

Kinetics of Oxidation and Antioxidation Processes in Biological Solutions Observed by NMR Relaxation

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Preliminary studies on the application of NMR relaxation measurements for observations of the kinetics of oxidation processes in tea samples were performed. Tea is a rich source of flavonoids and other compounds with great antioxidant capacity and is therefore beneficial for human health. Its antioxidant potential depends on the type of tea and the method of preparation. Green and black tea prepared from tea bags brewed in water at 90 °C for 20 and 60 min were examined. Oxidation processes were induced in tea samples by adding 3% H₂O₂ in 1:10 proportion. Observed time courses of spin-lattice T_1 relaxation time and parameters obtained from fitting of combined exponential and compressed-exponential function, depended on kind of tea, brewing time, and milk addition. The obtained results showed the usefulness of the NMR relaxation measurements for observation of oxidation processes and antioxidant activity.

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PACS/topics: NMR relaxation, tea, oxidation processes, antioxidants' activity

1. Introduction

Tea, one of the most popular beverage in the world, is regarded to have many benefits on human health, e.g., prevention from cancer, cardiovascular, neurodegenerative diseases and others. Tea infusions contain catechins, theaflavins, oxiaromatic acids, flavonols, tannins, etc., which can act, *in vivo* and *in vitro*, either as pro- or antioxidants. They can act as antioxidants directly scavenging free radicals or chelating transitional metals, but on the other hand, they can lead to free radicals' formation. Each tea is different in terms of composition and concentration of antioxidant compounds thus may exert different benefits on human health. Also the manner of brewing tea may influence activity of antioxidants. Many researches, involving different physical and chemical methods, are provided to find out the best kind of tea and manner of preparation [1, 2].

The antioxidant potential of tea is usually assessed by spectrophotometric methods as free radical scavenging method (DPPH or ABTS) or by measuring the reduction of oxidation state of some metal ions by antioxidants (FRAP or CUPRAC methods) [3]. In this communication the NMR relaxation method for observation of oxidation and antioxidation processes was proposed and tested.

2. Materials and methods

Black tea in 1.6 g tea bags and green tea in 1.75 g bags, commercially available were used to prepare tea infusion samples with 200 ml of tap water at 90 °C and brewing time of 20 and 60 min. Fresh cow milk was added in 1:10 proportion to black tea infusions.

The 3% hydrogen peroxide (purchased at pharmacy) as an initiator of oxidation process was added to tea infusions in 1:10 proportion and well mixed with investigated solution immediately before measurements.

Proton NMR relaxation time T_1 was measured using a Minispec Bruker spectrometer, operating at 1.4 T (60 MHz) at stabilized room temperature (25 °C), using an inversion recovery (IR) method. The values of τ at IR sequence ranged from 50 to 20000 ms. The 90° pulse length was 1.3 μ s, and the repetition time between scans was at least 5 times longer than T_1 .

Data were analyzed using a mono-exponential function by means of the standard Bruker procedure. Measurements errors were 2%.

3. Results and discussion

Water proton spin-lattice relaxation time T_1 as measured at 25 °C in distilled water after addition of 3% H₂O₂ in 1:10 proportion remains unchanged because of a lack of species which could be oxidized. However, after addition of hydrogen peroxide to drinking water, reactive oxygen species are successively produced. They are paramagnetic and cause exponential drop of T_1 . Time courses of T_1 which illustrate kinetics of oxidation processes in distilled and drinking water are presented in Fig. 1.

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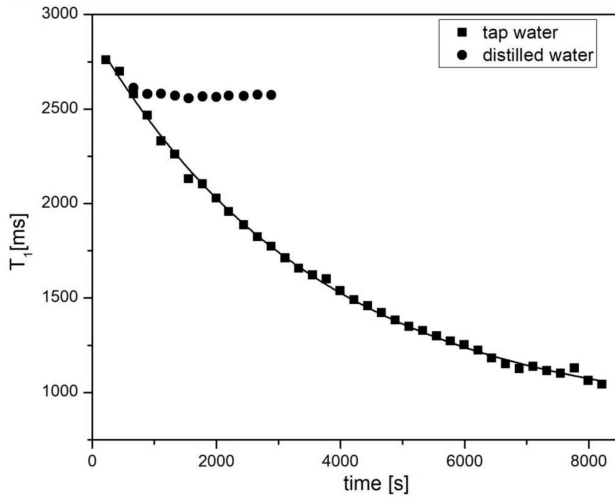


Fig. 1. The time courses of T_1 relaxation time after adding 3% H_2O_2 in a ratio of 1:10 to distilled and drinking water. T_1 values before adding H_2O_2 were 2957 ± 8 ms and 2920 ± 10 ms, respectively.

If drinking water was used to prepare tea extracts, the T_1 run in time is different. Figure 2 shows time courses of T_1 obtained after addition of 3% H_2O_2 to black tea samples taken after 20 min and 60 min of brewing, as well as a green tea sample after 20 min of brewing. The time course of T_1 for black tea infusion (20 min) is initially an exponential decay, thus shortening of T_1 indicates oxidation processes. After reaching the minimum, T_1 starts to increase as a result of antioxidants' activity. This curve is similar to the time course for protein solutions with the addition of vitamin C [4] and may be interpreted as an action of antioxidants present in tea. In aqueous solutions of proteins

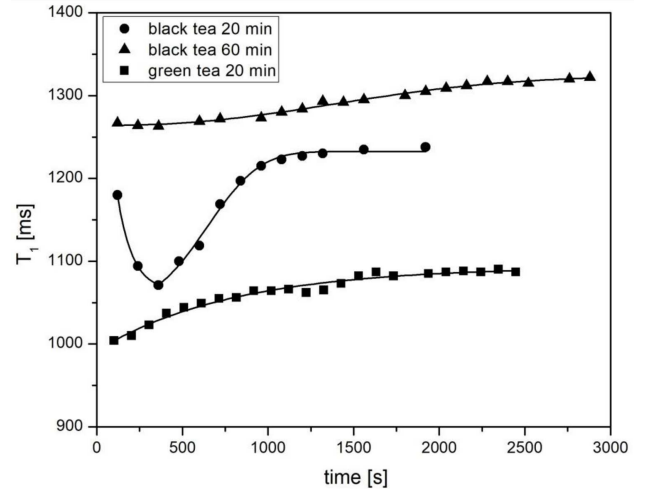


Fig. 2. The time courses of T_1 relaxation time after adding 3% H_2O_2 to black tea after brewing at $90^\circ C$ for 20 min and 60 min and to green tea after 20 min of brewing. T_1 values before adding of H_2O_2 were 1184 ± 5 ms, 1018 ± 6 ms and 792 ± 5 ms, respectively.

(like egg white or bovine serum albumin) the time courses, obtained after the addition of hydrogen peroxide, were well fitted by exponential decays with parameters depending on the type and concentration of proteins. Adding an antioxidant (e.g., ascorbic acid or glutathione) to protein solutions resulted in a different behavior — after exponential decay and reaching a minimum — an increase of the relaxation times was observed [4]. Similar time courses were measured also in native blood serums which contained the endogenous antioxidants [5].

The time courses of T_1 were well fitted by combining the exponential and compressed-exponential function according to the formula:

$$T_1 = \begin{cases} T_{1,0} + T_d \exp\left(-\frac{t-t_c}{t_d}\right) & \text{for } t < t_c, \\ T_{1,0} + T_d + T_g \left(\exp\left(-\left(\frac{t_c}{t_g}\right)^n\right) - \exp\left(-\left(\frac{t}{t_g}\right)^n\right)\right) & \text{for } t > t_c, \end{cases} \quad (1)$$

where $2 \leq n < 4$, $T_{1,0}$ is equilibrium value of T_1 relaxation time, T_d and T_g are the loss and gain in value of T_1 , t_d and t_g are time constants, t_c is turning time constant. Values of selected parameters of time courses obtained by fitting data presented in Fig. 2 are shown in Table I.

Kinetics of free radical scavenging processes in extracts derived from plants, among others in tea, measured by spectrophotometric methods, are usually described by exponential functions [6]. NMR relaxation time of water is affected by a series of interactions, which are: strong interaction of water with free radicals (paramagnetic ions) that shorten T_1 value (the first term in formula (1))

and interaction with radicals quenched by antioxidants and with antioxidants themselves (second term in formula (1)). The first described process may be very fast and thus not measurable at all (Fig. 2 and Table I). The second process, due to variety and amount of antioxidant compounds present in tea infusions [3], may result from a series of higher-order chemical reactions [7], and hence this, unusual compressed-exponential relationship ($n > 1$) was proposed. This two-step relationship was only observed for the tea brewed for 20 min.

Time courses of T_1 changed if the time of brewing of black tea was longer (60 min) or when green tea was used (Fig. 2). There was no clear decay and no clear minimum

TABLE I

Selected parameters of time courses presented in Fig. 2

	T_d [ms]	t_d [s]	t_c [s]	n	T_g [ms]	t_g [s]
black tea 20 min	8.0	89.4	359	3.1	179.4	729
black tea 60 min	0	–	–	2.5	59.5	1763
green tea 20 min	0	–	–	1	101.3	805

for them and only the second part in formula (1) was used as a match to the data (Table I). For green tea time course only an exponential increase of T_1 was observed ($n = 1$ and $t_c = 0$ and $T_d = 0$), which may suggest that because of a greater antioxidant capacity of this green tea in comparison to black tea for the same brewing time, an antioxidant action is faster. Therefore, a characteristic decrease (exponential decay) in T_1 , which corresponds to the oxidation process, was not observed after the addition of H_2O_2 during the experimental time.

Apparently, extending the time of brewing of black tea also causes that only an increase in the time course of T_1 was observed over time. The nature of changes and fitted parameters are also different (Table I). They may suggest faster antioxidant action immediately after adding of hydrogen peroxide in comparison to tea brewed for 20 min. The shortest t_g time and the largest increase in T_g parameter were observed for black tea brewed for 20 min as compared to other samples (Table I). It may indicate that this tea contained antioxidants with high efficiency with respect to the green tea. However, their effect was significantly reduced when the brewing time increased to 60 min (T_g — lower, t_g — higher), which was probably associated with the process of degradation of bioactive substances.

Moreover, the addition of milk to the infusions of black tea caused a disturbance of the regular time course of T_1 (data not shown). Therefore, it was not possible to match any functional dependence to these measurements. This is probably related to the fact, known in the literature, that milk inhibits anti-oxidation processes [8].

4. Conclusions

The presented results and earlier made measurements of T_1 time courses in blood serum of various origins indicate the possibilities of using the NMR relaxation method to study the kinetics of oxidation and anti-oxidation processes in biological samples. It can be seen that regardless of the type of sample, if it contains any antioxidant compounds, the addition of H_2O_2 induces a specific kinetics of T_1 , the course which most likely depends on the type and amount of antioxidant. Changes in T_1 time courses, described by the proposed model, showed both the effect of free radical formation and the oxidation process (shortening of T_1 in time) and the antioxidant activity of bioactive compounds in tea extracts (increase of T_1 in time). The parameters of the T_1 kinetics model allowed to assess oxidation rates and the effectiveness of antioxidants. Thus, the proposed NMR relaxation method can be useful to study the nature of oxidation and antioxidant processes.

References

- [1] A. Yashin, Y. Yashin, B. Nemzer, *Am. J. Biomed. Sci.* **3**, 322 (2011).
- [2] S.P.J. Namal Senanayake, *J. Function. Foods* **5**, 1529 (2013).
- [3] I. Peluso, M. Serafini, *Brit. J. Pharmacol.* **174**, 1195 (2017).
- [4] D. Wierzuchowska, L.W. Skorski, B. Blicharska, *Acta Phys. Pol. A* **129**, 226 (2016).
- [5] D. Wierzuchowska, M. Witek, B. Blicharska, *Acta Phys. Pol. A* **133**, 289 (2018).
- [6] A. Fadda, M. Serra, M.G. Molinu, E. Azara, A. Barberis, D. Sanna, *J. Food Compos. Anal.* **35**, 112 (2014).
- [7] K.Y. Luna-Ramirez, S. Arellano-Cardenas, S. Garcia-Pinilla, M. Comejo-Mazon, *Rev. Mexic. Ingen. Quim.* **16**, 121 (2017).
- [8] S.C. Langley-Evans, *Int. J. Food Sci. Nutr.* **51**, 181 (2000).