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NEW SPECTROPHOTOMETRIC METHOD FOR WINE QUALITY CONTROL

KONTROLA KVALITETA VINA PRIMENOM NOVE SPEKTROFOTOMETRIJSKE METODE

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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.*

REZIME

Danas je poznato da korišćenje namirnica bogatih antioksidantima u ishrani može imati pozitivan efekat na zdravlje. Antioksidansi, kao što su polifenoli, tokoferoli, askorbinska kiselina i karotenoidi, reaguju sa slobodnim radikalima, sprečavajući oksidaciju lipida, lipoproteina i nukleinskih kiselina, tako da pokazuju antimutogeno i antikancerogeno dejstvo. Najveći doprinos ukupnom antioksidativnom kapacitetu voća, povrća, voćnih sokova i vina daju polifenoli. U ovom radu je predložena nova, brza, osetljiva i jevtina spektrofotometrijska metoda za određivanje antioksidativne aktivnosti uzoraka vina. Predložena spektrofotometrijska metoda je zasnovana na reakciji kalijum-bromat – hloridna kiselina – metiloranž. Reakcija obezbojavanja metiloranža u ovom sistemu je veoma brza i praćena je merenjem apsorbance na talasnoj dužini od 505 nm na svakih 30 sekundi tokom 10 minuta. Zapaženo je da prisustvo fenolnih jedinjenja, poput galne kiseline, znatno usporava reakciju razgradnje metil oranža. Smanjenje brzine indikatorske reakcije je utoliko izraženije, ukoliko je veća količina fenola u reakcionom sistemu. Eksperimentalni rezultati pokazuju da sistem kalijum-bromat – hloridna kiselina – metil oranž može se primeniti ne samo na model-sisteme, već i za određivanje antioksidativne aktivnosti realnih uzorak vina. Jedini aparat potreban za primenu ove metode je spektrofotometar, dok je dejonizovana voda jedini korišćeni rastvarač. Rezultati dobijeni predloženom metodom upoređeni su sa rezultatima dobijenim primenom α,α-difenil-β-pikrilhidrazil (DPPH) radikala za određivanje antioksidativne aktivnosti vina i pri tome nije uočena značajna razlika između ova dva niza rezultata.

***Ključne reči:** vino, antioksidativna aktivnost, bromat, metil oranž.*

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, as they can be used to control the quality of red and white wine and to determine the varietal origin by quantitative analysis of the flavonoid content (*Rodriguez-Diaz et al., 2006*). Very high amounts of polyphenols in fruits can have negative effects on the quality of grape juice and wine. Autoxidation of polyphenols to yellow- or brown-colour quinones leads to a reduction in quality. Furthermore, polyphenols can react with proteins, carbohydrates and minerals. This also leads to a decrease in quality. 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-ethylbenzothialozine-sulphonic acid) (ABTS assay), 2,2-diphenyl-1-picrylhydrazyl (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 at al. 2005*), and pharmaceuticals (*Basavaiah et al., 2006*). The produced bromine 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:

$$10\ Cl^- + 2\ BrO_3^- + 12\ H^+ \rightarrow 5\ Cl_2 + Br_2 + 6\ H_2O \qquad (1)$$

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 6×10^{-4} mol l^{-1} was prepared by dissolving methyl orange in water. Hydrochloric acid 2.33 mol l^{-1} was prepared by dilution of concentrated hydrochloric acid with distilled water.

Two selected wines, red wine Vranac (Rubin, Kruševac), and rose wine Rose (Rubin, Kruševac) from different grape cultivars grown in Serbia were analyzed.

UV/Vis spectra were recorded between 300 and 600 nm, employing a UV-1650PC Shimadzu UV/Vis spectrophotometer equipped with two matched 10 mm quartz cuvettes.

A Radiometer PHM 29 b pH-meter and a combined glass–calomel electrode, GK 2311C, were used to determine the pH-values of the solutions.

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:

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

where $A_{blank\ 5\ min}$ 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, Int_{blank} is intercept from regression equation of the control reaction, A_{sample} is the absorbance of the system with tested wine 5 min after addition of the last drop of the bromate solution, and Int_{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.

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. Inhibition of free radical by DPPH in percent (I%) was calculated in following way:

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

where A_{blank} is the absorbance of the control reaction (containing all reagents except the wine), and A_{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 chromophore 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.

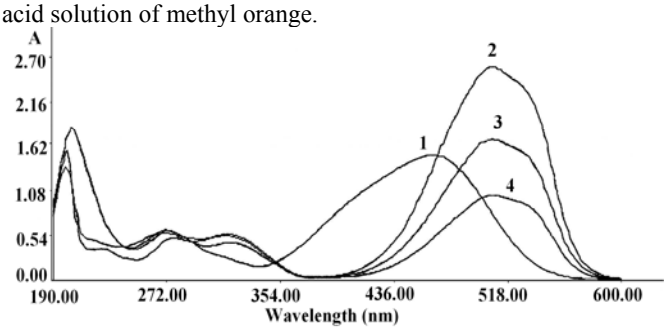

Fig. 1. Absorption spectra of methyl orange aqueous solution: on natural pH (pH = 5.9) (curve 1); in acid solution (pH = 0.6) (curve 2); in acid solution, 5 min after addition of potassium bromate (curve 4); in acid solution in presence of wine, 5 min after addition of potassium bromate (curve 3). Conditions: 6×10^{-4} mol dm^{-3} methyl orange, 1×10^{-4} mol $KBrO_3$, 0.23 mol dm^{-3} HCl. Reference, water

The absorbance changes of methyl orange at 505 nm as functions of reaction time in aqueous solutions, in the presence of wine, bromate ions at concentrations of 1×10^{-4} and 0.23 mol l^{-1} hydrochloric acid, are shown in Fig. 2. The decrease of absorption band at 505 nm during the reaction indicates a rapid degradation of methyl orange. The decrease is also meaningful with respect to the nitrogen double bond of the azo dye, as the most active site for oxidative attack. The complete discoloration of solution was observed after 10 min. At the same time, a mild increase in the absorbance at 312 nm is observed. As the change in the absorbance at 312 nm is considerably less significant than

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

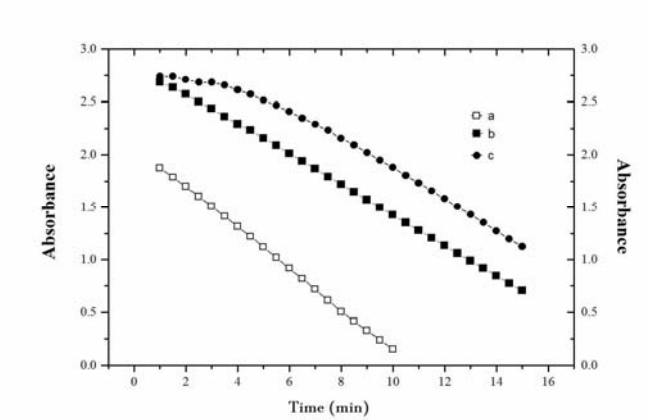

Fig. 2. Absorbance change of methyl orange–bromate–HCl−wine system: (a) blank reactions, (b) rose wine Rose, (c) red wine Vranac. Conditions: 6 × 10^{-4} mol dm^{-3} methyl orange, 1 × 10^{-4} mol dm^{-3} $KBrO_3$, 0.23 mol dm^{-3} HCl

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.

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REFERENCES

Basavaiah K., Chandrashekar U., Nage Govda P. (2005). Titrimetric and spectrophotometric assay of felodipine in tablets using bromate–bromide, methyl orange and indigo carmine reagents. Journal of the Serbian Chemical Society, 70, 969−978.

Basavaiah K., Chandrashekar U., Nage Govda P. (2006). Titrimetric, spectrophotometric and kinetic methods for the assay of atenolol using bromate–bromide and methyl orange. Journal of the Serbian Chemical Society, 71, 553−563.

Ensafi A. A., Rezaei B., Movahedinia H. (2002). Kinetic–spectrophotometric determination of ascorbic acid by inhibition of the hydrochloric acid–bromate reaction. Spectrochimica Acta Part A, 58, 2589–2594.

Harkensee, Daniela, Beutel, S., Young, M., Ulber, R. (2006). Development of a fast spectroscopic enzyme assay for on-site measurement of total polyphenol content in grapes and wine. Analytical Bioanalytical Chemistry, 384, 1013–1018.

Li, H., Wang, X., Li, Y., Li, P., Wang, H. (2009). Polyphenolic compounds and antioxidant properties of selected China wines. Food Chemistry, 112, 454–460.

Rodriguez-Diaz, R.C., Aguilar-Caballos, Maria Paz, Gomez-Hens, Agustina (2006). Determination of some hydroxybenzoic acids and catechins in white wine samples by liquid chromatography with luminescence detection, Journal for Separation Sciences, 29, 2772 – 2779.

Villano, D., Fernandez-Pachon, M.S., Moya, M.L., Troncoso, A.M., Garcıa-Parrilla, M.C. (2007). Radical scavenging ability of polyphenolic compounds towards DPPH free radical. Talanta, 71, 230–235.

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