NEW SPECTROPHOTOMETRIC METHOD FOR WINE QUALITY CONTROL

KONTROLA KVALITETA VINA PRIMENOM NOVE SPEKTROFOTOMETRIJSKE METODE

Blaga RADOVANOVIC*, Jorgovanka BOJIĆ**

*University of Niš, Faculty of Sciences and Mathematics, Department of Chemistry, Višegradska 33, 18000 Niš, Serbia
**High Polytechnic School, Kosančićeva 36, 37000 Kruševac, Serbia
e-mail: blaga_radowanovic@yahoo.uk.com

ABSTRACT

This work presents a development of a rapid, relatively sensitive, and low-cost spectrophotometric method enabling the determination of the antioxidant activity of wine. The method is based on the wine inhibition effect and the reaction between hydrochloric acid, bromate and methyl orange. The proposed method involves the addition of a known amount of bromate and methyl orange to a sample in an acid medium, and the measurement of absorbance at the wavelength of 505 nm, 5 minutes after the addition of the last drop of the bromate solution. The presence of wine in the medium causes a slower reaction. The reliability of the new assay was established by parallel determination by the reference DPPH method and no significant difference between the proposed and the standard method was noticed.

Key words: wine, antioxidant activity, bromate, methyl orange.

INTRODUCTION

Phenolic compounds are a group of biologically active compounds, which are involved in many metabolic routes of plants. This is a heterogeneous group including catechins, anthocyanidins, tannins, flavonones, flavones, flavonols and hydroxybenzoic and hydroxycinnamic acids, among others. These compounds present antioxidant properties which are thought to reduce the risks of coronary or cancer diseases, thus having a direct influence on human health. Phenolics may be present in different products of plant origin, such as fruit juices, olive oil and red or white wine. They play a key role as antioxidants due to the presence of hydroxyl substituents and their aromatic structure, which enables them to scavenge free radicals (Villano et al., 2007).

Flavonoids, as well as other phenols, and related compounds are also found in finished products, such as wine or beer. They are in part responsible for the colour and fragrance, and to some extent, taste and quality of the wine. The composition and amount of phenolic compounds depend on the sample, on the origin of raw material, elaboration processes and storage conditions. Hence, these compounds are significant to wine production. In addition to polyphenols, grapes also contain polyphenoloxidases, which catalyze the oxidation of polyphenols to quinones (Harkensee et al., 2006).

With increasing interest in the function and diversity of antioxidants in foods, several in vitro methods for measuring antioxidant activity of food, beverages and biological samples have been developed. The most commonly used antioxidant capacity assays include oxygen radical absorbance capacity (ORAC assay), reducing power, determination of total phenols, 2,2-azinodi-(3-ethylbenzthiazoline-sulphonic acid) (ABTS assay), 2,2-diphenyl-1-picrylhydrazil (DPPH assay), hydroxyl radical scavenger activity, superoxide radical scavenger activity and lipid peroxidation inhibition. These methods differ in terms of their assay principles and experimental conditions. Because multiple reaction characteristics and mechanisms are usually involved, no single assay will accurately reflect all antioxidants in a mixed or complex system (Li et al., 2009). Some of these methods are time-consuming and suffer from the lack of selectivity and short linear dynamic range. They involve long pretreatment steps to remove interfering species, require complicated and expensive instruments, and use reagents that are not commercially available.

*University of Niš, Faculty of Sciences and Mathematics, Department of Chemistry, Višegradska 33, 18000 Niš, Serbia
**High Polytechnic School, Kosančićeva 36, 37000 Kruševac, Serbia

e-mail: blaga_radowanovic@yahoo.uk.com

ABSTRACT

This work presents a development of a rapid, relatively sensitive, and low-cost spectrophotometric method enabling the determination of the antioxidant activity of wine. The method is based on the wine inhibition effect and the reaction between hydrochloric acid, bromate and methyl orange. The proposed method involves the addition of a known amount of bromate and methyl orange to a sample in an acid medium, and the measurement of absorbance at the wavelength of 505 nm, 5 minutes after the addition of the last drop of the bromate solution. The presence of wine in the medium causes a slower reaction. The reliability of the new assay was established by parallel determination by the reference DPPH method and no significant difference between the proposed and the standard method was noticed.

Key words: wine, antioxidant activity, bromate, methyl orange.

INTRODUCTION

Phenolic compounds are a group of biologically active compounds, which are involved in many metabolic routes of plants. This is a heterogeneous group including catechins, anthocyanidins, tannins, flavonones, flavones, flavonols and hydroxybenzoic and hydroxycinnamic acids, among others. These compounds present antioxidant properties which are thought to reduce the risks of coronary or cancer diseases, thus having a direct influence on human health. Phenolics may be present in different products of plant origin, such as fruit juices, olive oil and red or white wine. They play a key role as antioxidants due to the presence of hydroxyl substituents and their aromatic structure, which enables them to scavenge free radicals (Villano et al., 2007).

Flavonoids, as well as other phenols, and related compounds are also found in finished products, such as wine or beer. They are in part responsible for the colour and fragrance, and to some extent, taste and quality of the wine. The composition and amount of phenolic compounds depend on the sample, on the origin of raw material, elaboration processes and storage conditions. Hence, these compounds are significant to wine production. In addition to polyphenols, grapes also contain polyphenoloxidases, which catalyze the oxidation of polyphenols to quinones (Harkensee et al., 2006).

With increasing interest in the function and diversity of antioxidants in foods, several in vitro methods for measuring antioxidant activity of food, beverages and biological samples have been developed. The most commonly used antioxidant capacity assays include oxygen radical absorbance capacity (ORAC assay), reducing power, determination of total phenols, 2,2-azinodi-(3-ethylbenzthiazoline-sulphonic acid) (ABTS assay), 2,2-diphenyl-1-picrylhydrazil (DPPH assay), hydroxyl radical scavenger activity, superoxide radical scavenger activity and lipid peroxidation inhibition. These methods differ in terms of their assay principles and experimental conditions. Because multiple reaction characteristics and mechanisms are usually involved, no single assay will accurately reflect all antioxidants in a mixed or complex system (Li et al., 2009). Some of these methods are time-consuming and suffer from the lack of selectivity and short linear dynamic range. They involve long pretreatment steps to remove interfering species, require complicated and expensive instruments, and use reagents that are not commercially available.
Methyl orange, such as many acid dyes, are prone to oxidation, forming colourless products in an acid medium and providing a suitable analytical approach for the indirect assay of inorganic ions (Ensafi et al. 2002), organic compounds (Basavaiah et al. 2005), and pharmaceuticals (Basavaiah et al. 2006). The produced bromide and chlorine react with methyl orange and this reaction causes decolourization of the solution. However, no bromate–hydrochloric acid reaction has been developed for the determination of antioxidant activity of wine.

This paper describes a sensitive, simple, low-cost, and fast (requiring only 10 min) method for determination of antioxidant activity of wine based on the reaction of bromate with hydrochloric acid.

The method employed is based on a reaction between bromate and chloride ions in highly acidic media. Bromate can be reduced by hydrochloric acid, producing bromine and chlorine:

$$10 \text{Cl}^- + 2 \text{BrO}_3^- + 12 \text{H}^+ \rightarrow 5 \text{Cl}_2 + \text{Br}_2 + 6 \text{H}_2\text{O} \quad (1)$$

Decolourization of methyl orange by the reaction products was used to monitor the reaction spectrophotometrically at 505 nm.

**MATERIAL AND METHOD**

All chemicals and reagents were of analytical grade and were obtained from Sigma Chemical Co. (St. Louis, MO, USA), and Merck (Darmstadt, Germany).

A 0.01 mol l$^{-1}$ potassium bromate solution was prepared by dissolving KBrO$_3$ in water in a volumetric flask. A solution of methyl orange in water. Hydrochloric acid 2.33 mol l$^{-1}$ was prepared by dissolving hydrochloric acid, producing bromine and chlorine: 10 Cl$^- + 2$ BrO$_3^- + 12$ H$^+$ $\rightarrow$ 5 Cl$_2$ + Br$_2$ + 6 H$_2$O (1)

Decolourization of methyl orange by the reaction products was used to monitor the reaction spectrophotometrically at 505 nm.

All the solutions were kept at 20ºC. All experiments were carried out in triplicate for the reproducibility of results.

The reliability of the new assay was established by parallel determination by the reference DPPH method. The hydrogen atom or electron donation abilities of the corresponding wine were measured from the bleaching of the purple-coloured methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). This spectrophotometric assay uses the stable radical, DPPH as a reagent. One thousand microlitre of diluted wine were added to 4 ml of 0.004% methanol solution of DPPH. After a 15 min incubation period at room temperature, the absorbance was read against a blank at 515 nm. Absorbance of free radical by DPPH in percent (I%) was calculated in following way:

$$I\% = (1 - A_{\text{sample}}/A_{\text{blank}}) \times 100$$

where $A_{\text{blank}}$ is the absorbance of the control reaction (containing all reagents except the wine), and $A_{\text{sample}}$ is the absorbance of the tested wine (Villano et al., 2007). Tests were carried out in triplicate.

**RESULTS AND DISCUSSION**

The electronic absorption spectrum of methyl orange aqueous solution is shown in Fig 1: before (curve 1) and after the addition of hydrochloric acid (curve 2), after the addition of bromate (curve 4) and after addition of bromate and wine (curve 3).

It has been noticed that the absorption spectrum of methyl orange in water at pH = 5.9 (natural) is characterized by one band in the visible region, with maxima located at 464 nm, and by two bands in the ultraviolet region, located at 271 and 199 nm. The chromatophore containing azo linkage has absorption in the visible region, while the benzene ring and the naphthalene ring have absorptions in the UV region. The naphthalene ring absorption wavelength is higher than that of benzene ring. The spectrum recorded after addition HCl in aqueous solution of methyl orange, at pH = 0.6, was characterized by four bands at 505, 312, 272 and 205 nm. The same bands are present in the spectrum after addition of bromate and after addition bromate and wine in acid solution of methyl orange.

The inhibited reaction was monitored spectrophotometrically by observing the change in the absorbance of the reagents solution at 505 nm. An aliquot of the diluted wine was transferred into a 10-ml volumetric flask, and then 1 ml of 2.33 M hydrochloric acid was added, followed by a 1.0 ml methyl orange solution. The solution was diluted to ca. 5 ml with water. Then 1.0 ml bromate was added to the solution and the resulting solution was diluted to the mark with water. The solution was mixed and a portion of the solution was transferred to the spectrophotometric cell. The change in the absorbance with time was measured for 1–15 min from the initiation of addition of the last drop of the bromate solution.

Inhibition of methyl orange degradation in percent (I%) was calculated as follows:

$$I\% = (A_{\text{sample}} 5\text{ min}/\text{Im}_{\text{sample}} - A_{\text{blank}} 5\text{ min}/\text{Im}_{\text{blank}}) \times 100$$

where $A_{\text{blank}}$ 5 min is the absorbance of the control reaction (containing all reagents except the wine) 5 min after addition of the last drop of the bromate solution, $\text{Im}_{\text{blank}}$ is intercept from regression equation of the control reaction, $A_{\text{sample}}$ is the absorbance of the system with tested wine 5 min after addition of the last drop of the bromate solution, and $\text{Im}_{\text{sample}}$ is intercept from regression equation of the system with wine.

All the solutions were kept at 20ºC. All experiments were carried out in triplicate for the reproducibility of results.
Radovanović and Bojić, Jorgovanka / New Spectrophotometric Method for Wine Quality Control

The one at 505 nm, the band in the visible area is chosen for the spectrophotometric monitoring of the reaction.

![Fig. 2. Absorbance change of methyl orange–bromate–HCl–wine system: (a) blank reactions, (b) rose wine Rose, (c) red wine Vranac. Conditions: 6 × 10⁻⁴ mol dm⁻³ methyl orange, 1 × 10⁻⁴ mol dm⁻³ KBrO₃, 0.23 mol dm⁻³ HCl.](image)

The presence of wine in the medium causes a slower reaction which, in the absence of wine, is fairly fast. The inhibition effect of wine is due to its reaction with produced bromine and chlorine. This inhibitory effect on the reaction system depends on the wine sample (Fig 2). The higher antioxidant activity of wine, the more slowly the decolourization reaction proceeds.

Antioxidant activity results of wines determined by DPPH and MO methods under study. As expected, the red wines had significantly higher antioxidant activity compared to rose wine. This is due to a greater grape skin and seed contact time and temperature for the fermentation process for red wines. The percentage inhibition for red wine Vranac (diluted with water 1:10, v/v) was 61.8% for DPPH and 59.41% for MO assay, when the inhibition for diluted rose wine Rose, was 29.35% (DPPH assay) and 25.15% (MO assay).

As it can be observed, red wine values are higher than those of rose wine in both antioxidant test used. The compatibility of these two methods indicates successful applicability of the proposed method for the determination of antioxidant activity of wine samples.

**CONCLUSION**

It has been verified that the red wines have higher phenolic content levels than rose wines and the same result is obtained for antioxidant activity. The amounts of phenolic materials and antioxidant activity vary considerably in different types of wines, depending on the grape variety, environmental factors in the vineyard and the wine processing techniques. The compatibility between the results of DPPH assay and MO assay indicates successful applicability of the proposed method for the determination of antioxidant activity of wine.

**ACKNOWLEDGMENT:** The research was supported by the European Union, FP7 – REGPOT– 2007–3–01, KBBE: Food, Agriculture, and Biotechnology, Project «CHROMLAB-ANTIOXIDANT», No. 204756.

**REFERENCES**


Received: 30.04.2010. Accepted: 21.08.2010.