C. R. Chimie 15 (2012) 424–427

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Antioxidant activity determination in Sencha and Gun Powder green tea extracts with the application of voltammetry and UV-VIS spectrophotometry

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ARTICLE INFO

Article history:
Received 29 November 2011
Accepted after revision 3 January 2012
Available online 10 February 2012

Keywords:
Cyclic voltammetry
Green tea
Polyphenols
Antioxidants
UV-VIS spectroscopy

ABSTRACT

This article presents the electrochemical analysis of the oxidation of rutin and green tea extract solutions and the antioxidative properties of these substances. Flavonoids and other polyphenols contained in green tea have antioxidative properties due to the presence of various numbers of hydroxyl groups in different arrangements. The investigation of the oxidation of green tea extracts was performed to identify the most effective antioxidant. The cyclic and pulse voltammograms show that the extract of Gun Powder (GP) green tea contained rutin and other polyphenols, while the extract of Sencha (S) tea contained other antioxidants that are oxidised at a more positive potential. The GP extract showed slightly better antioxidative properties than did the S extract. The UV-VIS spectra show that, in addition to flavonoids extracts contain chlorophyll. The results obtained demonstrate that the tested tea extracts show very good antioxidative properties and therefore may be considered as potential stabilising agents that are able to reduce the rate of undesirable oxidation processes.

1. Introduction

Previously published reports have demonstrated that green tea is a rich source of polyphenol compounds. This type of tea mainly grows in Japan, China and India [1]. The most common method of obtaining polyphenols consists of extracting them with organic solvents. The world market offers a wide range of various green tea brands. Green tea contains about 36% polyphenol derivatives in addition to alkaloids and catechins. The remaining compounds include fats, vitamins, sterols and proteins. Undoubtedly, the largest group of compounds consists of flavonoid derivatives including flavones, flavonols, flavonones, catechins, anthocyanins, isoflavones and dihydroxyflavonols. Flavonoids are responsible for the colour and taste of tea [2,3]. The contents of flavonols and flavonones in green tea are very high compared to those in other foods. The most common flavonoid in tea is rutin hydrate. Tea treatment processes (fermentation or steam treatment) and the preparation of green tea drinks for consumption sometimes result in the degradation of the most important components. Therefore, it is of major importance to select proper tea treatment conditions, especially temperature [4,5]. The presence of ascorbic acid can sometimes protect antioxidants from decomposition [6]. The activity of particular antioxidants closely depends on their molecule structures, especially the number of hydroxyl groups [7]. The range of the effects of these bioactive compounds is very wide, and their most important activities include bactericidal and viricidal effects, free radical trapping (free radical scavengers) and the slowing down of oxidation reactions [8–11]. In addition, green tea extracts aid the therapy of many diseases, e.g., malignant diseases or angiopathy [12,13]. Because of these properties, green tea extracts are receiving growing interest among pharmacists [14]. Nowadays, phenol derivatives (flavonoids) are used

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doi:10.1016/j.crci.2012.01.005

to protect materials against ageing [15]. The addition of natural antioxidants to polymers could eliminate hazardous substances.

The flavonoids present, their mechanisms of oxidation [16] their electrochemical properties and their antioxidative capabilities were examined by means of electrochemical and spectrophotometric methods [2,17–19].

2. Materials and methods

2.1. Reagents

The pure flavonoid, rutin, was obtained from commercial source (Sigma-Aldrich) and used as received. The chemical structure of rutin is shown in Fig. 1.

Two types of Chinese green tea, Sencha (S) and Gun Powder (GP), in the form of dry leaves were obtained from Natur-Vit (Poland). Their extraction was performed in 90% ethanol using an OMC SER 148 apparatus from Envag, consisting of three extraction columns and three containers with thimbles, in which samples of powdered and dried tea were placed. The process was carried out in boiling ethanol (temperature, 78.5 °C) for 8 h.

The chemicals used for the preparation of the extract solutions were as follows:

- acetonitrile (CH_3CN) pure p.a. from POCh Gliwice, Poland;
- tetrabutylammonium perchlorate ($[C_4H_9]_4NClO_4$) from Fluka, which was used as a supporting electrolyte.

2.2. Measurement methods

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) measurements were performed using an Autolab analytical unit (Eco Chemie, Holland). A three-electrode system was used for the measurements with the platinum test and auxiliary electrodes. The potential of the tested electrode was measured versus a ferricinium/ferrocene reference electrode (Fc^+/Fc), the standard potential of which is defined as zero, independent of the solvent used. Prior to taking the measurements, all solutions were degassed with argon. During the measurements, an argon atmosphere was maintained over the solution. The effect of the polarisation rate on the electro-oxidation of rutin and green tea extracts in an anhydrous medium was assessed.

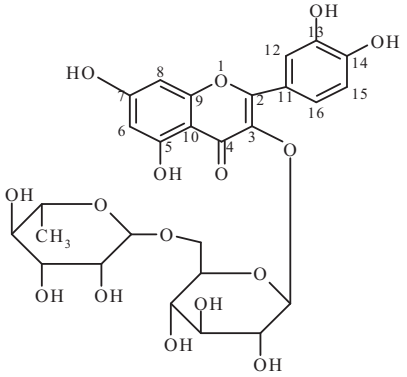

Fig. 1. Chemical structure of rutin.

UV-VIS spectra were recorded in the wavelength range from 190 to 800 nm using a UV-VIS spectrophotometer (Shimadzu UV-24001 PC).

3. Results and discussion

3.1. Cyclic and differential pulse voltammetry of rutin and green tea

The electrode reactions characterising the electrochemical oxidation of flavonoids on a platinum electrode were examined by means of cyclic and DPV. The cyclic voltammetric profile is an important tool for assessing the presence of homogenous chemical reactions following the electrochemical oxidation. DPV has a higher resolution, which enables better separation of the peaks characterising the subsequent steps of an electro-oxidation reaction. The half-wave potential of a peak in a cyclic voltammogram corresponds to the potential of a peak occurring in a differential pulse curve and is characteristic of each of the subsequent steps of the electrode reaction being investigated.

Select cyclic and differential pulse voltammograms recorded in the solution of the rutin, green tea extracts and the supporting electrolyte are presented in Fig. 2.

Within the potential range in which the flavonoid oxidation peaks appear, the supporting electrolyte (tetrabutylammonium perchlorate in acetonitrile – 0.1 mol L^{-1}) shows no characteristic peaks except for the charging of the electrical double layer (Fig. 2A, B, curve 4). However, a small wave appears in the supporting electrolyte within the potential range from 0.5 to 1.0 V in the voltammograms. This wave can be attributed to the presence of $(C_4H_9)_4NClO_4$ and perchlorate ion oxidation. Fortunately, this wave current is relatively low in comparison with the peak currents attributed to the flavonoid oxidation. Zieja, Gadowska-Trzos, and Stojek [20] have reported that this wave can also be caused by the oxidation of impurities such as water and other organic substances.

The voltammograms recorded by CV show that the oxidation of rutin on a platinum electrode proceeds in at least four electrode steps before the oxidation potential of the electrolyte (1.8 V). Four oxidation peaks (peaks I, II, III and IV) are observed at the potentials 0.36 V ($E_{1/2I}$ – half-wave potential), 0.69 V ($E_{1/2II}$), 1.06 V ($E_{1/2III}$), and 1.50 V ($E_{1/2IV}$) (Fig. 2A, curve 1). The electrode reactions investigated are irreversible reactions. The cyclic voltammograms of rutin and the extract of GP green tea are similar. GP is oxidised irreversibly, as is rutin, in at least in four electrode steps: 0.36 V ($E_{1/2I}$), 0.73 V ($E_{1/2II}$), 1.08 V ($E_{1/2III}$), and 1.56 V ($E_{1/2IV}$) (Fig. 2A, curve 2). In contrast, the extract of S green tea is oxidised in at least two electrode steps at potentials 1.11 V ($E_{1/2III}$) and 1.62 V ($E_{1/2IV}$) (Fig. 2A, curve 3). With the use of DPV, rutin and GP solutions are oxidised in at least in four electrode steps. Their DPV voltammograms show four peaks at the following potentials: for rutin, 0.33 V (E_{pI}), 0.69 V (E_{pII}), 1.04 V (E_{pIII}), and 1.50 V (E_{pIV}) (Fig. 1B, curve 1), and for GP extract, 0.32 V (E_{pI}), 0.70 V (E_{pII}), 0.98 V (E_{pIII}), and 1.56 V (E_{pIV}) (Fig. 2B, curve 2). The solution of

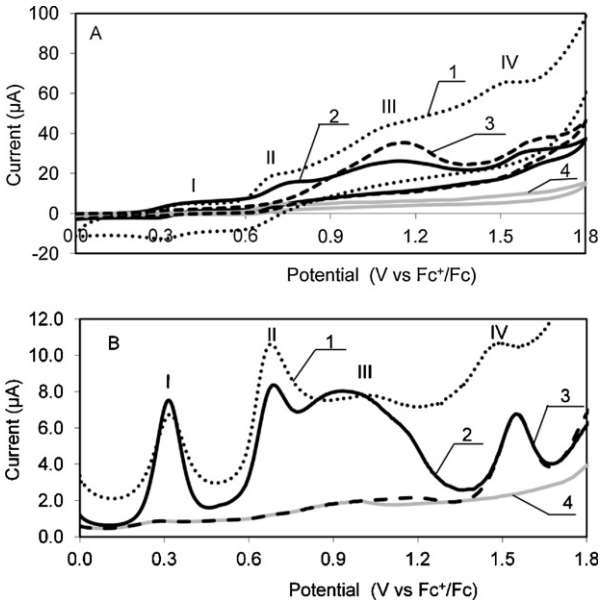

Fig. 2. A. Cyclic voltammograms (CV) of the oxidation of rutin and green tea extracts; $c = 1$ g dm^{-3} in 0.1 M $(C_4H_9)_4NClO_4$ in acetonitrile; $\nu = 10$ mVs^{-1}, electrode – Pt. B. Differential pulse voltammetry (DPV) of the oxidation of rutin and green tea extracts; $c = 1$ g dm^{-3} in 0.1 M $(C_4H_9)_4NClO_4$ in acetonitrile.

sample S is oxidised in at least one electrode step (Fig. 1B) at a potential of 1.56 V (E_{pIV}) (Fig. 2B, curve 3). It can be concluded that the extract of GP contains primarily rutin and other compounds, while the S extract contains no rutin but contains other phenol compounds.

3.2. UV-VIS analysis

The rutin and green tea solutions were examined by UV-VIS spectrophotometry, and the results are shown in Fig. 3. Three characteristic absorption bands of rutin were present at 202 nm and 252 nm (which correspond to the transfer of π-π* electrons in the benzene ring) and at 349 nm (which corresponds to the transfer of π-π* electrons in B and C rings). The intensity and the positions of these two bands depend on the number and positions of hydroxyl groups in the rutin molecule. The weaker band at

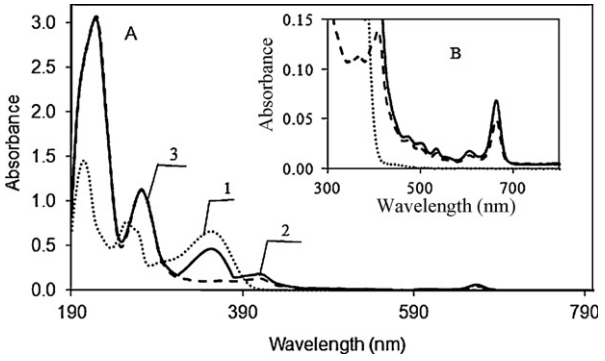

Fig. 3. UV-VIS spectrum of rutin and green tea extracts in acetonitrile: curve 1: rutin; curve 2: GP (Gun Powder); curve 3: S (Sencha).

296 nm corresponds to keto-hydroxyl tautomerism between the four and seven positions in the rutin molecule. (Fig. 3A, curve 1) [17]. These are characteristic bands of flavonoids. The spectra of GP and S extracts were not identical. There were absorption bands at 217, 274, 407, 598 and 667 nm. Spectrum of GP extract includes additional band at 350 nm, which is characteristic for rutin. It can be concluded that the extract of GP contains primarily rutin and other compounds, while the S extract does not contains rutin but contains other phenol compounds. In comparison to the spectrum of rutin, spectra of green tea extracts showed additional absorption bands at 407, 589 and 659 nm characteristic for chlorophyll (Fig. 3B, curve 2 and 3) [21–23]. Based on the analysis of the UV-VIS spectra, it can be concluded that, in addition to rutin and other phenol compounds, the samples of green tea contain chlorophyll, which imparts colour to both GP and S green tea.

4. Conclusions

The electrochemical examination of the oxidation of rutin and green tea extracts, which contain numerous phenol compounds, yielded essential information about the antioxidative properties of drinks consumed by people. The low oxidation potential of the compounds contained in the samples investigated indicates that they are excellent scavengers of free radicals. The cyclic and pulse voltammograms obtained demonstrate that the extract of GP green tea contained rutin and other polyphenols, while the extract of S contained other flavonoid derivatives that are oxidised at a more positive potential. The green tea extracts showed very good antioxidative properties. The analysis of the UV-VIS spectra of the rutin and green tea samples revealed that, in addition to flavonoids, green tea contains chlorophyll, which is responsible for the teas colour. The methods of cyclic and DPV and UV-VIS spectrophotometry allow faster and less expensive analyses than do chromatographic methods, which often require great amounts of toxic solvents and long determination times. The voltammetric methods are environmentally friendly and can be successfully used for the determination of polyphenol contents in tea samples in place of conventional tests estimating the antioxidative activity of the compounds.

The electroanalytical methods are useful and valuable tools for the investigation of flavonoids and intermediate products of their electro-oxidation under potentiostatic conditions. Moreover, electroanalytical analysis can be an important source of information useful in the determination of flavonoids potential applicability in the ageing and therapeutic processes.

Acknowledgements

The work was financially supported from the research project No. N N209 450639 and No. N N209 088038.

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