ESTIMATION OF ANTIOXIDATIVE PROPERTIES OF TEA LEAVES BY ABRASIVE STRIPPING ELECTROCHEMISTRY USING PARAFFIN-IMPREGNATED GRAPHITE ELECTRODE

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Received May 6, 2009
Accepted June 25, 2009

Dedicated to the memory of Professor Jaroslav Heyrovský on the occasion of the 50th anniversary of the Nobel Prize for polarography.

Electrooxidation potentials of powdered green and black teas were determined by abrasive stripping voltammetry. A circular surface of paraffin-impregnated graphite rod was contaminated with tea microparticles and used as a chemically prepared working electrode in square-wave voltammetric experiments. The responses are ascribed to electrooxidation of flavanols in teas. Low oxidation potentials of some green teas were observed. An advantage of the proposed method is that no extraction of electroactive components is necessary.

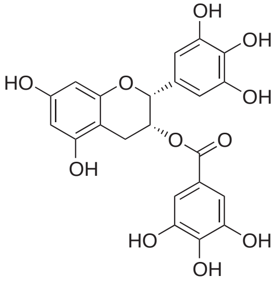
Keywords: Tea; (–)-Epigallocatechin gallate; (–)-Epigallocatechin; (–)-Epicatechin gallate; PIGE; Square-wave voltammetry; Electrooxidation; Antioxidants; Abrasive stripping voltammetry; Green tea.

Tea is an extract of leaves of the *Camellia sinensis* plant[1]. Depending on the degree of fermentation, green, oolong and black teas are produced. These beverages have beneficial effects on the consumer health, such as protection against cardiovascular diseases and cancer, lowering of hypertension, antioxidative activity and reduction of cholesterol[2–4]. Some of these effects are ascribed to flavanols (also known as flavan-3-ols or catechins), which account for 30–40% of the dry weight of solids in brewed green tea[5]. These compounds have shown antioxidant activity by quenching reactive oxygen species and chelating metal ions[6–8]. There are many methods for evaluating antioxidative activity of tea, such as radical scavenging determinations[9–11], measurements of inhibition of lipid peroxidation[12], cyclic voltammetry[13], flow-through column electrolysis[14] and the procedures using thiobarbituric acid[15], or oxygen electrode[16]. In this communication, a simple electro-

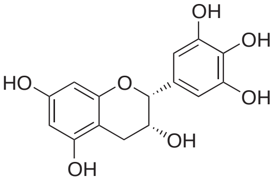
Collect. Czech. Chem. Commun. 2009, Vol. 74, No. 10, pp. 1467–1475

doi:10.1135/cccc2009062

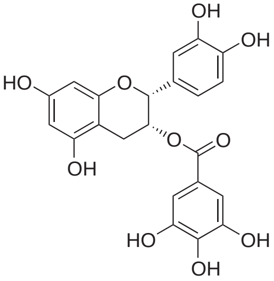
chemical method for estimation of the antioxidant activity of tea is described. The method is based on the voltammetry of microparticles[17–21]. A minute amount of tea powder is mechanically transferred onto the surface of paraffin-impregnated graphite electrode (PIGE) and the potential of maximum of the first oxidation of tea in aqueous electrolyte is measured by square-wave voltammetry[22]. Using the proposed method antioxidant activity of various teas can be quickly assessed as a function of their degree of fermentation. In the production of partially and fully fermented teas, the percentage of monomeric catechins in the *Camellia sinensis* leaves is reduced due to oxidation, resulting in the formation of catechin polymers[1,13].



(-)-epigallocatechin gallate (EGCG)



(-)-epigallocatechin (EGC)



(-)-epicatechin gallate (ECG)

EXPERIMENTAL

Analytical grade KNO_3 and HNO_3 (both Kemika, Zagreb, Croatia) as well as (–)-epigallocatechin gallate (≥95%), (–)-epigallocatechin (≥95%) and (–)-epicatechin gallate (>95%) (all Sigma–Aldrich, St. Louis, USA) were used as received. Water was purified by Millipore Milli-Q system (resistivity 18.2 MΩ cm). Ten green and three black teas were of commercial origin.

Square-wave voltammetric measurements were carried out using the computer-controlled electrochemical system Autolab PGSTAT 30 (Eco-Chemie, Utrecht, Netherlands). Voltammetric curves were recorded using a three-electrode system (Metrohm, Switzerland) with a spectral-grade paraffin-impregnated graphite rod as a working electrode (PIGE), a platinum wire auxiliary electrode and an Ag|AgCl|3 M KCl (Metrohm) reference electrode. PIGE was received from prof. Fritz Scholz as a gift[21]. Before each measurement, the graphite rod surface was rinsed with distilled water, polished with a wet polishing cloth, rinsed again and dried with fine-grade paper tissue. Tea leaves were prepared for measurement by grinding in an agate mortar with an agate pestle until a fine powder was obtained. The PIGE was pressed into the tea powder on a highly glazed ceramic tile and wheel motion was performed for about 5 s. By this procedure the surface of PIGE became contaminated with microparticles of tea or solid flavanols. Only less than 1 mm of the graphite rod was immersed into electrolyte. The electrolyte was 1 M KNO_3 and 10^{-2} M HNO_3 in aqueous solution. The solutions were degassed with high-purity nitrogen prior to electrochemical measurements. A nitrogen blanket was maintained thereafter.

RESULTS AND DISCUSSION

Figure 1 shows square-wave voltammograms of microparticles of tea leaves immobilized on the surface of PIGE and immersed into an aqueous electrolyte. The response consists of a wave between 0.1 and 0.4 V and a peak with the maximum at ca. 0.47 V. The two parts of the response are connected by the inflection at 0.36 ± 0.01 V. The potential of inflection was determined from the minimum of the first derivative of the current–potential curve.

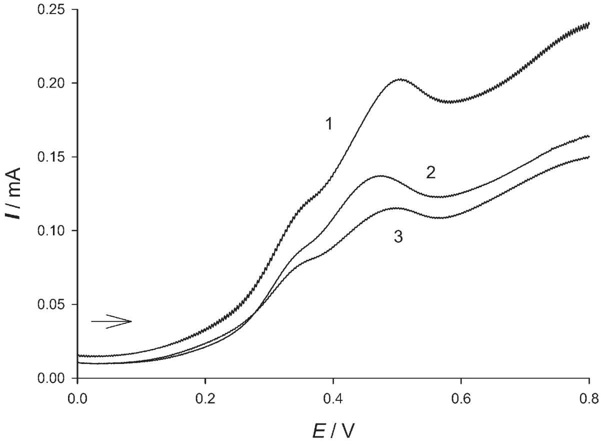


FIG. 1

Square-wave voltammograms of immobilized tea powders in 1 M KNO_3 (pH 2). Japanese green tea Bancha (*1*), Chinese green tea with no brand name (*2*) and Chinese black tea Biber (*3*). Square-wave amplitude E_{sw} = 50 mV, potential step dE = 2 mV, square-wave frequency f = 100 Hz

The inflection potential together with the peak potential of the second part of the response are reported in Table I for ten green and three black teas. These are average values of five independent measurements. The reproducibility of critical potentials is ±2 mV. In Fig. 2 it is shown that the first part of the response can be characterized by the maximum which is separated from the second part by the minimum (curve *1*), or the maximum is missing (curve *2*). These two parts of the response are two voltammetric peaks, which are more or less separated or completely merged. The inflection potential is related to electrooxidation of the most electroactive component of tea leaves. The lower is this potential, the higher is the antioxidative activity of tea[14,16,23]. The potential is the lowest for the Japanese green tea Hojicha (0.345 V) and rather low for three Chinese green teas (0.352

TABLE I
Characteristic potentials of tea microparticles by square-wave voltammetry

Commercial name	E_{infl}, V	E_{p}, V
	Japanese green teas	
Hojicha	0.345	0.478
Gabalong	0.360	0.465
Sencha	0.360	0.468
Kukicha	0.360	0.470
Bancha	0.362	0.484
Gyokuro	0.368	0.464
	Chinese green teas	
No name 1	0.352	0.452
No name 2	0.353	0.457
Yunnan	0.353	0.473
Gunpowder	0.360	0.468
	Black teas	
Darjeeling	0.360	0.461
Biber	0.368	0468
Earl Grey	–	0.454

and 0.353 V), while for the other investigated teas it is between 0.360 and 0.368 V, except for Earl Grey tea. The peak potentials of the second electrooxidation reaction are the lowest for the Chinese green teas with no brand names (0.452 and 0.457 V), which makes them the most redox-active of all the investigated teas. The peak potential of the Earl Grey tea is also rather low (0.454 V). All other samples exhibit peak potentials between 0.461 and 0.484 V.

The most abundant flavanols found in green tea are (–)-epigallocatechin gallate (EGCG), (–)-epigallocatechin (EGC) and (–)-epicatechin gallate (ECG)[1,4,5]. Their square-wave voltammograms are shown in Fig. 3. The response of EGCG consists of two peaks, with the maxima at 0.365 and 0.486 V. The forward, oxidative, and the backward, reductive, components of the response indicate a reversible, or quasireversible electrode reaction[22,24]. The peaks are ascribed to electrooxidation of pyrogallol moiety in the flavonoid B-ring of the molecule (at lower potential) and the galloyl moiety (at higher potential). This is confirmed by square-wave voltammetry of EGC, which is not an ester of gallic acid, but shares the pyrogallol moiety with EGCG (Table II)[24]. Electrooxidation of hydroquinone moiety in the B-ring of ECG and galloyl group of this molecule resulted in a single, broad square-wave voltammetric peak at 0.480 V (see Fig. 3c and Table II)[25]. Figure 4 shows a square-wave voltammogram of microparticles of Indian black tea. The net response consists of the wave and the peak, with the in-

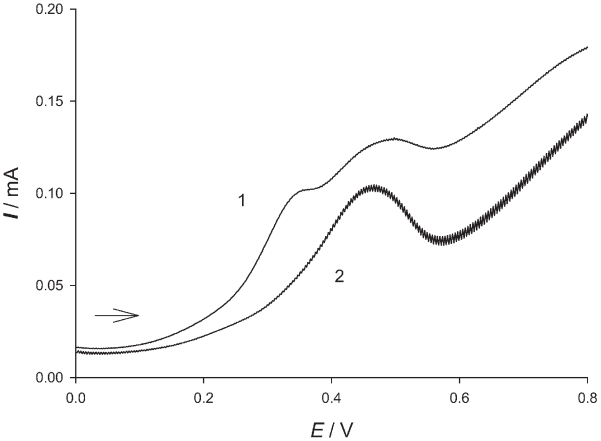


FIG. 2

Square-wave voltammograms of microparticles of Japanese green tea Hojicha (*1*) and Indian black tea Earl Grey (*2*). Experimental conditions are the same as in Fig. 1

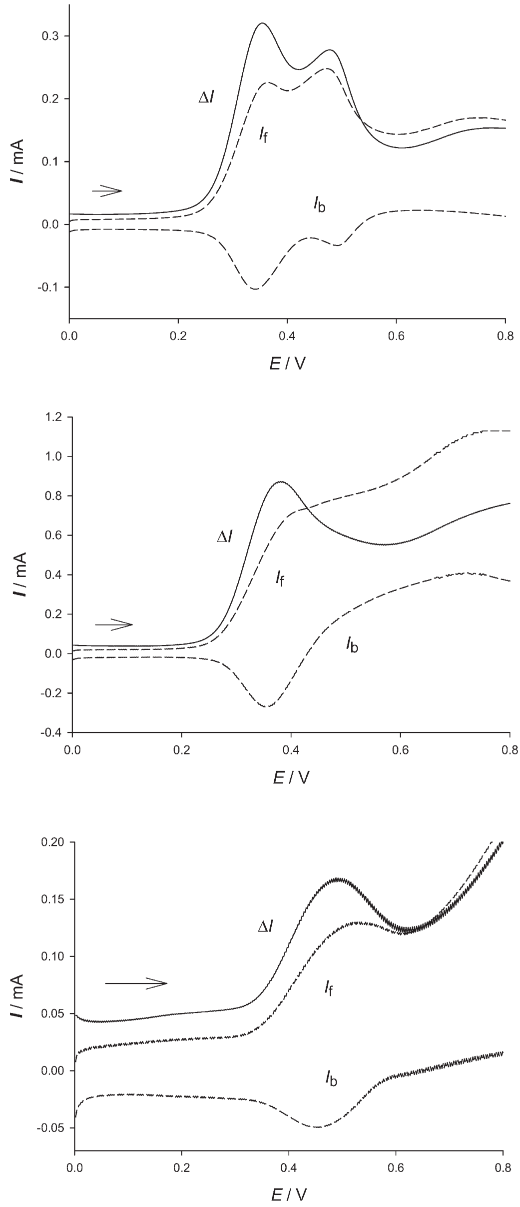


FIG. 3

Square-wave voltammograms of immobilized microparticles of epigallocatechin gallate (a), epigallocatechin (b) and epicatechin gallate (c). A net response (Δ*I*), and its forward (I_f) and backward (I_b) components are shown. Experimental conditions are the same as in Fig. 1

flection at 0.375 V and the maximum at 0.492 V. The backward component of the response is reductive, which means that the response of tea is qualitatively similar to those of catechins shown in Fig. 3. This is further confirmed by the similarity of the potentials listed in Tables I and II. The forward and backward components of the response of microparticles of the Bancha tea are compared to the components of the response of EGCG in Fig. 5. The latter are characterized by the maxima at 0.364 and 0.472 V, and the minima at 0.341 and 0.492 V. The components of the tea response consist of two small oxidative peaks with maxima at 0.36 and 0.50 V, and reductive peaks with minima at 0.34 and 0.495 V. Hence, we suggest that the responses of teas shown in Figs 1, 2 and 4 partly originate from electro-

TABLE II
Characteristic potentials of catechins by square-wave voltammetry

Catechin	$E_{p,1}$, V	$E_{p,2}$, V
EGCG	0.365	0.486
EGC	0.373	–
ECG	–	0.480

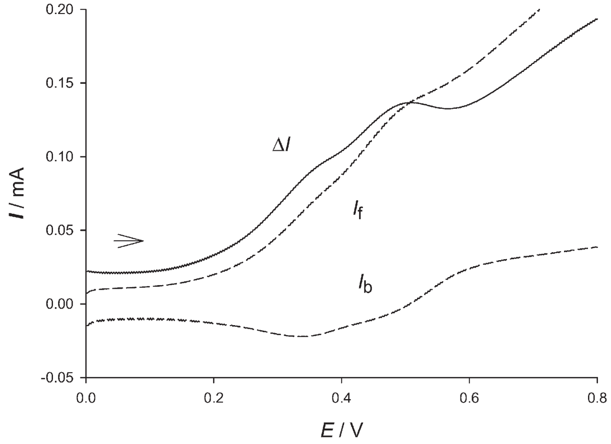


FIG. 4
Square-wave voltammograms of microparticles of Indian black tea. A net response (ΔI), and its forward (I_f) and backward (I_b) components are shown. Experimental conditions are the same as in Fig. 1

oxidation of catechins. However, the oxidation potential of a certain tea is an average of oxidation potentials of its electroactive components and depends on their concentrations. It seems that the observed high redox activity of Chinese and Japanese green teas can be partly ascribed to high concentrations of EGCG and EGC [1].

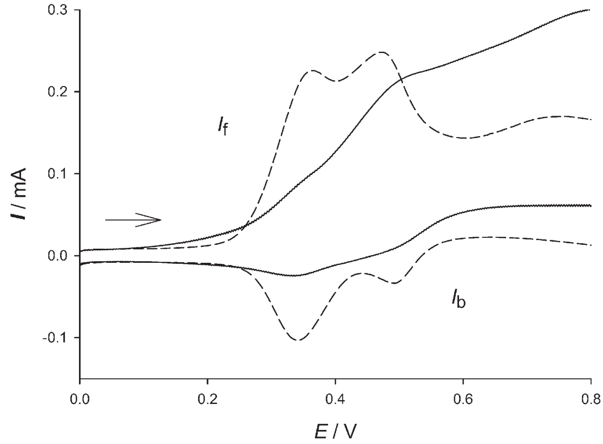


FIG. 5
The forward (I_f) and backward (I_b) components of square-wave voltammograms of microparticles of Japanese green tea Bancha (full line) and (–)-epigallocatechin gallate (broken line). Experimental conditions are the same as in Fig. 1

CONCLUSION

Square-wave voltammetry of microparticles is a useful method for the estimation of electroactivity of tea powders without extraction of its electroactive components. The advantage of this method is that there is no influence of adsorption of oxidation products on the electrode surface. These products block the electron transfer towards dissolved reactants[25]. A fast and simple method for the measurement of electrooxidation potential of tea is quite important because it is generally accepted that the tea with the lowest oxidation potential exhibits the highest antioxidant activity[14].

This work was supported by the Croatian Ministry of Science, Education and Sport under the project No. 098-0982904-2907.

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