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Evaluation of the antioxidant activity of ferulic, homovanillic and vanillic acids using the Briggs-Rauscher oscillating reaction method

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Abstract: In this study, the antioxidant activity of aqueous and ethanolic solutions of ferulic, homovanillic and vanillic acids were evaluated using Briggs-Rauscher oscillating reaction method. This method is based on the inhibitory effects of antioxidants on the oscillations of the Briggs-Rauscher reaction mixture. The inhibitory effect consists of an immediate quenching of oscillations, an inhibition time that depends on the amount and type of the antioxidant added, and a subsequent regeneration of oscillations. Flow oscillations in the Briggs-Rauscher reaction mixture were followed potentiometrically. In all samples, the inhibition times increased with increasing concentration and linearity was found in a wide concentration range of phenolic acid added. The antioxidant activity decreased in following order: ferulic acid > homovanillic acid > vanillic acid. It was also investigated the antioxidant activity for two-component and three-component mixtures of aqueous solutions examined phenolic acids.

INTRODUCTION

Phenolic acids are widely distributed in the plant world and have prominent antioxidant activity. They are ubiquitous in all plant organs and are therefore an integral part of the human diet (Ota *et al.*, 2011). It is estimated that humans consumed from 25 mg to 1 g phenolic acids a day depending on diet (Robbins, 2003). According to the basic structure, phenolic acids are divided into two classes: derivates of benzoic acid such as vanillic acid and derivates of cinnamic acid as ferulic acid (Rice-Evans *et al*., 1996).
Ferulic acid (FA) is a ubiquitous plant constituent that occurs primarily in seeds. Ferulic acid is found in wheat, maize, rye, barley, oats, spinach, sugar beet, and water chestnuts, generally esterified, and rarely as free form, such as in barley. Due to its phenolic nucleus and an extended side chain conjugation, it readily forms a resonance stabilized phenoxy radical that accounts for its potent antioxidant activity. Ferulic acid has been shown to be protective against oxidative stress *in vitro*. It is absorbed and excreted by humans. The low lipophilicity impairs its *in vivo* efficiency, bioavailability and stability (Fraga, 2010).

Homovanillic acid (HVA) is a major catecholamine metabolite and is produced by the consecutive action of monoamine oxidase and catechol-O-methyl transferase on dopamine. It is used as reagent for the fluorimetric determination of glucose oxidase and other oxidative enzymes (Curzon *et al*., 1970). In psychiatry and neuroscience, brain and cerebrospinal fluid levels of homovanillic acid are measured as a marker of metabolic stress caused by 2-deoxy-D-glucose (Marcelis *et al*., 2006). Homovanillic acid presence supports a diagnosis of neuroblastomas and malignant pheochromocytoma (Candido *et al*., 2002).
Vanillic acid (VA) is an oxidized form of vanillin produced during the conversion of vanillin to ferulic acid. The highest quantity of vanillic acid in plants has been found in the roots of *Angelica sinensis*. Vanillic acid is used as a flavoring agent. Various studies have provided evidence of the effectiveness of vanillic acid in the management of immune or inflammatory responses (Kim *et al*., 2010). Vanillic acid showed antigenic and genotoxic effects depending on the dose on human lymphocytes (Bival Štefan, 2015).

Many analytical methods have been developed for the determination the activity of antioxidants (Shalaby and Shanab, 2013). The Briggs-Rauscher oscillating reaction method is very useful and applicable method as a test for the activity of antioxidants because it is works at pH about 2 and partially mimics the physiological conditions similar to those of the fluids in the human stomach. When antioxidants are added to an active oscillating Briggs-Rauscher reaction mixture, there is an immediate quenching of the oscillations for a certain time, denominated as inhibition time. An inhibition time is linearly dependant on the concentration of the antioxidant added to the reaction mixture (Cervellati *et al*., 2001; Cervellati *et al*., 2002). The efficiency of the corresponding antioxidant is expressed as inhibition time before oscillations restart. A better antioxidant as well as higher concentration of it leads to a prolonged inhibiton time. Relative antioxidant activity with respect to a substance chosen as standard can then be determined on the basis of inhibition time (Hönor and Cervellati, 2002; Hönor *et al*., 2002). The Briggs-Rauscher oscillating reaction method can give useful *in vitro* information on the antioxidant activity at low pH values and has many advantages. Milos and Makota (2012) demonstrated some new possibilities of this method for determine the synergistic and antagonistic effects in mixture of compounds, which often poses a problem when using standard methods.

Oscillatory behaviour in the Briggs-Rauscher reaction system can be easily followed potenciometrically using a bright platinum electrode and a suitable reference electrode under thermostated and stirred conditions.

The aim of the present study is to evaluate the antioxidant activity of aqueous and ethanolic solutions of ferulic acid, homovanillic acid and vanillic acid using the Briggs-Rauscher oscillating reaction method.

EXPERIMENTAL

Reagents

All used reagents were of analytical grade and were used without further purification. Potassium iodate, sulfuric acid, hydrogen peroxide and ethanol were obtained from Semikem (Sarajevo, BiH), malonic acid, manganese(II) sulfate monohydrate and starch were obtained from Merck (Darmastadt, Germany), ferulic acid, homovanillic acid and vanillic acid were obtained from Sigma (St. Louis, USA).

Preparation of the solutions for the Briggs-Rauscher reaction

Three colourless stock solutions were prepared daily. Solution A: 43 g potassium iodate and 4.5 mL 96% sulfuric acid were dissolved in distilled water and diluted to 1 L; Solution B: 15.6 g malonic acid, 3.4 g manganese(II) sulfate monohydrate and 3.0 g starch were dissolved in distilled water and diluted to 1 L; Solution C: 500 mL of 30% hydrogen peroxide was diluted to 1 L.

Mixture of equal volumes of stock solutions (A, B and C) represents the Briggs-Rauscher reaction mixture, which is used for evaluation of antioxidant activity (Džomba and Gojak-Salimović, 2017).

Preparation of the solutions of phenolic acids

Solutions of acids where in the concentration range of 8-40 mg/L, 8-80 mg/L and 160-320 mg/L for ferulic, homovanillic and vanillic acids, respectively; all of phenolic acids are ethanol and water soluble.

Apparatus

The oscillating behavior of the Briggs-Rauscher reaction was followed visually and potentiometrically by recording the potential of the reaction mixture using a platinum wire electrode and $Ag/AgCl/KCl_{(sat)}$ reference electrode (+197 mV vs. SHE). The electrodes were connected to a pH multimeter (Phywe, Model 13702.93). The accuracy of the multimeter was ±1 mV. All measurements were conducted at temperature (25±0.5°C) using a suitable thermostating system. The reaction mixture was stirred by a magnetic stirrer (600 rpm).

The Briggs-Rauscher oscillating reaction method for the determination of antioxidant activity

The Briggs-Rauscher reaction mixture were prepared by mixing the appropriate amounts of stock solutions of reagents of A, B and C. For each measurement 10 mL of each solution A and B were mixed into the double-wall thermostated beaker equipped with a magnetic stir bar and placed on a stirring plate. The 10 mL of solution C was used to initiate the oscillations. After the third oscillation, 1 mL solution of phenolic acid at corresponding concentration was added to 30 mL of an active Briggs-Rauscher reaction mixture. The inhibition times were then measured from the recordings. Typical potentiometric recordings for a non-inhibited and an inhibited Briggs-Rauscher reaction mixture were shown in our previous works (Dacić and Gojak-Salimović, 2016; Džomba and Gojak-Salimović, 2017).

The inhibition time defined as the time elapsed between the end addition of the phenolic acid and first regenerated oscillation. The addition of 1 mL of ethanol or water, without phenolic acid does not interrupt the oscillations. The pH value of a non-inhibited-Briggs-Raucher reaction mixture was 1.56.

RESULTS AND DISCUSSION

Our previous works showed the ability of some selected phenolic acids (gallic, caffeic, chlorogenic, rosmarinic and *p*-coumaric acids) to inhibited oscillations of the Briggs-Rauscher reaction mixture (Džomba and Gojak-Salimović, 2017). In this study, the inhibitory effect of various concentrations of the ethanolic and aqueous solutions of ferulic acid, homovanillic acid and vanillic acid on the oscillatory system Briggs-Rauscher reaction were evaluted at 25°C.

Addition of a solution of ferulic, homovanillic or vanillic acids in the active Briggs-Rauscher reaction mixture causes an immediate effect of quenching of oscillations and after period inhibition start again because the reaction produces hydroperoxyl radicals that are quenched by antioxidants. The total antioxidant activity is measured as an inhibition time of Briggs-Rauscher oscillating reaction. The obtained values of inhibition time of aqueous and ethanolic solutions of ferulic, homovanillic and vanillic acids are presented in Table 1.

Table 1: Inhibitory effects of individual ferulic acid, homovanillic acid and vanillic acid at different concentrations on total antioxidant activity measured

Ferulic acid		
Concentration	t_{inhib} **(s)**	
(mg/L)	**Water**	**Ethanol**
8	20	16
16	177	133
24	319	251
40	550	483
Homovanillic acid		
Concentration	t_{inhib} **(s)**	
(mg/L)	**Water**	**Ethanol**
8	100	30
16	163	135
32	235	290
48	406	360
64	800	510
80	1144	670
Vanillic acid		
Concentration	t_{inhib} **(s)**	
(mg/L)	**Water**	**Ethanol**
160	20	64
200	314	410
240	660	640
280	829	710
320	1250	950

The results collected in Table 1 shown that the majority of the ethanolic solutions of phenolic acids have much less antioxidant activity than the corresponding aqueous solutions.

In all samples, the inhibition times increased with increasing concentration and linearity was found in a wide concentration range of phenolic acid added. Therefore, the requirement for the possibility of using Briggs-Rauscher reaction as a test for antioxidant activity was fulfilled. The linear behavior of the inhibition time versus concentration of ethanolic solutions of ferulic, homovanillic and vanillic acids added shown in Figure 1.

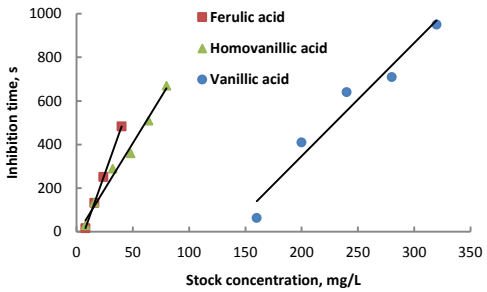

Figure 1: Straight lines of inhibition time versus concentration for the ethanolic solutions of phenolic acids studied

As shown in Figure 1, the slopes of the straight lines are different. The parameters of the straight lines and *R*-squared values are presented in Table 2.

Table 2: Parameters of straight-lines equations ($t_{inhib} = m$(antioxidant) + q) and R^2 values

Water solution	***m*** **(s L/mg)**	***q*** **(s)**	R^2
ferulic acid	16.42	–94.77	0.994
homovanillic acid	14.23	113.7	0.924
vanillic acid	7.482	–1170	0.987
Ethanol solution	***m*** **(s L/mg)**	***q*** **(s)**	R^2
ferulic acid	14.59	–100.4	1.00
homovanillic acid	8.409	–15.08	0.989
vanillic acid	5.195	–692.6	0.955

The antioxidant activity decreased in the following order: ferulic acid > homovanillic acid > vanillic acid, similar to the results previously shown in studies of other authors using Briggs-Rauscher oscillating reaction, TEAC and DPPH methods (Cervellati *et al*., 2001; Cervellati *et al*., 2002; Rice-Evans *et al*., 1996; Karamać *et al*., 2005).

The synergism between the antioxidants in the mixture makes the antioxidant activity not only dependent on the concentration, but also on the structure and the interaction between the antioxidants. In order to evaluate the impact of interactions among examined phenolic acids on their antioxidant activity, the inhibition time of ferulic acid, homovanillic acid and vanillic acid was compared with values obtained by mixing them in different combinations. The results obtained experimentally for nine two-component mixtures and one three-component mixture of aqueous solutions of phenolic acids were compared with the theoretical values by adding up the effects of two or three individual phenolic acids analyzed separately. The obtained results are presented in Table 3.

Table 3: Inhibitory effects of ferulic acid (16 mg/L), homovanillic acid (32 mg/L) and vanillic acid (200 mg/L) with various combinations on total antioxidant activity measured

Combination of compounds	t_{inhib} **(s)**
homovanillic acid + vanillic acid (50%:50%)	498 (275)
homovanillic acid + ferulic acid (50%:50%)	101 (206)
vanillic acid + ferulic acid (50%:50%)	300 (246)
homovanillic acid + vanillic acid (75%:25%)	560 (255)
homovanillic acid + ferulic acid (75%:25%)	504 (220)
vanillic acid + ferulic acid (75%:25%)	1502 (279)
vanillic acid + homovanillic acid (75%:25%)	1335 (294)
ferulic acid + vanillic acid (75%:25%)	1250 (255)
ferulic acid + homovanillic acid (75%:25)	580 (192)
ferulic acid + homovanillic acid + vanillic acid (33.3%:33.3%:33.3%)	320 (242)

*The values in parentheses are the summations of antioxidant activities of individual compounds at corresponding concentrations

Our results indicate that all combinations of phenolic acids demonstrated some level of discrepancy in antioxidant activity when compared to individual values of their constituents. Eight combinations of two and one combination of three phenolic acids showed more or less synergistic effect. The mixture of homovanillic acid and ferulic acid (50%:50%) showed a loss of antioxidant activity when compared to their individual values.

CONCLUSIONS

The Briggs-Rauscher oscillating reaction is suitable as an analytical method to determine antioxidant activity of the ethanolic and aqueous solutions of ferulic, homovanillic and vanillic acids. In all samples, the inhibition time of Briggs-Rauscher oscillating reaction increased with increasing concentration and linearity was found in a wide concentration range of phenolic acid added. The ethanolic solutions of examined phenolic acids had much less antioxidant activity than the corresponding aqueous solutions. The antioxidant activity decreased in following order: ferulic acid > homovanillic acid > vanillic acid. All combinations of phenolic acids demonstrated some level of discrepancy in antioxidant activity when compared to individual values of their constituents.

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Summary/Sažetak

U ovom radu ispitivana je antioksidacijska aktivnost vodenih i etanolnih rastvora ferulinske, homovanilinske i vanilinske kiseline primjenom metode Briggs-Rauscher oscilirajuće reakcije. Ova metoda se bazira na inhibitorskom efektu antioksidanasa na oscilacije Briggs-Rauscher reakcijske smjese. Inhibitorski efekat se sastoji od trenutnog gašenja oscilacija, vremena inhibicije koje zavisi od količine i vrste dodanog antioksidansa, i ponovne regeneracije oscilacija. Tok oscilacija Briggs-Rauscher reakcijske smjese praćen je potenciometrijskom metodom. Sa porastom koncentracije fenolskih kiselina linearno se povećavalo vrijeme inhibicije u širokom opsegu koncentracija. Antioksidacijska aktivnost je opadala prema sljedećem nizu: ferulinska kiselina > homovanilinska kiselina > vanilinska kiselina. Također je ispitana i antioksidacijska aktivnost dvokomponentnih i trokomponentnih smjesa vodenih rastvora fenolskih kiselina.