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Investigation of the Antioxidant Synergisms and Antagonisms among Caffeic, Ferulic and Rosmarinic Acids using the Briggs-Rauscher Reaction Method

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Abstract: Phenolic acids have been attracting huge attention over recent years due to their prominent antioxidant activity and potential health benefits. In this study, the antioxidant activity of caffeic, ferulic, and rosmarinic acids was evaluated using Briggs-Rauscher reaction method. In addition to single phenolic acids at different concentrations (50, 100, 200 and 250 μM), equimolar mixtures of two phenolic acids and all three phenolic acids were tested. The best ability to inhibit oscillations, i.e. the highest antioxidant activity showed rosmarinic acid at a concentration of 250 μM. Inhibition time of the Briggs-Rauscher oscillating reaction mixture was obtained experimentally for the different combinations of phenolic acids and compared with theoretical values calculated by adding up the effects of phenolic acids analyzed individually. The most of tested phenolic acids mixtures showed a difference in the antioxidant activity when compared to individual values of their constituents. The highest synergistic effect showed a mixture of caffeic acid and ferulic acid at a concentration of 250 μM while the highest antagonistic effect showed the mixture of caffeic acid, ferulic acid and rosmarinic acid at same concentration.

INTRODUCTION

Phenolic acids have been attracting huge attention over recent years due to their potential biological properties which makes them interesting to the food and pharmaceutical industries. They are widespread in all plant foods and are therefore an integral part of the human diet (Ota et al., 2011; Saxena et al., 2012). Phenolic acids have been associated with color, nutritional, organoleptic and antioxidant properties of foods. The food industry has investigated effect of phenolic acids on the fruit maturation, prevention of enzymatic browning and their roles as food preservatives (Robbins, 2003). Most of phenolic acids have a beneficial effect on health, or an active role in the treatment of a disease (Mota et al., 2008; Saxena et al., 2012).

Ferulic acid (FA) has received much attention among all phenolic acids (Karamać et al., 2005). It is the most abundant hydroxycinnamic acid in plants (Mota et al., 2008). It is found in many fruits and vegetables, seeds of rice, wheat and oats, coffee and olive oil (Rice-Evans et al., 1996; Mota et al., 2008; Kadoma and Fujisawa, 2008). Ferulic acid has significant health benefits and it serves as a precursor in the manufacture of useful aromatic compounds (Mota et al., 2008).

Caffeic acid (CA) is one of the most prominent naturally occurring hydroxycinnamic acids. It is found in many fruits, vegetables, seasonings, beverages and olive oil. Cafeic acid is highly predominant in sunflower seeds and greatly affects the solubility of plant proteins (Rice-Evans et al., 1996; Mota et al., 2008). It selectively blocks the biosynthesis of leukotrienes, components involved in immunoregulation diseases, asthma and allergic reactions (Robbins, 2003).

Rosmarinic acid (RA) is one of the more abundant ester of caffeic acid occurring in several members of the Lamiaceae family plants. Rosmarinic acid has antioxidant and anti-inflammatory effects and used to treat upper respiratory and allergic symptoms (Stansbury, 2014).

Each phenolic acid has a different antioxidant activity depending on its structure, number of aromatic and hydroxyl groups and their distribution in the structure. The phenolic acids in plant foods exist in the free, esterified, glycosidic, and insoluble-bound forms

(Karamać et al., 2005). However, interactions among phenolic acids may promote changes in overall antioxidant activity, which is difficult to predict on the basis of their individual antioxidant activities. Although the antioxidant activity of individual phenolic acids is widely described, information about their interactions and potential additive, synergistic or antagonistic effects is lacking (Peyrat-Maillard et al., 2003; Pinelo et al., 2004; Palafox-Carlos et al., 2012; Hajimehdipoor et al., 2014; Skroza et al., 2015; Sonam and Guleria, 2017).

In this study, the antioxidant activity of individual phenolic acids (caffeic acid, ferulic acid and rosmarinic acid) and equimolar mixtures of two or three phenolic acids were investigated using Briggs-Rauscher reaction method. The Briggs-Rauscher reaction method is very applicable method for measuring the activity of antioxidants because it works at pH which is similar to pH value of the fluids in the human stomach. Antioxidants added to an active oscillating Briggs-Rauscher reaction mixture cause an immediate cessation of the oscillations. After inhibition time the oscillations restart again. The inhibition time linearly depends on the type and amount of the added antioxidant. Better antioxidant results with longer inhibition time (Cervellati et al., 2001; Cervellati et al., 2002; Höner and Cervellati, 2002). It is easy to use Briggs-Rauscher reaction method for determine the potential synergistic, antagonistic or additive effects between antioxidants in the mixtures (Milos and Makota, 2012).

EXPERIMENTAL

Reagents

All used reagents were of analytical grade. 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), caffeic acid, ferulic acid, and rosmarinic acid were obtained from Sigma (St. Louis, USA).

Preparation of the solutions of phenolic acids

A stock solutions of caffeic, ferulic and rosmarinic acids, 1000 μM, were prepared daily by dissolving pure phenolic acid in 2 mL of ethanol and diluting with distilled water to 100 mL. Other solutions of phenolic acids in the concentrations of 50, 100, 200 and 250 μM were obtained by diluting the stock solution with the respective volumes of distilled water. Thus prepared solutions were used for the preparation of equimolar mixtures of two and three phenolic acids.

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

The antioxidant activity of individual phenolic acids and equimolar mixtures of two and three phenolic acids mixture have been evaluated using the Briggs-Rauscher reaction method described by Cervellati et al. (2001), with small modifications. In our study, three stock solutions (A, B and C) were prepared daily: solution A: 0.2 M potassium iodate and 0.08 M sulfuric acid; solution B: 0.15 M malonic acid, 0.02 M manganese(II) sulfate monohydrate and 0.03% starch; solution C: solution of hydrogen peroxide in distilled water concentration of 15%. The Briggs-Rauscher reaction mixture was prepared by mixing 10 mL of each stock solution (A, B and C). Kinetics of oscillating reactions were observed potentiometrically by recording the potential of the Briggs-Reaction reaction mixture using a platinum wire electrode as working electrode and Ag/AgCl electrode as reference electrode at 25±0.5°C. After the third oscillation, 1 mL solution of phenolic acid at corresponding concentration (or the mixture of two or three phenolic acids) was added to an active oscillating Briggs-Rauscher reaction mixture. The total antioxidant activity of the corresponding phenolic acids or their mixture was expressed as the inhibition time (t_{inhib}). The addition of 1 mL of distilled water, without phenolic acids does not interrupt the oscillations.

RESULTS AND DISCUSSION

Antioxidant activity of caffeic, ferulic and rosmarinic acids tested individualy

In this study, the antioxidant activities of caffeic, ferulic and rosmarinic acids were evaluated at different concentrations (50, 100, 200 and 250 μM) using the described Briggs-Rauscher reaction method. The obtained results are presented in Table 1. The longest inhibition time was detected with rosmarinic acid at a concentration of 250 μM (2107 s), and the lowest with caffeic acid at a concentration of 50 μM (30 s).

Table 1: Inhibitory effects of individual phenolic acids at different concentrations

Phenolic acid	t_{inhib} (s) at different concentrations (μM)			
	50	100	200	250
CA	30	60	326	491
FA	192	373	607	781
RA	58	151	1519	2107

*CA - caffeic acid, FA - ferulic acid, RA - rosmarinic acid

The inhibition time increased with higher concentration, and linearity was found in an tested concentration range of phenolic acid added. The parameters of the linearity are presented in Table 2.

Table 2: Parameters of linearity ($t_{inhib} = m$(antioxidant) + q) and R-squared values

Phenolic acid	m (μM^{-1} s)	q (s)	R^2
CA	2.376	-129.6	0.965
FA	2.824	64.65	0.993
RA	10.93	-681.1	0.965

*CA - caffeic acid, FA - ferulic acid, RA - rosmarinic acid

The obtained results confirmed that the antioxidant activity of the tested phenolic acids vary depending of their structure and concentration. The antioxidant activity decreased in the following order: rosmarinic acid > ferulic acid > caffeic acid, similar to the results in previous studies (Cervellati et al., 2001; Cervellati et al., 2002) obtained using Briggs-Rauscher reaction method.

Effect of combination phenolic acids on the total antioxidant activity

The obtained results for the inhibition time of the mixture of two and three combination phenolic acids are presented in Table 3. The results for the inhibition time ranged from 51 s for the mixture of rosmarinic acid and caffeic acid at a concentration of 50 μM to 1958 s for their combination at a concentration of 250 μM. To conclude about the antioxidant interactions between caffeic acid, ferulic acid and rosmarinic acid their values of the inhibition time were compared with the values obtained by mixing them in different combinations. The experimentally values for the different mixtures were compared with theoretical values calculated by adding up the effects of phenolic acids analyzed individually. Any significant difference values obtained from these comparisons indicate an sinergistic, antogonistic or additive effect.

Table 3: Inhibitory effects of the mixture of phenolic acids at different concentrations

Mixtures	t_{inhib} (s) at different concentrations (μM)			
	50	**100**	**200**	**250**
CA+FA	113 (111)	353 (216)	430 (466)	1881 (636)
FA+RA	126 (125)	**366 (262)**	**1241 (1063)**	1845 (1444)
RA+CA	51 (44)	102 (105)	699 (922)	**1958 (1299)**
CA+FA+RA	**193 (93)**	279 (195)	1206 (817)	679 (1126)

*CA - caffeic acid, FA - ferulic acid, RA - rosmarinic acid; The values in parentheses are the sum of antioxidant activities of individual phenolic acids.

The obtained results indicate that most of tested mixtures showed a difference in antioxidant activity when compared to their theoretical values. The highest antagonistic effect was found for mixture of rosmarinic and caffeic acid at a concentration of 200 μM, while the highest synergistic effect showed a mixture of caffeic acid and ferulic acid at a concentration of 250 μM. The mixtures of caffeic acid and ferulic acid, ferulic acid and rosmarinic acid at a concentration of 50 μM, and mixture of rosmarinic acid and caffeic acid at a concentration of 100 μM showed additive effect. The mixtures of three phenolic acids showed synergistic effect at concentrations of 50, 100 and 200 μM, while same combination mixture at concentration of 250 μM showed the high antagonism. In our previous study (Aljović and Gojak-Salimović, 2017) the antioxidant activity was investigated for two-component and three-component mixtures of ferulic acid, homovanillic acid and vanillic acid using Briggs-Rauscher reaction method. Most of the tested combinations of phenolic acids showed a sinergistic effect.

Peyrat-Maillard et al. (2003) investigated mixture effects between phenolic antioxidants in equimolar binary systems on the protection of linoleic acid from 2,2′-azobis (2-amidinopropane) dihydrochloride-induced oxidation. The slight synergy was observed between rosmarinic acid and caffeic acid. Hajimehdipoor et al. (2014) investigated synergistic antioxidant effects of binary and ternary combinations of methanolic solutions of caffeic acid, gallic acid, rosmarinic acid, chlorogenic acid, rutin and quercetin using FRAP method. The synergistic effect (37.5%) showed the combination of caffeic acid (600 μM) and rosmarinic acid (150 μM).

CONCLUSIONS

The obtained results support the fact that antioxidant activity of the phenolic acid mixtures cannot be predicted from the antioxidant activity for individual phenolic acids. Interaction of investigated phenolic acids (caffeic acid, ferulic acid and rosmarinic acid) in the mixtures depend on the concentrations of phenolic acids present. The most of tested phenolic acids mixtures showed a difference in antioxidant activity when compared to individual values of their constituents. Our future investigation will be focused on the potential synergistic or antagonistic effects among other phenolic acids using Briggs-Rauscher reaction method.

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Summary/Sažetak

Fenolske kiseline posljednjih godina privlače veliku pažnju zbog njihove značajne antioksidacijske aktivnosti i potencijalnih benefita po ljudsko zdravlje. U ovom istraživanju, antioksidacijska aktivnost kafene kiseline, ferulinske kiseline i ruzmarinske kiseline ispitivana je primjenom Briggs-Rauscher oscilirajuće reakcije. Osim pojedinačnih fenolskih kiselina pri različitim koncentracijama (50, 100, 200 i 250 μM), testirane su ekvimolarne smjese dviju fenolskih kiselina i sve tri fenolske kiseline. Najbolju sposobnost inhibicije oscilacija, tj. najveću antioksidacijsku aktivnost pokazala je ruzmarinska kiselina pri koncentraciji od 250 μM. Vrijeme inhibicije Briggs-Rauscher reakcione smjese dobijeno eksperimentalno za različite kombinacije fenolskih kiselina poređeno je s teorijskim vrijednostima izračunatim zbrajanjem efekata dvije ili tri fenolske kiseline pojedinačno analizirane. Većina ispitivanih smjesa fenolskih kiselina pokazala je određen stepen odstupanja u antioksidacijskoj aktivnosti u usporedbi s induvidualnim vrijednostima njihovih sastojaka. Najveći sinergijski efekat pokazala je smjesa kafene kiseline i ferulinske kiseline pri koncentraciji od 250 μM, dok je najveći antagonizam pokazala smjesa kafene kiseline, ferulinske kiseline i ruzmarinske kiseline pri istoj koncentraciji.