 show that all tested wines contain between 1.7 and 2.7 g/l polyphenols expressed as gallic acid. Merlot and Cabernet cultivats show higher phenolic concentrations (2,2 – 2.7 g/l excluding Mer4) than other grape cultivars. The cultivars might be arranged in descending order as follows: Merlot, Cabernet, Mavrud, Rubin, Melnik.

Considering the arrangement by regions, it should be mentioned that eastern region wines contain more polyphenols than these from western regions; south-east regions are richer than north-east regions, and the south-west region takes last place in this classification. It should be kept in mind that the polyphenol content depends on many factors and may vary significantly from year to year. The above speculations, however, are relevant to vintage 2006 only, and for the limited number of studied cases. Therefore it should be regarded as informative but not at all definitive.

Considering the second wine characteristic studied in this investigation, the antioxidant capacity, one should start with the credibility of the method applied. It was mentioned above, that the parameter IC50 was used instead of the simple inhibition capacity (IC) in order to avoid an indefinite long period for completing the radical scavenging process. Fig. 1 illustrates this phenomenon in case of wine samples Mer1, Mer3 and Mav1. Due to the numerous parallel chemical reactions of various polyphenols presented in the wine, even after 120 min the overall equilibrium was not reached.

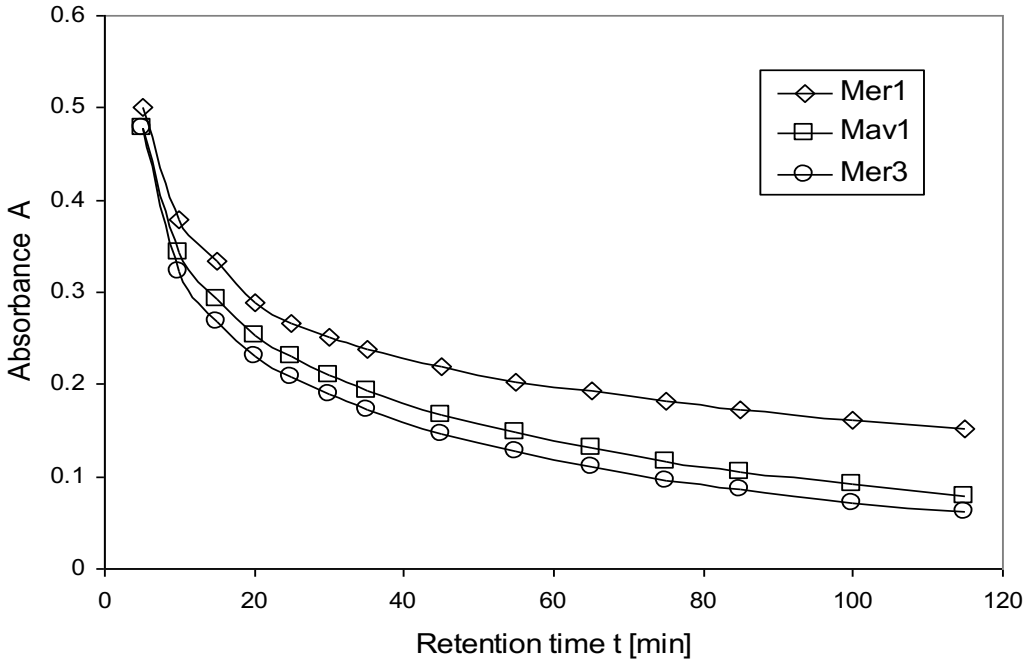


Fig.1. Kinetic curves representing the radical scavenging reactions in the course of time between the polyphenols presented in three of the tested wines and the source of free radicals - the dyestuff $DPPH^{+}$.

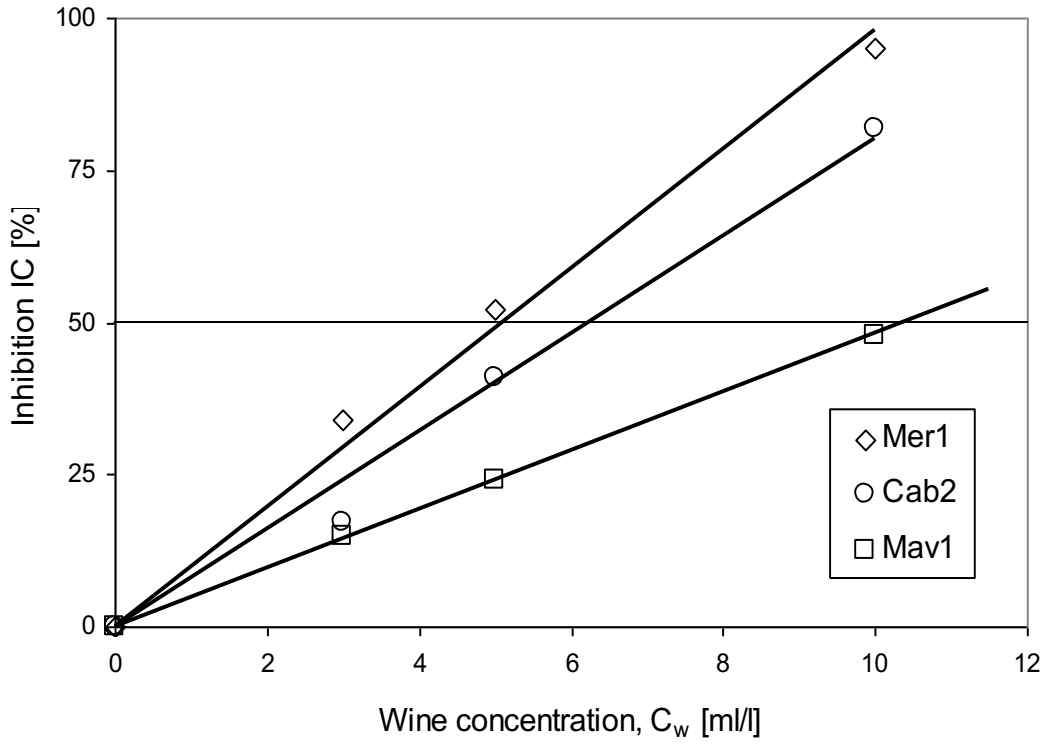


Fig.2. Graphical construction for determination of IC50 values of three tested wines. Wine samples dilution ratios (13% ethanol in water /wine) are 100:1, 200:1 and 330:1.

IC50 values of the wines were compared to IC50 value of a "standard" antioxidant, in this case ascorbic acid, obtained by the same procedure. The ratios $(IC50)_{AA}/(IC50)_{wine}$ are given in Table 2. They represent the ascorbic acid equivalent of the wine antioxidant capacity ($AOCE_{AA}$), i.e. the amount of ascorbic acid in grams equivalent to AOC of one liter wine.

Fig.3 represents a comparison between the content of polyphenols and antioxidant capacity. It is seen that in general there is a pronounced correlation between these two parameters – the higher the polyphenols concentration, the higher the wine antioxidant capacity. Moreover, the concentrations of polyphenols (TPC_{GA}) in gram per liter and the values of AOC in ascorbic acid equivalents ($AOCE_{AA}$) in gram per liter are rather close, the $AOCE_{AA}$ values being up to 20 % higher than the TPC_{GE} values. To avoid any confusion about this apparent coincidence it should be mentioned that AOC of gallic acid is 2.5 times higher than the AOC of ascorbic acid per unit weight. The samples of Mavrud cultivar demonstrate specific behavior showing about 25% lower AOC with respect to their polyphenolic content. This deviation from all other wine cultivars is probably due to different types of polyphenols, presented in this local cultivar.

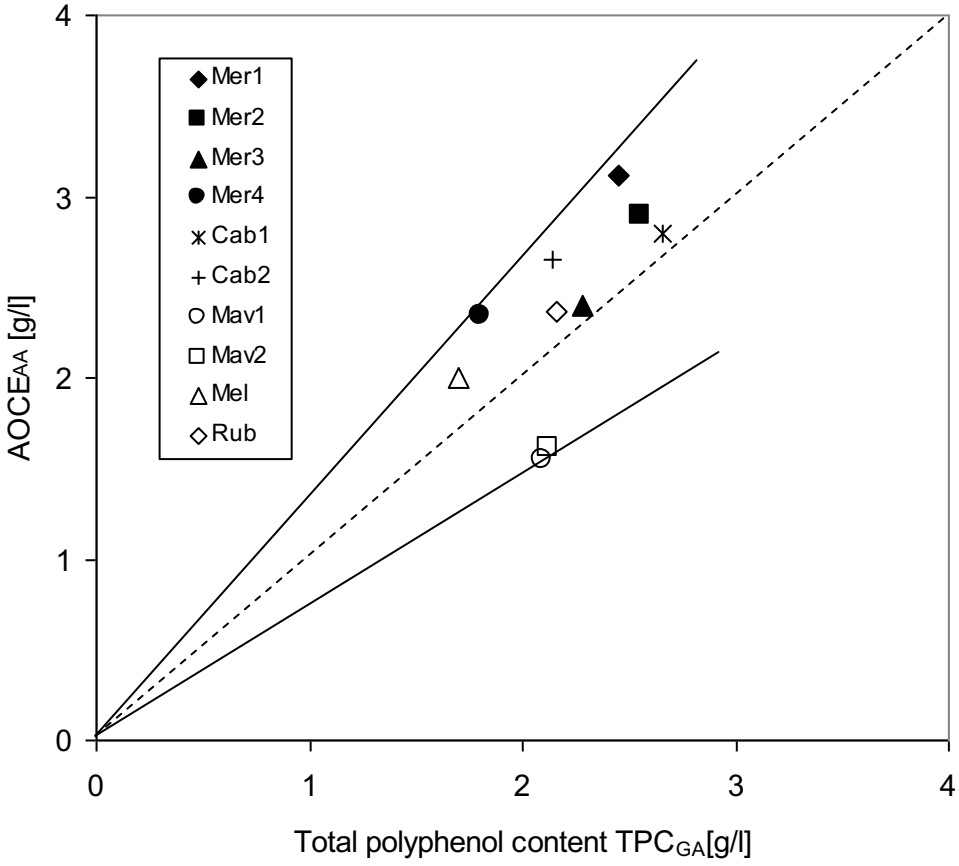


Fig. 3. Comparison of total polyphenol content (TPC_{GA} in g/l) of different wines to their antioxidant capacity ($AOCE_{AA}$) in g/l)

5. CONCLUSION

Ten Bulgarian red wines of five cultivars namely Merlot, Cabernet sauvignon, Mavrud, Rubin and Melnik from different country regions were studied in order to determine their total amount of polyphenols expressed as gallic acid concentrations, and their antioxidant capacity, expressed as vitamin C equivalents. It was found that Merlot, Cabernet and Rubin show higher values of these characteristics. Generally, (TPC_{GA}) values for the local cultivars Melnik and Mavrud are lower than these for the other tested cultivars.

It was shown that $DPPH^+$ method produces stable and reliable results at the condition to avoid the interference of reaction color with wine color by appropriate sample dilution. Also, the reaction time before light absorption measurement should be adequate. A procedure for antioxidant capacity objectification, making the results more comprehensible and comparable to other drinks and foods is proposed by introducing $AOCE_{AA}$ value, using as a standard the well known antioxidant - vitamin C.

Both methods, for determination of total polyphenols and antioxidant capacity, may be applied for analyses of other nutritional products.

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