APPLICATION OF BRIGGS-RAUSCHER REACTION FOR MEASUREMENT OF ANTIOXIDANT CAPACITY OF CROATIAN WINES

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Briggs-Rauscher (BR) reaction is one of the most commonly studied oscillation reactions which has been applied for measurement of antioxidant activity of water-soluble substances. By addition of free radicals (from fruits or vegetables), there is an immediate quenching of oscillations. The reaction is followed potentiometrically and the inhibition time (IT), or time of no oscillations, is proportional to concentration of antioxidant.

pH of BR reaction is about 2, what is similar to that of the fluids of the main digestive process (human stomach), giving in vitro information's on antioxidant activity at "real digestion conditions" and can help in assessment of nutrition for maintenance of health and prevention of diseases.

Antioxidant activities of different concentrations of native Croatian red and white wines are analysed by inhibition of BR reaction and determination of total phenols using galic acid as the calibration standard.

By use of mathematical models, relative antioxidant activities of antioxidants and amounts of total phenols are estimated. Second order polynomial calibration curve is estimated in the range of 150-2500 galic acid equivalent (GAE mg l^{-1}), with standard error of 84 GAE mg l^{-1}.

Keywords: Briggs-Rauscher reaction, white and red wines, antioxidant capacity, prediction of total phenols in wine

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Phenolic compounds of major dietary constituents are widely accepted as antioxidant substances which play important role in maintenance of human health and in prevention of diseases (ALONSO et al., 2004; CORDENUNSI et al., 2004; GALVANO et al., 2004; GONZALES-PARAMAS et al., 2004; CAI et al., 2004; VITAGLIONE & FOGLIANO, 2004). The phenolic OH group, present in fruits, vegetables and wine or other beverages derived from fruits, have radical scavenging properties and act as reducing agents (KIM et al., 2003; SINGH & RAJINI, 2004; KATSUBE et al., 2004).

Several testing methods as presented by DE LORIMIER (2000), DUENAS and co-workers (2003), BORBALAN and co-workers (2003) and XU and co-workers (2004) are available for measurements of the antioxidative capacity of drinks at pH about 7.4, what corresponds to pH of human blood, while other methods are used at even higher pH values (BRIGGS & RAUSCHER, 1973; SHAKHASHIRI, 1985). Generation of free radicals in a reaction mixture and their detection is the basis for all the methods.

HÖNER and CERVELLATI (2002) and HÖNER and co-workers (2002) reported a new method for measurements of antioxidative activity based on the inhibitory effects of antioxidants on the oscillatory Briggs-Rauscher reaction (BR). During BR reaction components go through periodic oscillations followed by colour changes. Presence of antioxidants inhibits the oscillations of hydrogen peroxide, acid iodate and malonic acid catalyzed by Mn(II). pH of reaction mixture is about 2 what is similar to pH of human stomach. BR reaction method works only for water-soluble antioxidants (CERVELLATI and co-workers, 2001, HÖNER and CERVELLATI, 2002, HÖNER and co-workers, 2002), for example it can not be applied for determination of vitamin E. Vegetable, fruit and beverage are consumed per os, and the first impact of antioxidants is against free radicals is in the stomach. Therefore, useful in vitro information on the antioxidant activity, at acid pH values can be obtained by the BR reaction which is the main advantage of this method. It is also shown that some antioxidants are more active and stable at strong acidic conditions than in alkali solutions, that makes BR method very important for the assessment of in vivo effects of digested antioxidants. A linear relationship has been proven experimentally between total phenolic content and the BR inhibition time described by CERVELLATI and co-workers. (2001), HÖNER and CERVELLATI (2002) and HÖNER and co-workers (2002).

The aim of this study was to evaluate a mathematical model for prediction of the total phenolic content in wines. Investigated samples were 6 Croatian wines (3 red and 3 white wines) which antioxidant constituents and status were known (BUDIĆ-LETO, 2003, KATALINIĆ and co-workers, 2004). The periodic variation of the concentrations of intermediates and catalyst (CERVELLATI and co-workers, 2001) was quenched by the addition of free radical scavengers from wine. Inhibition times (IT), defined as the time elapsed from the moment of addition of the antioxidant to the first regenerated oscillation, were measured and quadratic dependency of inhibition on concentration of the added antioxidant was applied for the estimation of total antioxidant activity.

1. Materials and methods

All used chemicals and reagents were of analytical grade and were obtained from Kemika (Zagreb, Croatia). Six selected wines from different grape cultivars grown in Croatia were analysed. Selected wines included 3 red (Plavac mali: 3 different products) and 3 white wines (Graševina: 3 different products).

1.1. Total phenol concentration

Total phenol content in selected wine samples was determined colorimetrically with Folin-Ciocalteus reagent using galic acid as a calibration standard (KATSUBE et al., 2004). The results are expressed as galic acid equivalent (GAE). Each sample is determined by three parallel tests.

1.2. Briggs-Rauscher mixture

Three colourless solutions were mixed: first solution was 8.6% hydrogen peroxide; second solution was prepared by dissolving 4.3 g potassium iodate in 100 ml distilled water with addition of 0.5 ml sulphuric acid; and the third solution was a mixture of 1.5 g malonic acid, 0.4 g manganese(II) sulphate monohydrate and 0.1 g starch dissolved in 100 ml of distilled water (SHAKHASHIRI, 1985; http). BR mixture was prepared by mixing the appropriate amounts of stock solutions to a total volume of 15 ml. Oscillations of the BR mixture were followed potentiometrically by recording the potential of a redox

electrode with accuracy of ±1 mV, and the electrode was on-line connected to PC with data sampling rate of 10 points per second (Fig. 1). All measurements were conducted at constant temperature, 25±0.1 °C, by use of a water thermostat. The mixture was stirred by a magnetic stirrer (300 r.p.m.).

Fig. 1.

1.3. Preparation of the aqueous dilutions of wines

Wine samples were prepared at room temperature, diluted with distilled water, and 0.5 ml was added to 15 ml of an active, well-stirred BR mixture, after the third oscillation. The inhibition times were measured before a regenerated appearance of the first peak. All samples were analysed in triplicates, and the results are expressed as the mean value with an associate standard deviation.

1.4. Antioxidant potential

To avoid possible subjectivity in the evaluation of inhibition time in determination of antioxidant potential (AP), a simple computer program, based on Newton method using *W.R. Mathematica*, was developed for calculation of the time delay between the third oscillation and appearance of regenerated oscillations (GAJDOŠ KLJUSURIĆ et al., 2004). Using the known data of total phenol content, calibration models were developed for prediction of total phenols in wine from the inhibition time (WOLFRAM, 1991).

2. Results and discussion

A non-inhibited BR reaction has approximately about 15 oscillations in approximately 10 minutes that can be observed by colour changes from colourless over yellow to dark blue and again the same changes. Oscillations occur between the evaluation of oxygen and carbon dioxide gasses and iodine and iodide ions. The yellow colour is attributed to the rise in I_2 concentration and the dark blue colour is attributed from the formation of the starch-iodine complex, and the colourless solution is caused by the decline in I_2

concentration and rise in I^- concentration (SHAKHASHIRI, 1985). The non-inhibited reaction of the BR mixture is presented with Fig.2.

In the studies presented by HÖNER and CERVELLATI (2002) and HÖNER and co-workers (2002) $NaIO_3$ as source of iodide ions and $HClO_3$ as acid were applied for preparation of BR mixtures. In our work, the iodide source is KIO_3 and the used acid is H_2SO_4. The changes from colourless to dark blue can be explained by oscillations of the concentrations of I_2 and I^- as followed:

$$IO_3^- + 2\ H_2O_2 + CH_2(CO_2H)_2 + H^+ \leftrightarrows ICH(CO_2H)_2 + 2\ O_2 + 3\ H_2O \quad (1)$$

This reaction (eq.1) is accomplished by two component reactions:

$$IO_3^- + 2\ H_2O_2 + H^+ \leftrightarrows HOI + 2\ O_2 + 2\ H_2O \quad (2)$$

$$HOI + CH_2(CO_2H)_2 \leftrightarrows ICH(CO_2H)_2 + H_2O \quad (3)$$

Reaction 2 can occur via two different processes, a radical process and a nonradical one. The domination of these two processes is determined by the concentration of iodide ions in the solution. The colour effect can be observed because reaction 3 takes place in two reactions:

$$I^- + HOI + H^+ \rightarrow I_2 + H_2O \quad (4)$$

$$I_2 + CH_2(CO_2H)_2 \rightarrow ICH(CO_2H)_2 + H^+ + I^- \quad (5)$$

The colour of BR solution turns amber from the I_2 produced trough the reaction 4, when the radical process maintains [HOI] greater than $[I^-]$. The solution turns to dark blue when $[I^-]$ becomes greater than [HOI], and the I^- can be combined with I_2 to form a complex with starch. When $[I^-]$ concentration is high, the reaction 2 switches to a slow nonradical process. The colour

then fades as reaction 3 consumes iodine faster than it is produced. By switching system back to the rapid radical process, the cycle is repeated.

Fig. 2.

Addition of a diluted wine, that contains free radicals, causes an immediate effect of quenching of oscillations, as shown in Fig. 3. The oscillations stop and start again after a period because the reaction produces hydroperoxyl radicals that are quenched by antioxidants. The quenching of oscillations is measured as an inhibition time which is correlated by a quadratic polynomial with concentration of the added antioxidant, presented on Fig. 3.

Fig. 3.

2.1. Model

As a regression model between inhibition time *IT* and a total phenol content *Tph*, a polynomial of the 2nd degree is proposed:

$$Tph = a \cdot IT^2 + b \cdot IT + c \quad (6)$$

In the model *Tph* presents the total phenol content, expressed as GAE (mg l^{-1}), and the inhibition time, IT is in seconds. The following values of the model parameters are estimated: a=5 10^{-4} GAE mg l^{-1} s^{-2}; b=0.6906 GAE mg l^{-1} s^{-1}; c=167.353 GAE mg l^{-1}, shown in Fig. 4. Standard error of *Tph* prediction and sensitivity threshold are estimated as 84 GAE mg l^{-1} and 150 *Tph*, respectively.

The total phenol content of the examined wines and the average values of the inhibition time for different wines are presented in Table 1 as well as the prediction of total phenol content calculated with quadratic

and linear models. The p-level for the linear model (p=0.046191) is higher than the p-level of a suggested quadratic model ($p \approx 0$) for prediction of total phenols, meaning that the quadratic model gives better accuracy.

Correlation between experimental data for total phenol content and predicted total phenol content according to the quadratic model is R^2=0.9993, while for the linear model is R^2=0.9941. This emphasizes that the measurement of inhibition time of wine samples and the use of quadratic calibration model enables to determine the antioxidative activity, based on total phenol content, with standard error of 84 GAE mg/L. Published data by CERVELLATI and co-workers, 2001, HÖNER and CERVELLATI, 2002 and HÖNER and co-workers, 2002, use a linear model with obtained R^2=0.88. In this work the proposed method with a second order polynomial and evaluation of the inhibition time by Newton algorithm and *W.R. Mathematica* software obtained is correlation R^2=0.94. Achieved is accuracy of IT determination of few seconds and improved estimation of the second order polynomial model by use of *Statistica* (Statistica , 2003) software leads to a more accurate total phenol content determination.

Fig. 4A.

Fig. 4B.

Table 1.

Samples with various dilutions were tested in order to estimate the effect of sample dilution on inhibition time and accuracy of total phenol determination. Wine was diluted with distilled water in volume fractions (1:10; 1:4; 1:2) and the relationship between the volume fraction of wines and inhibition time is presented on Fig. 5. The results show proportionality between volume fraction and inhibition time in the range of 100 to 7000 s without observed loss of accuracy. This is also in accordance with results presented by HÖNER and co-workers (2002), SINGH & RAJINI, (2004) and KATSUBE and co-workers (2004).

Fig. 5.

3. Conclusions

The BR reaction is suitable as an analytical method for in vitro determination of relative antioxidative potential of wines at a pH ≈ 2, similar of a human stomach. The analysis is inexpensive due to small quantity and inexpensive substances used. Small volumes (reacting mixture 15 ml, and wine sample 0.5 ml) provide better control of mixing and temperature regulation.

The measurement range of inhibition time is from 100 s to 7 000 s, which corresponds to total phenol GAE range from 200 to 2200 mg l^{-1}. A quadratic calibration model is derived with standard error of 84 GAE mg/L. Sensitivity threshold of 150 mg/L is estimated.

Experiments with diluted samples in the range of volume fractions 1:10 to 1:2 showed proportionality between inhibition time and volume fraction without loss of accuracy.

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Table 1.

Inhibition time of a BR reaction caused by some Croatian wines and phenolic content (Predicted[a]: according to linear model; Predicted[b]: according to the proposed quadratic mathematical model) and corresponding standard deviations

Fig. 1. Presentation of Briggs-Rauscher reaction for measurement of antioxidant activity.

Fig. 2. Recording of the potential for a non-inhibited reaction of an oscillating Briggs-Rauscher mixture.

Fig. 3. Recording of the potential for two inhibited reactions of an oscillating Briggs-Rauscher mixture when 0.5 ml white (A), Graševina or red wine (B), Plavac mali, was added.

Fig. 4A. The calibration curve by a second order polynomial between inhibition time (IT) and total phenol content (Tph) given as equivalent of galic acid (GAE).

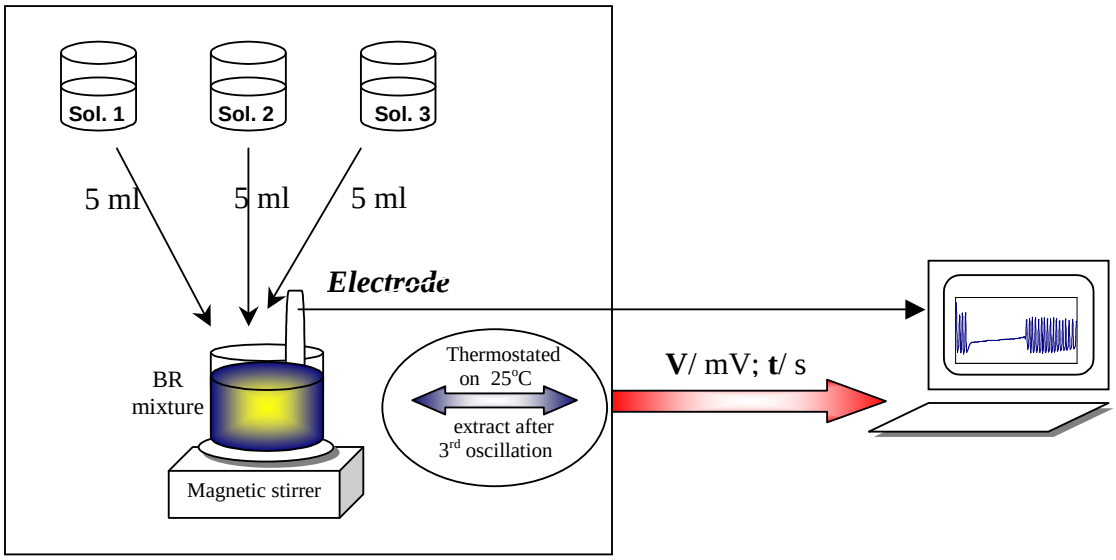
Fig. 4B. The 95% confidence interval for prediction of total phenol content (predicted vs. observed values).

Fig. 5. Changes of the inhibition time according to the volume fraction. ♦: Red wine 1; ■: Red wine 2; □: Red wine 3; ×: White wine 1; ⋄: White wine 2; +: White wine 3.

Table 1

Inhibition time of a BR reaction caused by some Croatian wines and phenolic content (Predicted[a]: according to linear model; Predicted[b]: according to the proposed quadratic mathematical model) and corresponding standard deviations

Samples	**Inhibition time** (s)	**Total phenols** (GAE mg l^{-1})	**Predicted[a]** (GAE mg l^{-1})	**Predicted[b]** (GAE mg l^{-1})
Red wine 1	1424.3±4.3	2156±7.6	2039.5±15.5	2131.1±23.1
Red wine 2	542.1±2.1	718±6.7	812.4±14.5	682.4±12.7
Red wine 3	1140.2±3.2	1532±5.0	1641.3±12.8	1578.6±16.6
White wine 1	123.5±1.6	253±4.7	233.4±2.1	259.8±1.3
White wine 2	100.4±1.1	241±3.2	201.6±1.8	241.5±1.0
White wine 3	109.7±1.0	241±4.6	213.1±2.8	248.0±1.6



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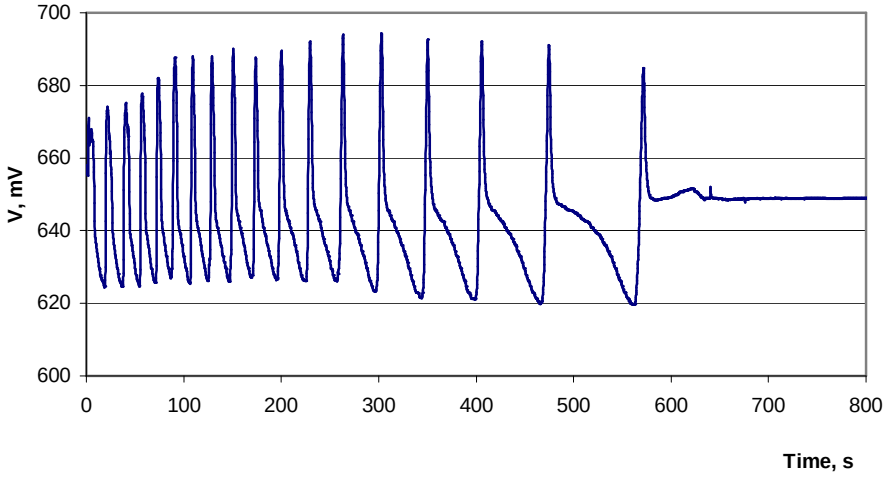


Fig. 2. Recording of the potential for a non-inhibited reaction of an oscillating Briggs-Rauscher mixture.

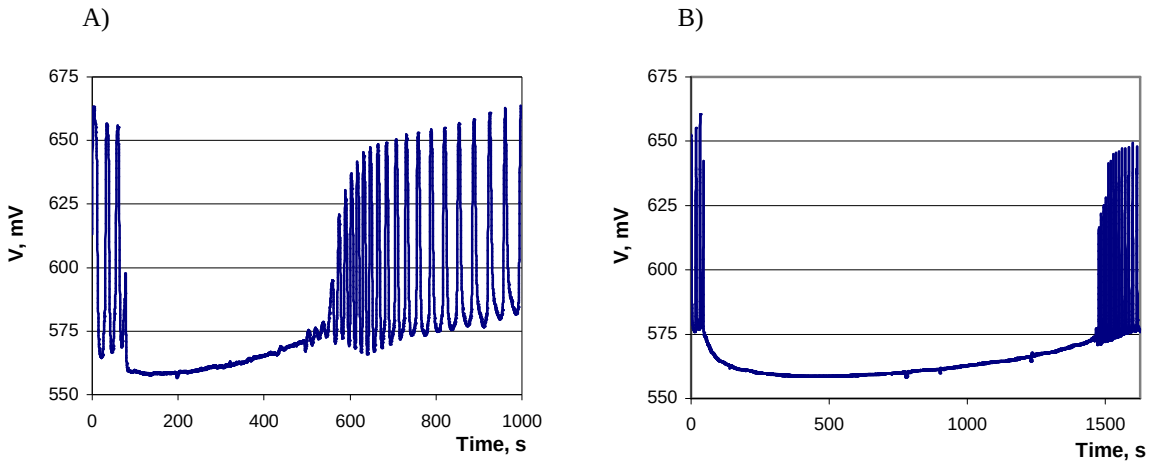
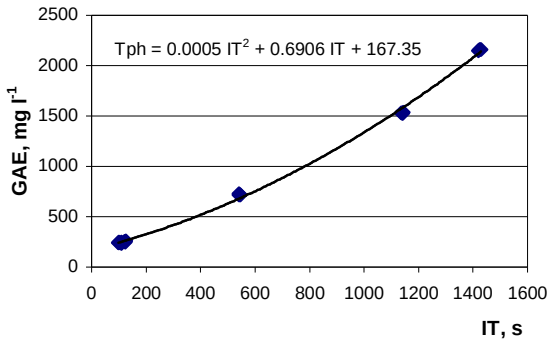


Fig. 3. Recording of the potential for two inhibited reactions of an oscillating Briggs-Rauscher mixture when 0.5 ml white wine, “Graševina” (A), or red wine, “Plavac mali”, (B), was added.

A)



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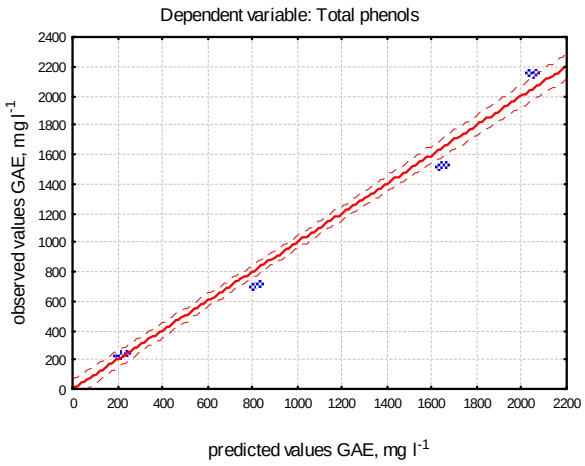

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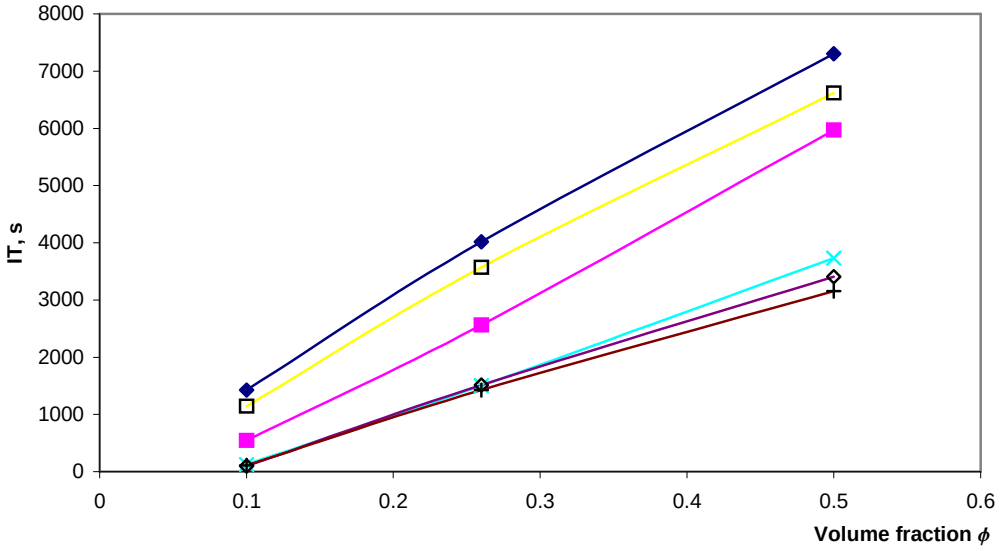

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