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THE ANTIOXIDANT ACTIVITY OF TEA INFUSIONS TESTED BY MEANS OF BRIGGS-RAUSCHER OSCILLATORY REACTION

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ABSTRACT. The antioxidant capacity of tea extracts was determined by means of Briggs-Rauscher oscillating system in batch conditions. This method consists in the measurement of the inhibition time caused by the addition of tea extract to the oscillating system. The inhibition time vs. the concentration of tea extract shows linear dependence.

Keywords: *Briggs-Rauscher oscillating reaction, inhibitory effect, analytical method, tea extract*

INTRODUCTION

The tea is one of the most popular worldwide beverages and a major source of dietary antioxidants.

In 2011, researchers have conducted a trial to test the effects of rooibos tea on various biological markers, considered to be indicative of risk for cardio-vascular disease and other degenerative diseases. A high intake of rooibos tea resulted in significant reductions in lipid peroxidation, LDL cholesterol, triglycerides, and an increase in HDL cholesterol levels compared with the control group. The researchers concluded that rooibos lowered the risk factor [18].

The main flavonoids found in fresh tea leaves are the catechins (flavan-3-ols, or flavanols) and the flavonols. These flavonoids represent usually more than 30% of the dry weight of the leaves. Two flavonoids were found in rooibos, the quercetin and luteolin, and they are used in cancer therapy [1]. The rooibos leaves do not contain the antioxidant catechins [2]. The differences between the white, green and black tea is the processing method of them. In order to prepare white tea the leaves are collected in spring, when the leaves are covered with white dust. White tea and green

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tea are produced using thermal processes, such as steaming or dry heating, to inactivate polyphenol oxidase that oxidize catechins to more complex oligomeric flavonoids characteristic of oolong and black teas.

During the manufacture of black and red teas, the colorless, monomeric catechins are converted to orange-yellow- and red-brown-colored oligomeric flavonoids. Additionally, oxidation of amino acids and lipids occurs with the generation of numerous volatile flavor compounds. These oxidative changes are reflected in the red-amber color, reduced bitterness, and increased astringency and more complex flavor of black teas. Green and white tea beverages contain 30–130 mg Epigallocatechin gallate (EGCG) per cup of tea, while black tea beverages 0–70 mg EGCG per cup of tea. The flavonols, such as quercetin, kaempferol, myricetin, and their glycosides, are present in much lower concentrations than the catechins and are found in comparable quantities in black, green, and oolong tea beverages (5–15 mg/cup) [3-6].

Tea polyphenols, especially the catechins, are possible antimicrobial and antioxidant agents, with positive effects on human health. The antioxidant activity can be determined by some well known analytical methods. All these methods are based on the generation of free radicals in the reaction mixture followed by their detection. In the presence of antioxidants, the amount of the free radicals detected is much less in comparison with that of a reference mixture. These are for example: *Franckel, Pryor* rapid screening, TEAC (trolox equivalent antioxidant capacity), TRAP (total radical-trapping antioxidant parameter) method, the FRAP (ferric reducing-antioxidant power) method [7].

The Briggs-Rauscher (BR) reaction, one of the few reactions showing long lived oscillations in batch conditions, was discovered by Briggs and Rauscher. Its classical version is the oscillatory oxidation and iodination of malonic acid (MA) by hydrogen peroxide and iodate, catalyzed by Mn^{2+} ions in acidic medium. The reaction was studied by many research groups, including that lead by Noyes, Furrow, Cervellati and Sørensen [8-10]. Recently a new method was developed based on the inhibition of the well known oscillatory system, the BR reaction [14]. An oscillating reaction is very sensitive -because it is far away from chemical equilibrium - and this behavior is used in analytical determinations.

Oscillations can be demonstrated by vivid color changes in the presence of a starch indicator or usually they are monitored by recording platinum or iodide selective electrode potentials vs. a reference electrode. Kinetically important intermediates are I_2, I^-, I_3^-, O_2, CO_2, CO, HOI, HOO° and iodomalonic acid (IMA) [10-13].

The basis of this analytical method consists in the fact that various antioxidants (mono- and polyphenolic compounds), change the dynamics of the BR reaction fundamentally by suppressing the oscillations even in a surprisingly low concentration (usually in a few micromoles/liter).

This kind of micro-analytical technique is used to determine the antioxidant capacity of various chemical compounds or plant extracts. Cervellati *et al*.[14] reported that the addition of mono- and polyphenolic compounds to the active mixture causes a temporary but instant cessation of oscillations. The time elapsed between the cessation and the subsequent regeneration of the oscillatory regime is the so called inhibition time. A linear correlation was found between the concentration and the inhibition time for numerous phenolic substances added to the BR-mixture

The inhibitory effect was accounted for a fast reaction involving the phenol compound and HOO• radical. Since polyphenols are known free radical scavengers, they can reduce the concentration of HOO• in the BR-mixture [15-17].

According to the suggested mechanism, as soon as the antioxidant is consumed, the HOO• concentration rises to a critical level where the oscillations can reappear.

In this study was made a comparison of white, green, black and rooibos tea's activity. The first three were prepared from the processed young leaves of *Camellia sinensis*, and the last one from the leaves of *Calicotome villosa*.

The study presented in this article is divided in two parts; first the development of the optimal composition of the BR mixture, and in the second part is presented the determination of antioxidant activity of white, green, black and rooibos tea.

It is worth mentioning that, no studies have been published so far, in which the antioxidant capacity of tea was determined by means of BR method.

RESULTS AND DISCUSSION

The optimal composition of the BR mixture

By varying the concentrations of iodate, hydrogen peroxide and H_2SO_4, we expected to obtain a better composition of the mixture with higher amplitude of the oscillations and a longer inhibition time.

These two factors are the most important characteristics of the oscillations; the higher the values of these parameters, the larger the domain of the applications of oscillatory reactions.

Increasing the amplitude of the oscillations the switch between oscillation and inhibition is sharper. On the other hand, the oscillation period is a limitative factor for the inhibition time. In the figure 1 are presented the variations of these parameters vs. the concentration of one of BR mixture's component, and the optimal ones are encircled. The concentrations of the other components in the reactor was kept constant and they were $[Mn^{2+}]$ = 65 mM, [MA] = 50 mM, $[H_2SO_4]$ = 25 mM, $[KIO_3]$ = 67 mM, if it's not mentioned otherwise.

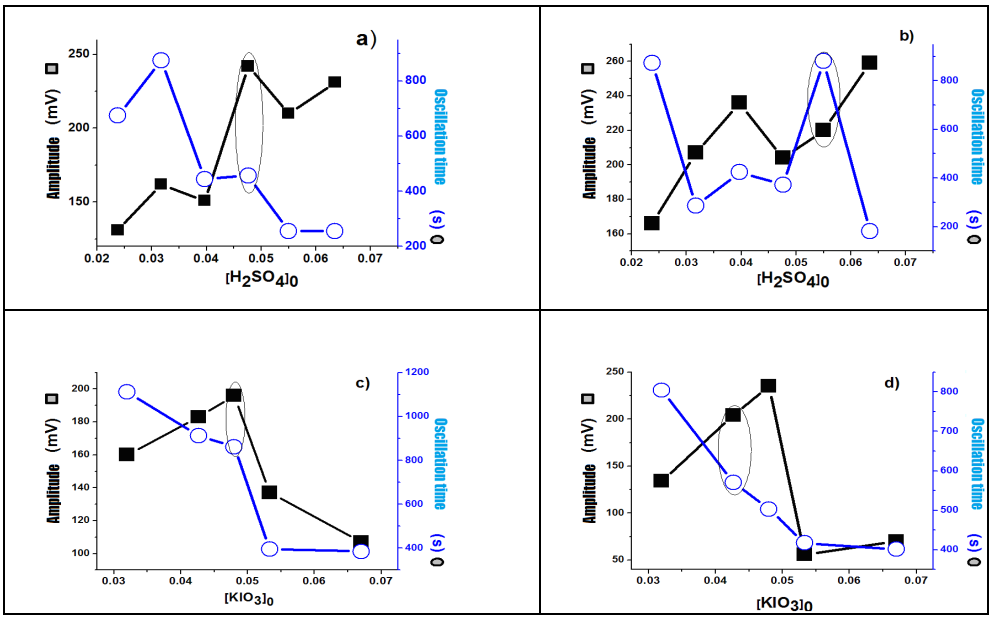

Figure 1 The values of amplitude and the duration of the oscillations as a function of initial concentration of H_2SO_4, figs. a) and b), The values of amplitude and the duration of the oscillations as a function of initial concentration of KIO_3 figs. c) and d) The H_2O_2 concentration was 0.65 M in figs. a) and c) and in figs. b) and d) it was 1.32 M

It can be concluded, that the optimal concentrations for BR method are $[H_2SO_4]_0$= 55 mM, $[KIO_3]_0$= 45 mM, $[MA]_0$= 50 mM, $[MnSO_4]_0$= 65 mM, $[H_2O_2]_0$= 1.32 M

The antioxidant activity of teas

Perturbation of the oscillatory BR system with a tea extract causes the immediate cessation of the oscillations; the time elapsed between the cessation and returns of the oscillations, the so called inhibition time is illustrated in Figure 2.

Using the optimal composition for the BR, the different tea extracts antioxidant activity was determined for several dilutions and the calibration curves were drawn for each of them. Variation of the inhibition time in function of the antioxidant concentration was found to be linear as can be seen in figure 3.

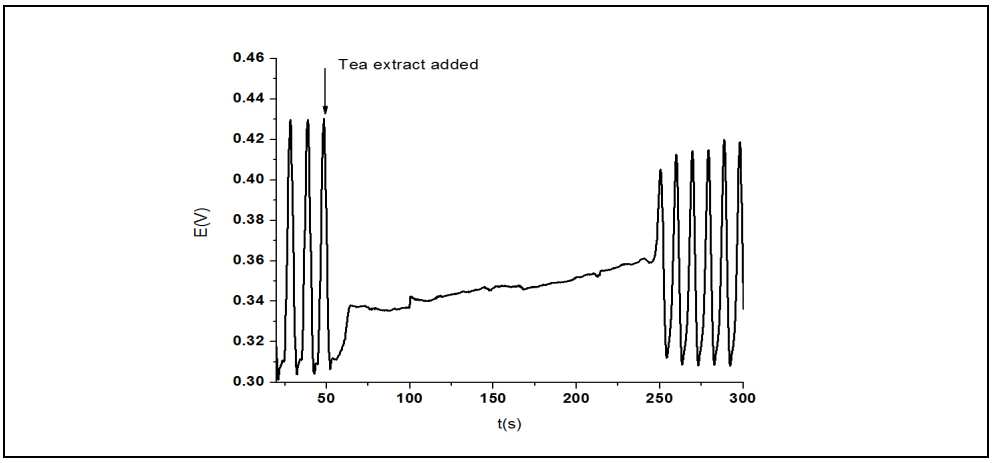

Figure 2. The effect of the roiboos tea extract on the active BR mixture. At the moment indicated by the arrow 0.35 ml of extract was added to the mixture

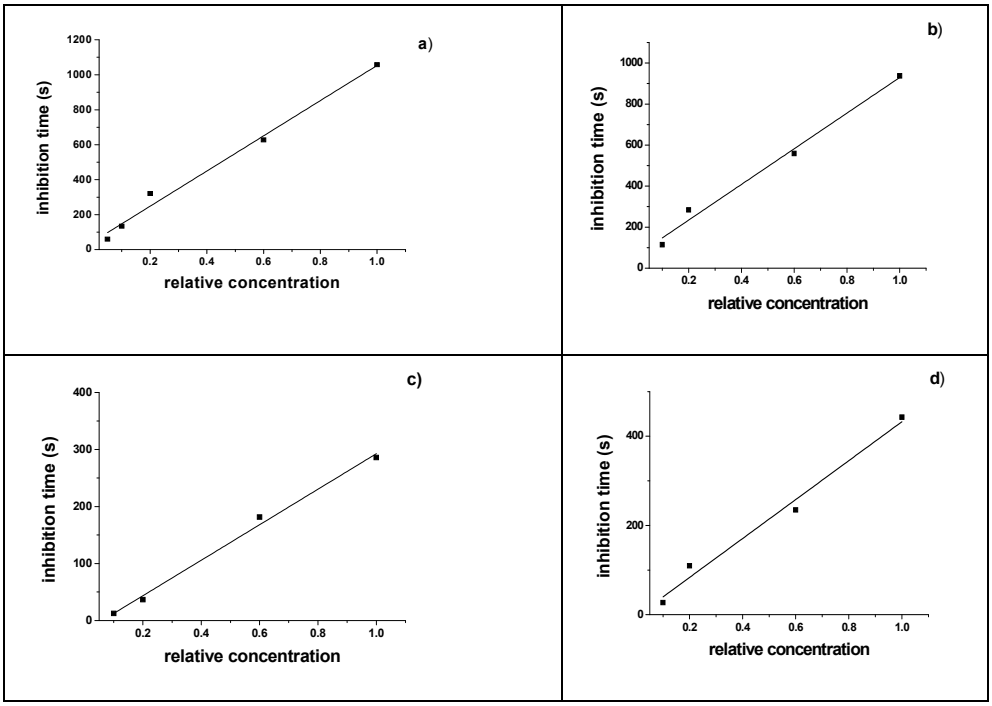

Figure 3. The calibration curves of the tea extracts: a) green tea; b) white tea; c) black tea; d) roiboos tea;

The following equations can be achieved after applying the linear fitting.

t_{inh}=47(±3.29) +1006.6(±6.18) *[green tea]

t_{inh}=60(±3.80) +868.9(±6.41) *[white tea]

t_{inh}=-3.8(±2.28) +436.5(±3.84) *[black tea]

t_{inh}=-18.8(±0.98) +311.4(±1.63) *[roiboos tea]

The antioxidant activity of the extracts was compared with that of the chosen standard (green tea) and R.A.C (relative antioxidant activity related to concentration) was calculated: the ratio between the concentrations of a sample and that of the chosen standard (*green tea*) that give the same inhibition time.

R.A.C. = [standard]/[sample]

R.A.S (relative activity with respect to slopes): the ratio between the slope of the straight line of the sample and that of the chosen standard (*green tea*).

R.A.S. = slope(sample)/slope(standard).

The results are presented in table 1.

Table 1. Values R.A.C and R.A.S for investigated sorts of tea

	R.A.C			
Relative concentration	**Green tea**	**White tea**	**Black tea**	**Roiboos tea**
0.1	1	0.85	0.20	0.10
0.2		0.88	0.34	0.10
0.6		0.89	0.37	0.29
1		0.89	0.42	0.27
R.A.C medium		**0.88**	**0.33**	**0.19**
R.A.S		***0.86***	***0.43***	***0.31***

CONCLUSIONS AND OUTLOOK

The active BR system was used as an analytical method to determine the antioxidant activity of some tea extracts. The analytical sign was the inhibition time which shows linear dependence vs. the concentration. Using the calibration curves two type of relative antioxidant activity was calculated. The R.A.C value show us the sample amount with the same antioxidant activity as the chosen reference (in our case the green tea), the R.A.S show to us the increase of the antioxidant activity with the concentration. The following order of antioxidant activity was found:

Green tea >white tea>black tea> rooibos tea

It can be concluded (using the R.A.C medium value) that approximately three cups of black tea or five cups of roiboos tea has the same antioxidant activity as one cup of green tea, white tea has almost the same activity as green tea. However the advantage of the roiboos tea is due to the absence of caffeine in its composition and because of this can be consumed by peoples with cardio-vascular disease history.

It is to be mentioned that the same order was established by means of TEAC method of determining the antioxidant capacity.

Our future investigation will be focused on the synergetic effect of them.

EXPERIMENTAL SECTION

The instrumental set-up used to implement the proposed method consisted of a double walled glass vessel of 10 mL capacity. Connection to a FALC FA 90 thermostat ensures a constant temperature by water circulation through the temperature jacket. We have chosen a value of 20°C.

Oscillations were monitored with a Pt electrode and an Ag/AgI indicator electrode, both handmade. In the BR reaction both the Platinum and the Ag/AgI electrodes are so called “indicator “ electrodes i.e. their potential oscillate with respect to a reference electrode.

Such a reference electrode should be connected to the system via a double junction salt bridge. To fit a double junction salt bridge into the reactor, however, increases the reactor volume considerably.

Moreover the liquid–liquid junction of a salt bridges always a source of contamination and “memories”.

To keep the reactor volume at a minimum and to avoid memory effects we applied two indicator electrodes. The voltage between these electrodes in the BR reaction was found to be still oscillatory thus the dynamic state of the reactor can be followed by recording that voltage. Potentiometric traces recorded this way were quite reproducible. [19]

They were connected to a PC through a PCI 6036 E data-acquisition interface.

Tea extracts were made using boiled distilled water. Each plant (0.15 g) was mixed with 100 mL of boiling water for 5 min, with constant shaking and the samples were filtered through filter paper. These extracts were considered the stock solutions, and different dilutions were made (for example 10 mL of the stock solution was diluted to 100 mL). The concentration of the diluted solutions –presented in table 1- is related to these ones.

Chemicals and procedure

All chemicals were of analytical grade and were used without further purification. Stock solutions with the following concentration were made: $[H_2SO_4]_0$=220 mM, $[KIO_3]_0$= 180 mM, $[MA]_0$=200 mM, $[MnSO_4]_0$=260 mM, $[H_2O_2]_0$=5.28 M by using double distilled water. In the reactor they were diluted 4 time.

The mixing order was: malonic acid, $MnSO_4$, H_2SO_4, KIO_3, and H_2O_2. Oscillations start after the addition of H_2O_2. At the third oscillation 0.35 mL of tea extract was added to the reactor using a micropipette. The experimental dates were processed by Origin 8 software.

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