ELECTROCHEMICAL BEHAVIOUR OF FLAVONOIDS ON A SURFACE OF A CARBON PASTE ELECTRODE

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ABSTRACT

Recent papers discuss relation of flavonoid compounds and tumour diseases. In addition it is impossible to say that effect of flavonoids is positive or negative without epidemiological studies. That is why it is necessary to suggest a simple, low cost and sensitive technique for determination of these compounds in biological material. The electrochemical techniques are very suitable for these purposes. Here we suggested the electrochemical technique for the determination of rutin, quercetin, quercitrin, chrysin and diosmin by square wave voltammetry using carbon paste electrode as a working electrode. The limits of detection of the studied flavonoids were about tens of nM, except diosmin (tens of μM).

INTRODUCTION

It is common knowledge that compounds contained in food considerably affect our health. People living in developed country have not usually problems with insufficient food and nutrient intake, contrarywise, they consume more than need[1]. Therefore there is needed to take care of compounds which are present in minor amount in food as well. For example vitamins or mineral elements belong to the minor food component, but there are many other constituents which have both positive and negative affect on health of the consumer. In relation to this, some authors speak about foodstuffs called nutraceutics[2]. Nutraceutics represent foodstuffs that could positively affect physiological functions of a human organism. To be specific, it was proved that they influence general state of health and could significantly support a physiological capacity or decrease a possibility of a disease appearing[3]. Different groups of metabolites such as phenolic acids, lignans, phytosterols, carotenoids, glucosinolates and also flavonoids influence human health as described above[2]. Flavonoids comprise a wide-ranging group of plant phenols. Up to now, more than 4,000 of flavonoid compounds are known and new ones have been still discovering. Flavonoids are derived from heterocyclic oxygen compound, flavan, which is formed by two benzene rings linked by heterocyclic pyran (Fig. 1). All three rings could be substituted by hydroxy- or methoxy-groups and particular derivates differs only in degree of substitution and oxidation. We distinguish following basic structures of flavonoids: catechins, leukoanthocyanidins, flavanons, flavononols, flavons and anthocyanides. Natural flavonoids occur mostly in form of *O*-glycoside. Existence of free aglycones is rare, but it was observed that concentration of free aglycones could increase in specific conditions (during technological processing at high temperatures and also in acidic medium) when hydrolysis of glycosides may proceed[4,5].

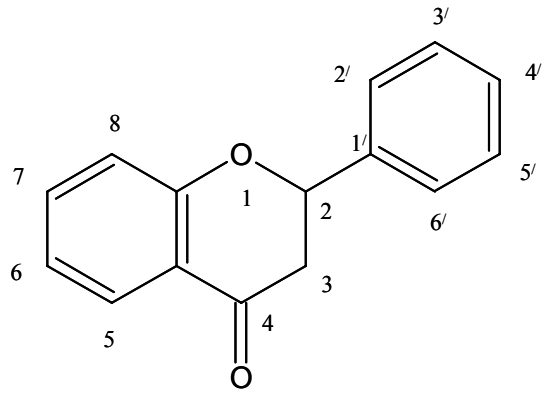

Fig. 1. Flavonoid structure made of two benzene rings linked by heterocyclic pyran.

Antioxidative activity of flavonoids, which means e.g. capability to scavenging of reactive oxygen species or protection of LDL fractions of lipids against their oxidative modification (crucial during atherosclerosis progress), has been studying. Moreover, flavonoids have other effects – anticoagulant, antiestrogenic, antiinflammatory (digestive tract), antimicrobial, or spasmolytic (Fig. 2). In addition opinions of the scientists markedly vary in case of the relation of flavonoids with tumour diseases. Number of authors assign to them anticancer effect which is ascertained in *in vitro* tests and on animal models. On the other hand, this effect has not been proved in epidemiological studies[2,6–8]. The same scientists allege that an anticancer activity of flavonoids could be deduced from theirs chemical structure and that the activity appear in both initiatory and promotional phase of carcinogenesis. In connection with this activity, the following mechanisms are discussed: **i)** inhibition of enzymes of phase I (PIE) encouraging oxidation, **ii)** induction of conjugating and detoxificating enzymes of phase II, **iii)** direct interaction

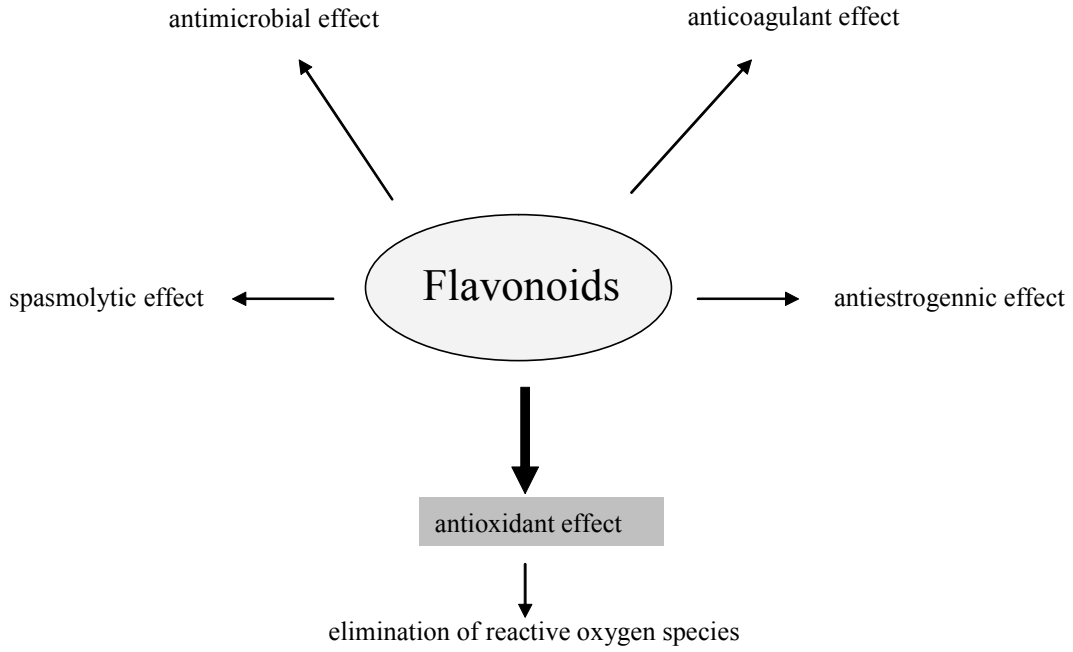

Fig. 2. Scheme of possible effects of flavonoids.

with DNA, **iv)** induction of apoptosis (programmed cell death), **v)** inhibition of cell proliferation, **vi)** antioxidant action, **vii)** modulation of immune system.

On the contrary, there are also authors which are convinced of carcinogen affecting of some flavonoids[9, 10]. They assert that applying of high dose of flavonoids, for example diosmin, on the one hand effectively protects formation of certain adducts with DNA but on the second hand has simultaneously genotoxic influence. It could be taken an exception that as high amount of flavonoid is not able to achieve without food supplements. But it was found out that flavonoids concentration can increase to mmol.l^{-1} due to limited resorption of them in small intestine and water resorption in large intestine[6, 7].

In this work we were concerned with suggestion of electroanalytical technique for determination of quercetin, quercitrin, rutin, diosmin and chrysin on the surface of a carbon paste electrode.

MATERIAL AND METHODS

Chemicals

Methanol, NaH_2PO_4, diosmin, chrysin, quercetin dihydrate, carbon powder, and mineral oil were purchased from Sigma Aldrich Chemical Corp. (St. Louis, MO, USA). Rutin trihydrate and quercitrin dihydrate were from Roth GmbH (Karlsruhe, Germany). Na_2HPO_4 was purchased from Merck (Darmstadt, Germany). Solutions were prepared using ACS water from Sigma Aldrich.

Electrochemical measurements

Electrochemical measurements were performed with AUTOLAB Analyser (EcoChemie, Netherlands) connected to VA-Stand 663 (Metrohm, Switzerland), using a standard cell with three electrodes. The working electrode was a carbon paste electrode. The reference electrode was an Ag/AgCl/3M KCl electrode and the auxiliary electrode was a graphite electrode. The supporting electrolyte was prepared by mixing buffer components. For smoothing and baseline correction the software GPES 4.4 supplied by EcoChemie was employed. SWV experiments were performed at room temperature. The measurements were performed using the following parameters: initial potential = 0.1 V, end potential = 1.2 V, pulse amplitude = 49.85 mV, step potential = 1.95 mV, and frequency = 180 Hz. Phosphate buffer (0.1 M NaH_2PO_4 + 0.1 M Na_2HPO_4, pH 7.0) was used as a supporting electrolyte. The carbon paste (about 0.5 g) was made of graphite powder (Aldrich) and mineral oil (Sigma; free of DNase, RNase, and protease). The ratio of the graphite powder and mineral oil was 70/30 (*w*/*w*). This paste was housed in a Teflon body having a 2.5-mm-diameter disk surface. Prior to measurements, the electrode surface was renewed by polishing it with soft filter paper.

RESULTS AND DISCUSSION

Using of an electroanalytical approach for detection of flavonoids is rare[11, 12]. Analytical determination of flavonoids is currently maintained mainly by chromatographic procedures[13–17] and capillary electrophoresis[15, 18, 19]. Electrochemical procedures represent advantageous possibility of miniaturisation and simultaneous analysis *in vivo* without disruption of living organism in comparison with these commonly used methods.

Recently, method for electrochemical determination of quercetin using glassy carbon electrode was published[12]. A renewing of the electrode surface (polishing by alumi-

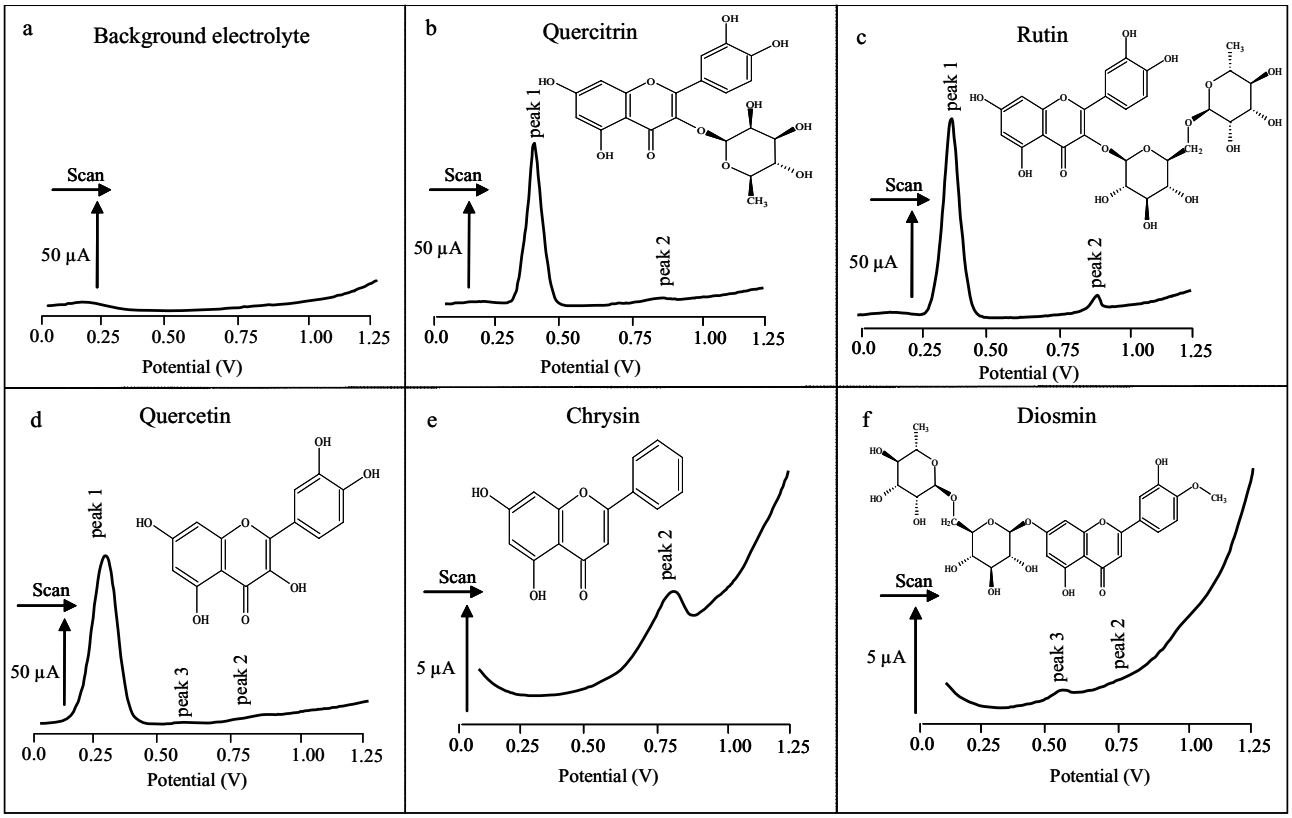

Fig. 3. Square-wave voltammograms of the studied flavonoids at 10 µM concentration – a) background electrolyte, b) quercitrin, c) rutin, d) quercetin, e) chrysin and e) 100 µM of diosmin. The SWV parameters were as follows: initial potential = 0.1 V, end potential = 1.2 V, pulse amplitude = 49.85 mV, step potential = 1.95 mV, and frequency = 180 Hz. Phosphate buffer (0.1 M NaH_2PO_4 + 0.1 M Na_2HPO_4, pH 7.0) was used as a supporting electrolyte. For other details see Materials and Methods section.

na, ultrasound) is the main disadvantage of this approach. Therefore we suggested the method utilising carbon paste electrode (CPE) for determination of quercetin, rutin, quercitrin, chrysin, and diosmin by square wave voltammetry (SWV) in the presence of phosphate buffer. The surface of the CPE is renewed by polishing using filtration paper prior to this purpose, which is much easier in comparison with glassy carbon electrode.

Four oxidative signals were observed on the obtained voltammograms. The signals were called as peak 1, peak 2, peak 3 and peak 4 (Fig. 3b, c, d, e, f). Brett[12] described two of these signals – peak 1 and peak 2 as electrochemical responses of oxidation of hydroxyl group in position 7 (Fig. 1). We assume that two other signals (peak 3 and peak 4) probably correspond to oxidation of hydroxyl group in position 5 according to Janeiro and Brett[20] (Fig. 1). Moreover, the influence of glycoside part of the flavonoids on the resulted electrochemical signal was studied. We observed the peak 1 potential of quercetin at potential of 0.15 V and signals of glycosided quercetin (rutin and quercitrin) at potential 0.25 V (Fig. 3b, c, d). The potential shifting (more than 100 mV to positive potential) could be caused by presence of glycoside part covalently bound on the quercetin (see in Fig. 3). Electrochemical signal of chrysin and diosmin were lower in comparison with signal of quercetin and its glycosylated derivatives (Fig. 3d, e). The observed decrease of electrochemical response is probably caused by different chemical structure of the studied compounds. The highest decrease of peak height was noticed in case of diosmin, which contain two glycoside molecules in its structure. In addition the detection limits (3 S/N) of studied analytes were calculated, see Table 1.

Table. 1. Limits of detection (LOD, 3S/N) of the studied flavonoids

Flavonoids	LOD (nM)
Quercetin	1.04
Quercitrin	0.73
Rutin	0.85
Chrysin	56
Diosmin	2663

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STRUCTURE-ACTIVITY RELATIONSHIP OF *TRANS*-RESVERATROL AND ITS ANALOGUES

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ABSTRACT

Cancer is one of the main reasons of death in both men and women, claiming over 6 million people each year worldwide. Chemoprevention in combination with anticancer treatment is therefore important to reduce morbidity and mortality. *trans*-Resveratrol is a naturally occurring phytoalexin present in grapes and many other plants. It was found that this compound possesses a variety of biological activities. One of the most striking biological activities of *trans*-resveratrol soundly investigated during the late years has been its cancer-chemopreventive potential. To improve the beneficial effects of *trans*-resveratrol it is necessary to know the structure-activity relationship of *trans*-resveratrol and its analogues. This gives us useful information for further chemopreventive drug design.

trans-Resveratrol (3,4',5-*trans*-trihydroxystilbene; t-RES) is a polyphenolic compound accounting to the stilbene class (Fig. 1). It has been found in high concentrations in a wide variety of plants, including grapes, peanuts, berries, pines and traditional oriental medicine plants[1]. Thus, relatively high concentrations of this compound are present in grape juice and, especially, in red wine[2,3]. In plants t-RES is synthesized in response to stress conditions such as trauma, UV irradiation, exposure to ozone and fungal infection, and thus it can be considered to be a phytoalexin, a class of antibiotics of plant origin[4,5]. t-RES has been reported to be a phytoestrogen due to its structural similarity to the estrogenic agent diethylstilbestrol[6]. In recent years, it has been shown to exhibit estrogenic activity in mammals[7,8]. t-RES has been reported to have both anti-carcinogenic and cardioprotective activities, which could be attributed to its antioxidant and anti-coagulant properties[9,10]. Besides these effects, t-RES has been reported to be effective in inhibiting platelet aggregation and lipid peroxidation, altering eicosanoid synthesis, modulating lipoprotein metabolism[11–13], and exhibiting vasorelaxing and anti-inflammatory activities[3,5]. In different rodent species as well as in humans, t-RES is well absorbed, distributed to various organs, and metabolized to *trans*-resveratrol-3-*O*-glucuronide and *trans*-resveratrol-3-*O*-sulfate[14–16].