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The effect of chlorogenic acid on the Briggs-Rauscher oscillating reaction

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Abstract: The Briggs-Rauscher oscillating reaction can be used as test for antioxidant activity of pure compounds or food extracts. Antioxidants are substances that have the ability to neutralize free radicals, which are harmful to human health. Adding the substances with antioxidant ability to the reaction mixture, oscillations temporarily stops, and after a certain time the oscillating reaction starts again. The period without oscillations is known as inhibition time, and it's proportional to the quantity of antioxidant species in reactive mixture. In this study the Briggs-Rauscher reaction was used to measure the antioxidant activity of chlorogenic acid. Inhibition time, duration of reaction and the number of oscillations was determined varying the concentration of chlorogenic acid and solvent (water, ethanol, dimethyl sulfoxide). Flow of oscillations in the Briggs-Rauscher reaction mixture was monitored as a change in potential between the platinum and silver-silver chloride electrodes at room temperature. With increasing concentrations of chlorogenic acid in all three solvents the inhibition time of oscillations is increased.

INTRODUCTION

Oxidative stress is the result of excessive production of free radicals, due to disturbances in the balance of oxidation-reduction processes in biological systems. Exposure to free radicals may be inhibited by the compounds that have antioxidant properties. Antioxidants have the ability to stabilize or deactivate free radicals before they can damage cells in a way to donate an electron or a hydrogen atom to free radicals (Brewer, 2011).

Various phenolic compounds have antioxidant properties and important role in the prevention and/or the development of various diseases caused by the action of free radicals. From the chemical structure of phenolic compounds depends on their antioxidant activity (Shalaby and Shanab, 2013).

Antioxidant activity of phenolic acids depends on the number and position of hydroxyl groups to the carboxyl functional group. Monohydroxy benzoic acids with a hydroxyl group in ortho- or para- position relative to the carboxyl group does not show antioxidant activity, while the meta-hydroxybenzoic acid show. Antioxidant activity of phenolic acids increases with the degree of hydroxylation. Trihydroxy gallic acid shows a high antioxidant activity (Rice-Evans *et al.*, 1996).

The antioxidant activity of food of plant origin stems from the cumulative and synergistic effects of a large number of antioxidants such as vitamins C and E, antioxidants, mainly phenolic acids and flavonoids, terpenoids, carotenoids and trace minerals (Rice-Evans *et al.*, 1996; Robards *et al.*, 1997).

Chlorogenic acid, ester of caffeic and quinic acid, a major phenolic compound in coffee is known as a powerful natural antioxidant. As coffee (with or without the presence of caffeine), chlorogenic acid acts as an antioxidant in neurons against hydrogen peroxide-induced stress. In one liter of coffee, chlorogenic acid is found in quantities of 500 to 800 mg. Significant amounts of chlorogenic acid is found in fruits and vegetables such as apples, pears, strawberries, eggplants, tomatoes and potatoes. Daily intake of coffee, can provide sufficient

intake of chlorogenic acid, however, the same input by using dietary supplements may be better (Beregi *et al*., 2003; Kim *et al.*, 1997; Kono *et al.*, 1997; Sondheimer, 1964).

For measurements of the antioxidant activity of pure compounds and food extracts, several different methods are used (Shalaby and Shanab, 2013). Cervellati *et al.* (2001) have developed a method for measurements of antioxidant activity based on the inhibitory effect by antioxidants on the oscillations of the Briggs-Rauscher reaction. The Briggs-Rauscher oscillating system consists of the iodination and oxidation of an organic substrate by acidic iodate it the presence of hydrogen peroxide with Mn(II) ion as catalyst (Gurel and Gurel, 1983; (Gajdoš-Kljusurić *et al.*, 2005). Oscillations occur between the periodic variation of the concentrations of intermediates and catalyst. By adding antioxidants in the Briggs-Rauscher reaction mixture the oscillations are temporarily interrupted. Inhibition time is in proportion to the amount and properties of added antioxidants. pH value of the Briggs-Rauscher reaction mixture is about 2, which is similar to that of the fluids in the human stomach. Therefore, useful information on the *in vitro* antioxidant activity under acidic conditions can be obtained using the Briggs-Rauscher reaction which is a great advantage compared to other methods for measurements of antioxidant activity (Cervellati *et al*., 2002; Hönor and Cervellati, 2002; Hönor *et al*., 2002).

An important application of the Briggs-Rauscher reaction is found in many studies concerning human health (Pejić *et al.*, 2012). Electromagnetic waves that travel through the muscle tissue similar oscillations Briggs-Rauscher reaction system, which is why these waves can help to understand of complex phenomena such as the heart beats and nerve tissue.

In this work, the antioxidant activities of different concentrations of chlorogenic acid in different solvents (water, ethanol and dimethyl sulfoxide) were analysed by inhibition of the Briggs-Rauscher reaction.

EXPERIMENTAL

Reagents

All used chemicals and reagents were of analytical grade: potassium iodate, Semikem; sulphuric acid, 96%, Semikem; hydrogen peroxide, 30%, Semikem; malonic acid, Merck; manganese(II) sulphate, Merck, starch, Merck; ethanol, 95%, Semikem; dimethyl sulfoxide, Merck and chlorogenic acid, Acros Organics.

Preparation of the solutions for the Briggs-Rauscher reaction

Three solutions (A, B and C) were prepared.

Solution A: Solution of potassium iodate (0.2 mol dm^{-3}) in sulfuric acid (0.43%).

Solution B: Solution of hydrogen peroxide (15%).

Solution C: Solution of malonic acid (0.15 mol dm^{-3}), manganese(II) sulphate (0.02 mol dm^{-3}) and starch (0.03%).

Mixture of equal volumes of the solutions A, B and C represents the Briggs-Rauscher reaction mixture, which is used for measurements of antioxidative activity (Marković and Talić, 2013).

Preparation of the solutions of chlorogenic acid

Exactly 10 mg of chlorogenic acid was dissolved in 100 cm^3 of solvent, distilled water, ethanol and dimethyl sulfoxide. From stock solution of chlorogenic acid (100 mg dm^{-3}) solutions of different concentrations: 75; 50; 25 and 10 mg dm^{-3} were prepared.

Evaluation of antioxidant activity using the Briggs-Rauscher reaction

Oscillations of the Briggs-Rauscher reaction mixture were followed potentiometrically by recording the potential of a platinum electrode and $Ag/AgCl/KCl_{(sat)}$ reference electrode (+197 mV vs. SHE). The electrode was connected to a pH multimeter (Phywe, Model 13702.93). The accurance of the multimeter was ±1 mV. All measurements were conducted at room temperature, 20±0,5°C. The mixture was stirred by a magnetic stirrer (600 r.p.m.). The Briggs-Rauscher reaction mixture were prepared by mixing the appropriate amounts of stock solutions (A, B and C) in beaker to a total volume of 30 cm^3. The order of addition was: solution A, solution C solution and B. Oscillations begin after the addition of solutuon B. The pH value of the Briggs-Rauscher reaction mixture is 1.56. Solutions of chlorogenic acid (1 cm^3) was added to 30 cm^3 active the Briggs-Rauscher reaction mixture after the third oscillation.

RESULTS AND DISCUSSION

A non-inhibited Briggs-Rauscher reaction had about 20 oscillations that could be monitored and visually based on changes in the color of the reaction mixture from colorless through yellow to dark blue and again the same changes. The color change is explained by the oscillation of the concentration of I_2 and I^- as in reaction (Gajdoš-Kljusurić *et al.*, 2005):

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

Reaction (1) is derived from the following two reactions:

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

$$HOI + CH_2(COOH)_2 \rightarrow ICH(COOH)_2 + H_2O \quad (3)$$

The reaction (2) occurs through two different processes, radical and non-radical.

During these process, changes in concentration of iodide ion in solution and the color change can be observed because the reaction (3) takes place in two steps:

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

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

Yellow occurs due to the formation of I_2. The emergence of I_2 is caused by the rapid production of HOI during radical process. Over time, it creates more HOI than can be utilized at any given moment. Certain amount of HOI is reduced with hydrogen peroxide to I^-. The concentration of I^- is growing, and the resulting yellow

color of the Briggs-Rauscher reaction mixture is changed to dark blue when I^- dominates more than HOI. Then I^- is combined with I_2 to form a complex with starch. When the concentration of I^- is high, reaction (2) is switched to slow non-radical process, and the color begins to fade, and the cycle is repeated. Sample of recording of the potential for non-inhibited oscillating Briggs-Rauscher mixture is shown in Figure 1.

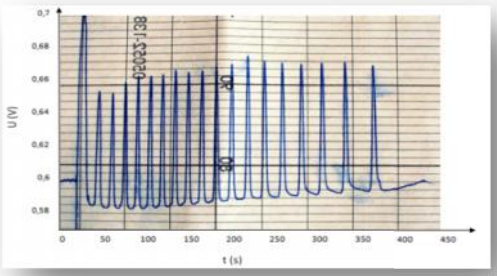

Figure 1. Oscillations for a non-inhibited Briggs-Rauscher reaction mixture

Addition of the solution of chlorogenic acid in theactive Briggs-Rauscher reaction mixture causes an immediate effect of quenching of oscillations. 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 with contents of the added antioxidant. Sample of recording of the potential when 1 cm^3 of aqueous solution of chlorogenic acid (50 mg dm^{-3}) was added to 30 cm^3 of an oscillating Briggs-Rauscher mixture after third oscillation is shown in Figure 2.

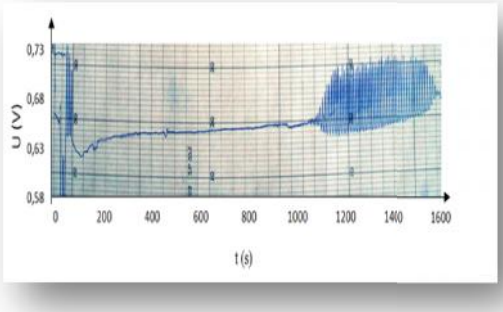

Figure 2. Oscillations for inhibited Briggs-Rauscher reaction mixture

Estimated inhibition time for measurements of the antioxidant potential is performed by subtracting the time appearance regenerated oscillations of the third oscillation. In this way, the determined value of inhibition time for all the tested solutions of chlorogenic acid (Table 1).

Table 1. Inhibition time of the Briggs-Rauscher reaction caused by different concentration of chlorogenic acid

Concentration of chlorogenic acid (mg dm^{-3})	Inhibition time (s)		
	Water	Ethanol	Dimethyl sulfoxide
10	35	25	20
25	110	135	70
50	1030	1210	855
75	1935	1635	1515
100	2395	2400	2080

An inhibition time of the Briggs-Rauscher reaction increases with increasing concentrations of chlorogenic acid in the case of all three solvents. The aqueous solutions of chlorogenic acid showed the best antioxidant activity, followed by ethanolic, and dimethyl sulfoxide solutions. Inhibition time depends linearly on the concentration of chlorogenic acid between 10 and 100 mg dm^{-3}. The correlation coefficient between the inhibition time and concentration of chlorogenic acid in aqueous and ethanolic solutions was 0.977 and 0.983 in dimethyl sulfoxide solutions.

With increasing concentrations of chlorogenic acid increases the total time of oscillations of the Briggs-Rauscher reaction, as expected (Table 2).

Table 2. Time of oscillations of the Briggs-Rauscher reaction caused by different concentrations of chlorogenic acid

Concentration of chlorogenic acid (mg dm^{-3})	Time of oscillations (s)		
	Water	Ethanol	Dimethyl sulfoxide
0	355	250	280
10	520	435	420
25	850	640	375
50	1655	2005	1125
75	2545	2275	2035
100	2800	2990	3300

Addition of 1 cm^3 of pure solvent (water, ethanol and dimethyl sulfoxide) in the Briggs-Rauscher reaction mixture had no significant effect on the number of oscillations in the system, as in the case of adding a solution of chlorogenic acid (Table 3).

Table 3. Number of oscillations of the Briggs-Rauscher reaction caused by different concentrations of chlorogenic acid

Concentration of chlorogenic acid (mg dm^{-3})	Number of oscillations		
	Water	Ethanol	Dimethyl sulfoxide
0	22	18	20
10	31	28	28
25	53	30	30
50	61	66	66
75	51	53	53
100	51	56	56

Number of oscillations of the Briggs-Rauscher reaction is increased by the addition of chlorogenic acid in higher concentrations of all three cases to a point, after which the number is reduced. It has been noted that the chlorogenic acid concentration of 50 mg dm^{-3} increased the number of oscillations, after which further increase the concentration reduces the number of oscillations.

CONCLUSIONS

The Briggs-Rauscher oscillating reaction is suitable as an analytical method for measuring the relative *in vitro* antioxidant activity of pure compounds and extracts of food at a low pH, like the pH in the human stomach. In this study a linear relationship is confirmed between the inhibition time of oscillation in the Briggs-Rauscher reaction mixture and the concentration of pure chlorogenic acid in three solvents (water, ethanol and dimethyl sulfoxide), ranging from 10 to 100 mg dm^{-3}. Application of the Briggs-Rauscher reaction has many advantages over other methods for measurements of antioxidant activity. Analysis is cheap, quick and necessary reagents and devices are commonly used in all chemical laboratories.

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Summary/Sažetak

Briggs-Rauscher oscilirajuća reakcija se primjenjuje kao nova metoda za testiranje antioksidacijske aktivnosti čistih spojeva i ekstrakata hrane. Antioksidansi su spojevi koje imaju sposobnost neutralizirati slobodne radikale, koji su štetni za ljudsko zdravlje. Dodatkom spoja koji ima antioksidacijska svojstva u Briggs-Rauscher reakcijsku smjesu, oscilacije se privremeno prekidaju, da bi se nakon određenog vremena ponovo nastavile. Vrijeme prekida oscilacija naziva se vrijeme inhibicije i proporcionalno je količini dodanog antioksidansa. U ovom radu Briggs-Rauscher reakcija je primijenjena za dokazivanje antioksidacijske aktivnosti hlorogenske kiseline. Praćeno je vrijeme inhibicije, vrijeme trajanja i broj oscilacija u zavisnosti od koncentracije hlorogenske kiseline i rastvarača (voda, etanol, dimetilsulfoksid). Tok oscilacija u Briggs-Rauscher reakcijskoj smjesi praćen je kao promjena potencijala između platinske i srebro-srebrohloridne elektrode na sobnoj temperaturi. Sa porastom koncentracije hlorogenske kiseline u sva tri rastvarača produžavalo se vrijeme inhibicije oscilacija Briggs-Rauscher reakcijske smjese.