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Dedicated to prof. dr. I. C. Popescu
on the occasion of his 70th anniversary

COMMONLY USED RAW FRUIT AND VEGETABLE JUICES OVERALL ANTIOXIDANT ACTIVITY DETERMINATION BY MEANS OF BRIGGS-RAUSCHER REACTION

NORBERT MUNTEAN[a], GABRIELLA SZABÓ[a,*]

ABSTRACT. The antioxidant capacity of commonly used raw fruit and vegetable juices was determined by means of the Briggs-Rauscher oscillating system in batch conditions. Though it is very important to know the composition of a juice mixture sometimes it is not enough to predict the behavior, because of the interaction of the different components. Antioxidant mixtures can present discrepancy in their activity compared to the individual values of the different components. Inhibition times at different concentrations were determined for a number of juices; they presented a linear dependence *vs.* their concentration. The relative antioxidant activity of a mixture of juices was measured and compared to that of the individual juices and a synergistic effect was found.

Keywords: *Briggs-Rauscher oscillating reaction, inhibitory effect, analytical method, raw fruit juice antioxidant capacity*

INTRODUCTION

The Briggs-Rauscher (BR) oscillating reaction was described for the first time four decades ago [1] and represents the iodination and oxidation of malonic acid by iodate and hydrogen peroxide, catalyzed by manganous ion in acidic media. Several papers reported on the investigations regarding the mechanism of it [2-5]. According to their proposed skeleton mechanism the global reaction is the sum of a radical and a non-radical path.

[a] *Department of Chemistry and Chemical Engineering of Hungarian Line, "Babeş-Bolyai" University, Cluj-Napoca, 11 Arany Janos Str, Romania, RO-400028.*
[*] *Corresponding author: gszabo@chem.ubbcluj.ro.*

The existence of free radicals in the BR system was observed by Franz [6] and was evidenced by Cervellati and Furrow [7] through the interaction of them with soy antioxidants. They reported that the inhibitory effect caused by the addition of aqueous extracts of soy flour to an active BR mixture, consisted in the immediate cessation of oscillation. After the consumption of antioxidants contained in the extract, the oscillatory regime was regenerated.

It was developed a new method for monitoring the relative antioxidant activity [8] based on the inhibition time, namely the time elapsed between the cessation and the regeneration of the oscillatory regime. Cervellati et al. determined the relative antioxidant activity for several vegetable and fruit extracts [9], some natural polyphenols [10, 11] the effect of ascorbic acid [12] of hydroalcoholic solutions of antioxidants [13] and of the German red wine [14]. The influence on the active BR mixture of some other species, like lipoic acid [15], tea infusions [16] and some medicinal plants [17], e.g. Balanites aegyptiaca [18], Leontopodium alpinum [19], Cynara scolymus [20], was also studied. Pasteurized and sterilized commercial red orange juices [21] antioxidant activity was also determined with this method. The antioxidant components of tart cherries [22], sweet grapefruit and white grapefruit [23] were characterized. Temperature dependence of the overall antioxidant activity of red wine [24] was investigated.

The BR reaction under flow conditions is very sensitive to small perturbations caused by the analyte addition to the system resulting in the modifications of the oscillation parameters, such as amplitude or period. The determination of the resorcinol antioxidant activity was reported in continuous stirred tank reactor (CSTR) [25].

Kinetically important intermediates of the BR system are I^-, I_3^-, oxyiodine species like HOI, HIO_2, HOO• and iodomalonic acid (IMA); their concentration oscillates between a maximum and minimum value. The free radicals play a key role in the oscillating behavior, and their interaction with antioxidants changes the dynamics of the BR reaction. Recently CO_2 and CO evolution was observed in the BR system [26] and it was reported that this is also the consequence of a radical path [27]. The inhibitory effect was accounted for the fast reaction between the antioxidants and the main intermediates of the BR system, e.g. the hydroperoxyl radicals [9, 11].

Antioxidants are defined as molecules that, in low concentrations compared to those of a substrate, significantly delay or prevent the oxidation of that substrate. In plants they act as chemical self-protective agents against pathogens, also providing defense against various forms of environmental stress such as the harmful UV-B radiation and the reactive oxygen species. Phytochemical investigation of several plant extracts

demonstrated that free radical scavenger components are mainly the anthocyanins, flavonols, flavanols, folates and carotenoids. Plant phenolics are characterized as aromatic compounds that possess one or more phenolic hydroxyl groups.

Dietary antioxidants may affect human health, possibly by acting as antioxidants, are scavengers of reactive oxygen species (ROS), anticarcinogens and cardio protective agents. The plant based antioxidants are believed to have a better biological effect than the synthetic ones [28]. The fruits like dark-purple berries, raspberries, red currant and pomegranate have a high level of hydrolysable tannins, quercetin, gallic acid, anthocyanins, and cyanidins [29]. Apples are a significant source of flavonoids. It was noticed that the apple peels contain more antioxidant compounds, especially quercetin, than the apple flesh [30]. The citrus species are source of phytochemicals such as flavanones, hesperidin and naringenin, vitamin C and carotenoids [31-32]. The beetroot is one of the few vegetables that contain a group of highly bioactive pigment known as betanins, which have high antioxidant and anti-inflammatory capabilities [33].

Recently, it was reported [34] that antioxidant mixtures presented some discrepancy in their antioxidant capacity in comparison with the individual values. In some cases synergetic effects were observed, while in others antagonistic ones were seen.

In the past decades, several analytical methods were developed to determine the antioxidant activity [35]. All the methods are based on the generation of free radicals and their detection. The chemical reaction between these species is monitored usually spectrophotometrically (TEAC assay, DPPH assay, B-carotene bleaching assay) or by measuring chemo luminescence (PCL assay) or electron spin resonance (ESR assay).

The testing system of these assays differs in the pH value (3.3–10.5) of the medium and in the nature of the radicals. Some assays are suitable only for hydrophilic antioxidants, others for hydrophobic and lipophilic ones. The antioxidant activity of the most substances depends on pH so it’s difficult to make a comparison of the obtained values. The activity of the examined antioxidants gives different ranking orders from assay to assay.

In our study the used method was that based on the BR reaction. It works for water soluble antioxidants in acidic medium at a low pH value. Some of the free radicals of the BR reactions are present in the human body too so the conclusions regarding the antioxidant activity of the juices can be useful for a healthy diet.

In this paper, the antioxidant activity of several raw fruit and vegetable juice was studied by the BR method. Selected for testing were the most commonly used fruit juices, like orange, apple, and pomegranate.

Also two vegetable juices were examined (beetroot and carrot), because they are the most recommended by medical staff as a dietary supplement. (It is daily practice that for patients who are under chemotherapy is recommended the red beet consumption). The behavior of the apple, orange, beetroot and carrot mixture against BR free radicals was studied. The inhibition time obtained for the mixture was compared with that of the individual values of the mixture components. Four berries: blackberry, raspberry, blueberry and red currant were also studied. It was reported that berries are considered "super fruits" because of their rich nutrition values. It is to be mentioned, that so far none of these berries were studied by the BR method.

RESULTS AND DISCUSSION

The antioxidant activity of fruit juices

Perturbation of the oscillatory BR system with a diluted fruit juice causes the immediate cessation of the oscillations; the time elapsed between the cessation and the return of the oscillations is the so called inhibition time (Figure 1). The first arrow indicates the addition of the hydrogen-peroxide to the BR system and the start of the oscillatory regime. The second arrow marks the addition of the antioxidant that causes the quenching of the oscillations.

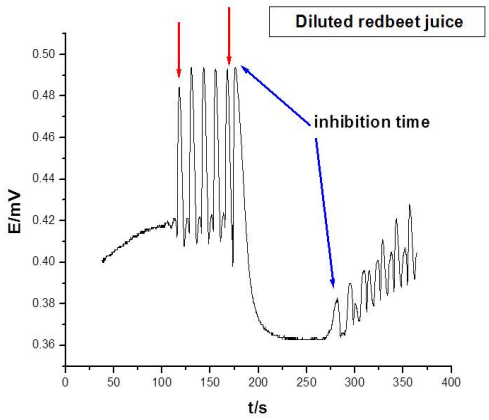

Figure 1. The effect of the red beet juice on the active BR mixture.

The antioxidant activity of the different fruit juices was obtained for several dilutions and the calibration curves were drawn for each of them by linear fitting. Variation of the inhibition time with respect to antioxidant concentration was found to be linear (Figure 2).

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

t_{inh}=-201.0(±80.7) +1.69*10^5(±781.9) * [blueberry]

t_{inh}=-270.2(±11.9) +1.35*10^5(±562.2) * [raspberry]

t_{inh}=-87.4(±66.0) +0.91*10^5(±639.8) * [red currant]

t_{inh}=-191.9(±75.5)+1.67*10^5(±731.0) * [blackberry]

These equations evidenced that the blueberry and the blackberry have almost the same activity.

Beside of the berries, the antioxidant activity of orange, apple (peeled and unpeeled) and pomegranate was studied. The results are presented in the Figure 3. The calibration curve of the unpeeled apple juice has a steeper slope than the peeled one. Evidently, the antioxidant activity varies in the same way.

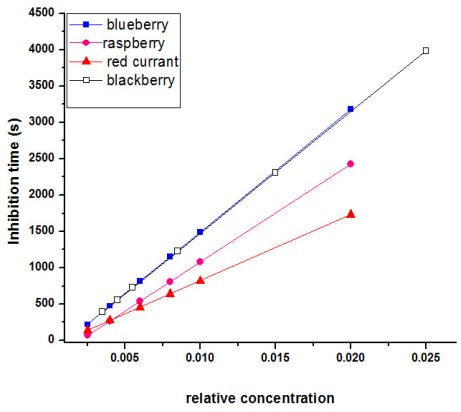

Figure 2. The calibration curves of the berries: a) blueberry(■), b) raspberry(●); c) red currant(▲); d) blackberry(□)

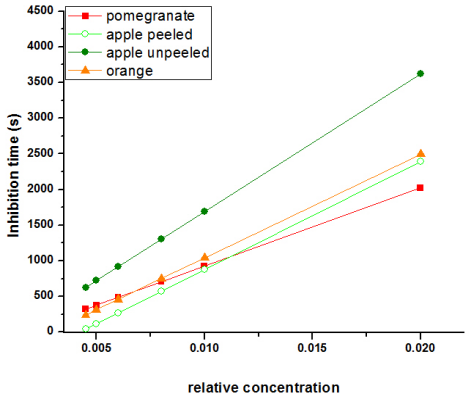

Figure 3. The calibration curves of the a) apple unpeeled (●), b) apple peeled (○); c) orange (▲); d) pomegranate (■)

The obtained equations of the calibration curves are given below.

t_{inh}=-243.3(±53.2)+1.93*10^5 (±657.8) * [apple, unpeeled]

t_{inh}=-644.2 (±23.9)+1.51*10^5 (±172.2) * [apple, peeled]

t_{inh}=-414.6 (±66.0)+1.45*10^5(±567.8) * [orange]

t_{inh}=-170.7(±19.2)+1.10*10^5(±748.4) *[pomegranate]

The effect of the mixture

The antioxidant activity of an unpeeled apple, beetroot, orange and carrot juice mixture was investigated and compared with the individual activities. The inhibition effect of this mixture on the BR reaction was measured and plotted.

In the Figure 4, the calibration curves for the individual juices and the mixture are plotted.

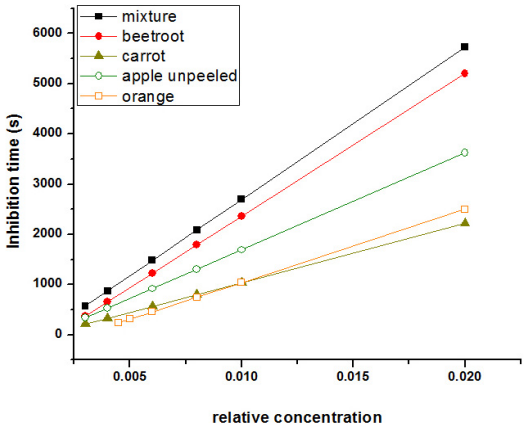

Figure 4. The calibration curves of the: a) mixture (■); b) beetroot (●); c) apple, unpeeled (○); d) orange (□); e) carrot (▲).

The calibration curves were obtained by linear fitting:

t_{inh}=-338.5(±32.4)+3.03*10^5(±6346.0) * [mixture]

t_{inh}=-479.1(±17.1)+2.84*10^5(±351.3.0) * [beetroot]

t_{inh}=-139.9(±27.4)+1.18*10^5(±961.5) * [carrot]

The inhibition time (at a certain concentration) of the mixture is higher than as expected considering the individual inhibition times of the components.

There are three different ways for calculating the relative antioxidant activities, with respect to concentration, to inhibition time and to the calibration curve slope [8].

First of all in order to determine a relative activity there must be chosen a standard. In our case this is the beetroot. From the calibration curve equation of the substance chosen as standard was determined the concentration that should give the same inhibition time as the sample. The ratio between this value and the concentration of the sample gives the relative activity, *i.e.*

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

The concentration of the standard that gives a certain inhibition time can be calculated from its calibration curve.
Relative activity with respect to slopes (R.A.S.): is the ratio between the slope of the calibration curve of the sample and that of the standard; *i.e.*

$$R.A.S. = slope_{(sample)}/slope_{(standard)}.$$

Relative activity with respect to inhibition times (R.A.T.): is the ratio between the inhibition time of the sample and that of the standard at the same concentration:

$$R.A.T. = t_{inhib(sample)}/t_{inhib(standard)}.$$

The chosen concentration must be specified together with the R.A.T. values. These relative antioxidant activities were determined for the studied juices.The obtained results are presented in table 1.

Table 1.Values R.A.C and R.A.S and R.A.T. for investigated fruits

	R.A.C ($c_{standard}$=0.01)	R.A.S	R.A.T (c_r=0.01, t_{inh}=2368 s)
Mixture	1.12	1.07	1.14
Beetroot	1	1	1
Apple unpeeled	0.76	0.68	0.71
Apple peeled	0.48	0.53	0.37
Blackberry	0.68	0.58	0.62
Blueberry	0.69	0.59	0.63
Raspberry	0.54	0.47	0.46
Carrot	0.53	0.41	0.44
Orange	0.53	0.51	0.44
Pomegranate	0.49	0.39	0.39
Red currant	0.48	0.32	0.34

The R.A.T. values reveal which of the juices with the same concentration provide longer inhibition time. The R.A.S. values explain the development of the antioxidant activity related to the concentration of the juice. The R.A.C values compare the sample's concentration with that of the chosen standard for a certain inhibition time.

The best antioxidant activity was obtained for beetroot and it was chosen as the standard. The anthocyanin rich blueberry and blackberry obtained the highest rank, not only among the berries, but also among the other most commonly consumed fruit juices. Despite of the fact that pomegranate is called “super fruit” its antioxidant activity is relatively poor compared to those of the above mentioned berries. The raspberry and the pomegranate obtained a lower position in this rank.

CONCLUSIONS AND OUTLOOK

The active BR system was used as an analytical method to determine the antioxidant activity of some raw juices. The analytical signal was the inhibition time which shows linear dependence vs. the relative concentration. In order to compare the antioxidant activity, the R.A.C., R.A.T. and R.A.S. values were calculated. According to the R.A.C orders of antioxidant activity rank is:

Beetroot > unpeeled apple > blueberry > blackberry > raspberry > carrot = orange > pomegranate > red currant = peeled apple

The unpeeled apple, beetroot, orange and carrot mixture presented synergetic effect.

The BR analytical method is an excellent tool to measure the antioxidant activity of water soluble antioxidants because it is inexpensive and quick.

EXPERIMENTAL SECTION

The measurements were performed in a 20 mL double-walled glass batch reactor, connected to a FALC FA 90 thermostat (accuracy ± 0.1°C) in order to maintain the constant 20°C temperature. Continuous stirring was provided by a FALC 60 magnetic stirrer at a constant stirring rate. Oscillations were monitored with a double-junction saturated calomel electrode (SCE) as a reference electrode and a Pt electrode. They were connected to a computer through a PCI 6036 E data-acquisition interface. The oscillations were recorded through the LabView data-acquisition program and were processed by means of Microcall Origin 6.0 program.

For the preparation of the BR active mixture malonic acid (MA) (Fluka, puriss.), KIO_3 (Fluka, puriss. p.a.; ≥99.5%), KI (Riedel-deHaën, puriss. p.a.), H_2SO_4 (97%, Merck, p.a.), $MnSO_4*H_2O$ (Reanal) were used as received and the H_2O_2 (Fluka, puriss. p.a. ACS; ≥30%) was standardised by manganometric analysis. The stock solutions were obtained from doubly distilled water and they had the following concentrations: $[KIO_3]_0$=0.134 M,

$[H_2SO_4]_0$=0.358 M $[MA]_0$=0.2 M, $[MnSO_4]_0$=0.02 M, $[H_2O_2]_0$=2.6 M. They were kept in the thermostat at a constant temperature (20ºC). The BR systems were obtained by mixing the appropriate amounts of stock solutions (2 mL of each component) in such a way that finally resulted the initial composition $[KIO_3]_i$=0.0268 M, $[H_2SO_4]$=0.0716 M [MA]=0.04 M, $[MnSO_4]$=0.004 M, $[H_2O_2]$=0.52 M. The order of mixing was: malonic acid, $MnSO_4$, H_2SO_4, KIO_3, and H_2O_2. Oscillations start after the addition of the H_2O_2. After the fifth oscillation 0.5 mL diluted raw juice was added to the reactor using a micropipette.

The following fruits and vegetables were selected for inclusion in the study: pomegranate, orange, apple, blackberry, raspberry, blueberry, red currant, carrot and red beet (beetroot). Except the orange and pomegranate, all the fruits and vegetables were cultivated in the region of Cluj. Orange, pomegranate, beetroot, apple and carrot was peeled to remove the outer skin, chopped into small pieces and passed through a juice extractor to produce the fresh, raw fruit or vegetable juice. For comparison was made an unpeeled apple juice too which presented a different behavior compared with the peeled apple juice. Several stock solutions of the fresh raw juices were made by the dilution of 5 mL of each sample to 10 mL with distilled water. Further and consecutive dilutions were made.

During the experiment, the appropriate dilutions of each juice were taken to give inhibition times which were of a reasonable length of time. Juices which were perceived as being low in antioxidant activity from the initial testing were examined at a lower dilution than juices with a higher antioxidant activity.

A sample which combined apple, orange, carrot and red beet (beetroot) was prepared by pouring equal quantities of these juices and was also tested at different dilutions.

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