DETERMINATION OF RED WINES TOTAL ANTIOXIDANT CAPACITY BY ELECTROMETRIC AND SPECTROPHOTOMETRIC METHODS

DETERMINAREA CAPACITĂȚII ANTIOXIDANTE TOTALE A UNOR VINURI ROŞII PRIN METODE ELECTROMETRICE ŞI SPECTROFOTOMETRICE

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Abstract. *Phenolic compounds from various food matrices can be investigated with different analytical techniques such as spectrophotometric, cromatographic or electrochemic methods. Cupric Reducing Antioxidant Capacity method (CUPRAC) is a convenient assay for determining total antioxidant capacity in food substrates. In this work, electrochemical and spectrophotometrical approaches were used to estimate the total antioxidant capacity by means of an alternative of the CUPRAC assay, as the concentrations of the participating species ($[Cu(Nc)_2]^{2+}$ and $[Cu(Nc)_2]^{1+}$) can be evaluated by electrochemical and spectrophotometrical methods. The electrometric–CUPRAC methods have been applied to measure total antioxidant capacity of different local wines and validated against the spectrophotometric CUPRAC assay. The electrochemical methods proved to be easy, fast and with low detection limits. A good correlation for the total antioxidant capacity determined by CUPRAC spectrofotometric and electrometric method versus total phenol content (determined by Folin-Ciocalteu Method) was recorded, wich highlights that wines antioxidant activity results mainly from their phenolics content.*
Key words: *wines, antioxidant capacity, electrometric methods, spectrophotometric methods*

Rezumat. *Compuşii fenolici din diverse substraturi alimentare pot fi investigați prin tehnici analitice variate. Metoda CUPRAC reprezintă a tehnică analitică convenabilă pentru determinarea capacității antioxidante totale a substraturilor alimentare. În această lucrare tehnicile electrochimice şi spectrofotometrice utilizate au folosit o variantă a metodei CUPRAC, pentru determinarea concentrației speciilor participante ($[Cu(Nc)_2]^{2+}$ şi $[Cu(Nc)_2]^{1+}$) din vinurile locale. Metodele electrochimice s-au dovedit a fi precise, simple, rapide şi reproductibile. Corelația buna între capacitatea antioxidantă totală (masurată electrochimic şi spectrofotometric) şi conținutul de polifenoli totali (măsurat prin metoda Folin-Ciocalteu) a evidențiat rolul predominant al acestora în determinarea activității antioxidante totale a vinurilor.*
Cuvinte cheie: *vinuri, capacitatea antioxidantă, metode electrometrice, metode spectrofotometrice*

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INTRODUCTION

Phenolic compounds play one of the most important roles in the quality of wines, as they strongly contribute to the color, mouth feel and palatability of red wines. Phenolic compounds can be classified into phenolic acids (including hydroxybenzoic acids and hydroxycinnamic acids), flavonols, anthocyanins and stilbene derivatives. Phenolic acids are considered to possess the ability to scavenge excess radicals and maintain the balance of reactive oxygen species in the human body (Jiang and Zhang, 2012).

Positive correlations between total phenolics and antioxidant capacity have been reported. According to many authors, antioxidant activity of wines results mainly from their phenolics, whereas the phenolic content and composition depend on the grape variety, vineyard location, cultivation system, climate, soil types, vine cultivation practices, harvesting time, production process and ageing (Lopez-Velez *et. al.,* 2003).

The antioxidant compositions of wine samples are fairly complex, usually involving multiple reaction characteristics and mechanisms, so no single assay will accurately reflect all antioxidants in a complex system, therefore different antioxidant capacity assays may be needed (Badarinath *et. al.,* 2010). The aim of this work was to estimate the total antioxidant capacity by means of an alternative of the CUPRAC assay, as the concentrations of the participating species ($[Cu(Nc)_2]^{2+}$ and $[Cu(Nc)_2]^{1+}$) can be evaluated by both electrochemical and spectrophotometrical methods.

MATERIAL AND METHOD

Wine Samples

Red wine samples (Cabernet Sauvignon, Fetească neagră, Merlot, Pinot Noir) were purchased from local stores. Samples were opened, protected against sunlight and stored at 4˚C. Analyses were carried out within a few days. Each wine was analyzed 5 times.

Determination of Total Phenolic Content

Spectrophotometric determination of the TPC was carried out with Folin-Ciocalteu method as adapted for wine analysis using gallic acid as the standard. This method is based on the reduction of a phosphotungsten-phophomolybdate complex by phenolics to blue reaction products. For the preparation of calibration curve, 0.1 mL aliquots of 50, 100, 150, 200, 250 and 300 mg/L aqueous gallic acid solutions or 0.1 mL 20-fold dilution samples of red wines (diluted with 13% (v, v) ethanol) were introduced into a test tube and then 2 mL of 2% sodium carbonate was added. After incubation for 2 min, 0.1 mL of Folin-Ciocalteu's reagent (diluted with water 1:1, v/v) was added. After a further 30 min the absorbance was measured at 750 nm using a spectrophotometer. Results were expressed as mg gallic acid equivalents per litre of wine (mg GAE/L) were estimated.

Cupric Reducing Antioxidant Capacity (CUPRAC)

Cupric reducing antioxidant capacity assay was carried out according to Apak et. al., 2004, method:1 ml each of 10 mmM Cu(II), 7.5 mM neocuprine, 1 M ammonium acetate buffer (pH 7) solutions and 0.6 mL water were mixed in a test tube. 0.5 mL of diluted wine or Trolox standard solutions were added to the initial mixture. The absorbance was

measured at 450 nm after 30 minutes. Trolox standard solutions were prepared at a concentration range from 40 to 400 µM.

Electrochemical investigations:DPV−CUPRAC Assay (Proposed Method)

Differential pulse voltammetry was performed using a potentiostat model VOLTALAB 40(Potentiostat/Galvanostat/) in a three-electrode configuration, employing two platinum wires as the working/counter electrode, and saturated calomel electrod as the reference electrode. The required volume of antioxidant standards and samples (20^{th} fold wine diluted samples) was added into a mixture solution of the supporting electrolyte and Cu(II)−Nc chelate in the electrochemical cell. The reaction mixture was left to react for 1 min on a stirrer at room temperature. The voltammograms were recorded immediately to minimize adsorption of polyphenols. Work conditions were as follows: scan range, from +0.6 to −0.25 V; pulse size, 50 mV; step size, 4 mV; pulse time, 0.1 s; and sample period, 0.5 s. The cyclic voltammograms were recorded by scanning the potential from +0.8 to −0.2 V at a scanning rate of 40 mV/s. The total antioxidant capacity values of wine samples were expressed as millimoles of trolox equivalents per gram of wine (mmol TE/g wine). The standard calibration curves of trolox and other phenolics were obtained by measuring the currents (Ip) at the cathodic reduction peak potential of the $Cu(Nc)_2^{2+}$ / $Cu(Nc)_2^{+}$ redox couple before and after reaction with antioxidants, taking the difference (ΔIp), and plotting this difference versus concentration of antioxidant standard (Cárdenas et. al. 2014)

Statistical Analysis

Statistical analyses were realized using Excel software (Microsoft Office 2007) for calculating the means and the standard errors of the means. Results were expressed within 95% confidence interval as mean ± SD. The results were interpreted by two-way analysis of variance (ANOVA).

RESULTS AND DISCUSSIONS

Total antioxidant capacity determined values of the tested wine samples measured by the developed voltammetric method (fig. 1) were in good agreement with those of spectrophotometric-CUPRAC method (fig. 2B).

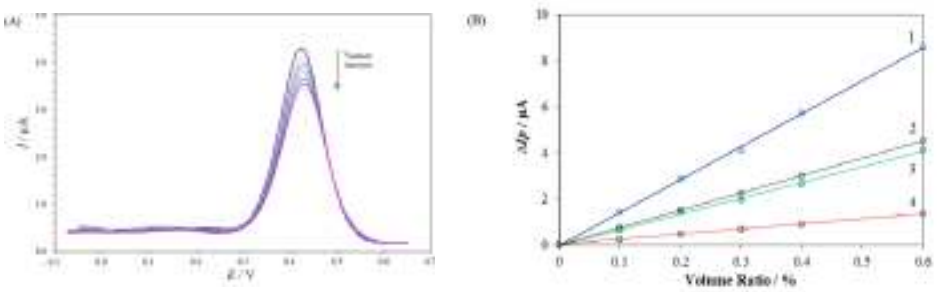

Fig. 1 - (A) Differential pulse voltammograms of $Cu(Nc)_2^{2+}$ reagent showing the effect of increasing wine volume parts.(B) Dependence of ΔIp on the volume ratio (V/Vtotal, %) of wines

Total phenols content (fig. 2A) of the antioxidant capacity wines samples were in good agreement with those of spectrophotometric-CUPRAC method (fig. 2B).

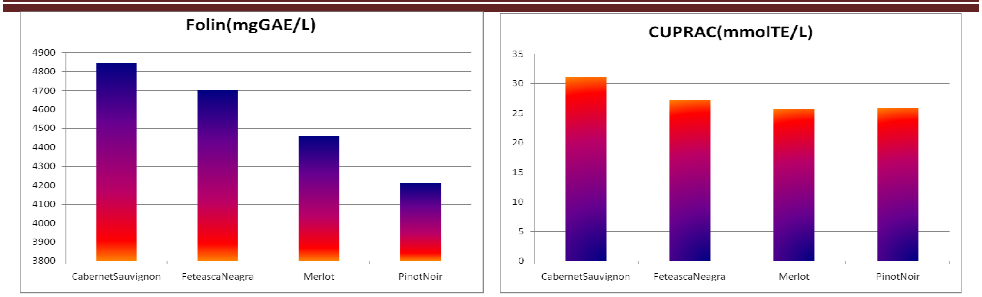

Fig. 2 - (A)Total phenols content of red wines measured by FOLIN–CIOCALTEU method. (B) Total antioxidant capacity of red wines measured by CUPRAC spectrophotometric method

CONCLUSIONS

1. Total antioxidant capacity values of the tested wine samples measured by the developed voltammetric method were in good agreement with those of spectrophotometric-CUPRAC investigation.

2. Electrochemical results showed that total antioxidant capacity of wine samples were in the order: Cabernet Sauvignon > Feteascã neagrã > Merlot > Pinot Noir, in compliance with the results of the spectrophotometric−CUPRAC assay and total phenols content, respectively.

3. A good correlation (0.88) for the total antioxidant capacity determined by CUPRAC spectrofotometric and electrometric method versus total phenols content (determined by Folin-Ciocalteu Method) was recorded.

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