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Flavonoid electrochemistry: a review on the electroanalytical applications

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Review

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Abstract: Flavonoids are polyphenolic compounds widespread in vegetal kingdom. They present a C-15 skeleton, which is divided into three units A, B and C. Unit C is an oxygen containing heterocyclic, whose oxidation state and saturation level define major subclasses. Units A and B are aromatic rings, in which four major types of substituents, *i.e.* hydroxyl, methoxyl, prenyl and glycosides, lead to over 8000 different flavonoids. The great healthy-protecting value of these phytochemical biomarkers has attracted the attention of scientific community. Their main biological actions include anticancer and anti-inflammatory properties, which are strictly linked to antioxidant activities. So that, electroanalysis have been extensively applied on mechanistic studies and also for analytical determinations. This review presents the state of the art regarding the main applications of electroanalysis on the flavonoid research. The approaches on redox behavior characterization leading to a better understanding of structure antioxidant activity relationships are highlighted.

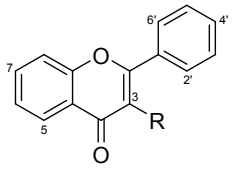
Introduction

Flavonoid is a generic name for a large group of plant metabolites, which as other polyphenols are mostly derived from the biosynthetic route of shikimic acid. They have a C_6-C_3-C_6 general core, which is represented by three units, A, B and C (**1**). Units A and B are essentially aromatic rings of phenolic nature, whilst unit C is an oxygen containing hetereocycle, benzo-γ-pirone, whose level of oxidation, namely the presence or absence of: C_2=C_3 double bond; C_3-OH hydroxyl group; and C_4-keto group defines their subclasses and also the controversial nomenclature (Cook & Samman, 1996; Tsao, 2010). Accordingly to these narrow structural differences they have been divided in six major classes, flavones (**1**), flavonols (**1**), flavanones (**2**), flavanonol (**3**), catechins, flavonols and flavan-3-ols (**4**) and anthocyanins (**5**), which can be extended to ten by the inclusion of isoflavonoids (**6**), neoflavonoids (**7**) and also the open ring chalcones (**8**) and aurones (**9**) (Cook & Samman, 1996; Rice-Evans et al., 1996; Harborne & Williams, 2000; Narayana et al., 2001; Erlund, 2004; Taylor & Grotewold, 2005; Tsao, 2010; Hidalgo et al., 2010; Mülazımoğlu et al., 2011).

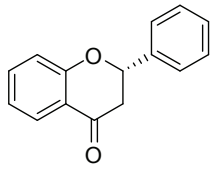
Furthermore, the hydrogen atoms in each unit A, B or C, can be replaced by three main types of substituent, hydroxyl (-OH), acetyl (-OR), or sugar ($C_xH_yO_z$). Whereas, the low molecular weight aglycones can coexist with numerous glycosides, dimeric, oligomeric and polymeric derivatives (Cook & Samman, 1996; Erlund, 2004; Tsao, 2010). Hence, despite the lack of diversity of recognized substituents, it is already known the existence of almost thousand flavonoids (Tsao, 2010; Mülazımoğlu et al., 2011).

Among such subgroups, flavones, flavonols, flavanones and flavanonols are almost ubiquitous in the plant kingdom. They can be isolated from fruits, seeds, flowers and other vegetal tissues of uncountable number of plants (Cook & Samman, 1996; Rice-Evans et al., 1996; Harborne & Williams, 2000; Erlund, 2004; Taylor & Grotewold, 2005; Tsao, 2010; Hidalgo et al., 2010; Mülazımoğlu et al., 2011). In turn, anthocyanins and their precursors catechins, also named as flavan-3-ols or flavanols, are mostly found in the peel of red fruits, tea leaves, coffee and cacao bean. Yet, isoflavones are typical biomarkers in soybeans and other leguminous plants (Tsao, 2010). On the other hand, the open ring chalcones have been detected in apples, hops and beers, whereas the scarcer occurrence neoflavonoids is mainly represented by dalgerbin, a 6-hydroxy-7-methoxy-neoflavonoid (Tsao, 2010).

The relevance of such widespread class of phytochemicals refers to the chemical properties of phenol and benzenediol isomers (Cook & Samman, 1996; Rice-Evans et al., 1996; Harborne & Williams, 2000; Tsao, 2010). In fact, they have been explored as precursors in organic synthesis of drugs, antioxidants and other complex molecules (Cook & Samman, 1996; Rice-Evans, et al., 1996; Harborne & Williams, 2000).



R=H Flavones
R=OH Flavonols or flavon-3-ols



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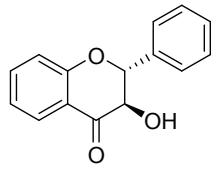

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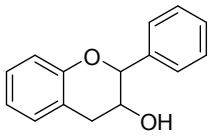

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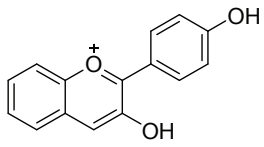

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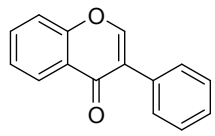

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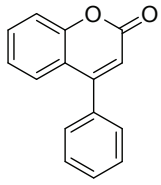

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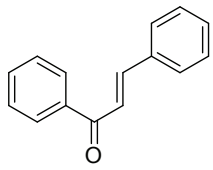

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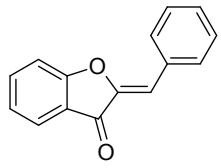

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Indeed, flavonoids are crucial for the plant survival, playing important physiological roles, which include UV protection, antifungal and phytoalexin properties, as well as some contributions as plant regulators (Harborne & Williams, 2000; Taylor & Grotewold, 2005). On the other hand, the sensorial characteristics of flavonoids, *i.e.* color and tasting properties have also key functions on the plant life cycle. For instance, the attractive colors of flowers and fruit peel are useful for pollination and seeds dissemination, yet in leaves such coloring properties are hidden by the intense green of chlorophylls; meanwhile the bitter taste preserves the photosynthesis level by repulsing herbivorous (Harborne & Williams, 2000; Taylor & Grotewold, 2005).

In turn, flavonoids have proved to be essential for animal kingdom, though they must be supplied by vegetal intake. Furthermore, the biological relevance of flavonoids has driven the nutritional human habits, leading to upsurge of consume of natural foodstuffs (Cook & Samman, 1996; Rice-Evans et al., 1996; Harborne & Williams, 2000; Erlund, 2004; Tsao, 2010; Hidalgo et al., 2010). Hence, many plant derivatives containing high levels of such healthy-protecting biomarkers have been extensively commercialized as phytopharmaceuticals, whereas some isolated compounds, *i.e.* rutin and diosmin have been classified as vitamins and drugs (Hidalgo et al., 2010).

Some pharmacological actions that highlight the health benefits of flavonoids include antibacterial, antiviral, antineoplasic, antiinflammatory, antiallergic, vasodilactory and cardioprotective activity (Cook & Samman, 1996; Rice-Evans et al., 1996; Harborne & Williams, 2000; Narayana et al., 2001; Tsao, 2010; Chaves et al, 2011; Mülazımoğlu et al., 2011).

It was demonstrated that the plant phenolic compounds have protective effects against liver, colon and tongue carcinogenesis. According to some data, onion, lettuce, apples and red wine are important sources of dietary flavonoids, which are probably responsible for the anti-mutagenic activity associated with food and beverages. It was further suggested that smokers ingesting dietary flavonoids are partly protected against harmful effects of tobacco carcinogens within their bladder mucosal cells (Cook & Samman, 1996; Rice-Evans et al., 1996; Harborne & Williams, 2000; Narayana et al., 2001; Nijveldt et al., 2001; Erlund, 2004; Taylor & Grotewold, 2005; Hidalgo et al., 2010; Tsao, 2010; Mülazımoğlu et al., 2011).

Perhaps, the mechanism of action for all these biological actions, as a rule, lay on their antioxidant and/or radical scavenging properties, which is centered on their phenolic groups (Rice-Evans et al., 1996; Narayana et al., 2001; Nijveldt et al., 2001; Montoro et al., 2005). In fact, the electron/hydrogen donor and metal chelator ability of flavonoids depend on the number and position of their oxygenated substituent (Rice-Evans et al., 1996; Nijveldt et al., 2001; Montoro et al., 2005).

These compounds donate hydrogen and the mechanism of their action as antioxidants involves the ability of flavonoids to scavenge radicals by an H-atom or electron transfer process. The resulting antioxidant free radical does not lead to formation of another free radical due to the stabilization by delocalization of radical electron,

thus terminating the chain reaction (Cook & Samman, 1996; Rice-Evans et al., 1996; Harborne & Williams, 2000; Erlund, 2004; Taylor & Grotewold, 2005; Hidalgo et al., 2010; Tsao, 2010; Mülazımoğlu et al., 2011).

Furthermore, it has been found that they can inhibit some redox active enzymes, such as cyclooxygenase, lipoxygenase and NADPH oxidase (Nijveldt et al., 2001; Tsao, 2010). Indeed, it is well known that the extension of enzymatic inhibition lay on chemical interaction strengthens, which in this case is again enhanced by electrostatic interactions, *i.e.* hydrogen bonds, ionic interactions, and once more favored by oxygenated groups (Cook & Samman, 1996; Rice-Evans et al., 1996; Harborne, & Williams, 2000; Narayana et al., 2001; Nijveldt et al., 2001; Montoro et al., 2005; Tsao, 2010).

In spite of that, all these antioxidant mechanisms can contribute positively to the stress oxidative balance. It is well established that upon determined physiological conditions, an antioxidant can act as pro-oxidant, generating free radicals, which in turn can exert dual functions, as defense or deleterious agents, *i.e*. apoptosis in immunological defense and aging process (Narayana et al., 2001; Nijveldt et al., 2001; Erlund, 2004; Montoro et al., 2005; Mülazımoğlu et al., 2011).

Therefore, the understanding of redox behavior of flavonoids, as well their analytical determination is quite clearly of great relevance. In this context, the electroanalysis comes to supply data for both purposes (Hendrickson et al., 1994; Romani et al., 2000; Escarpa et al., 2007; Simic et al., 2007; Reis et al., 2009). Thus, the electrochemical characterization of flavonoids is an useful approach to evaluate the mechanisms involved in the stability, antioxidant activity, pro-oxidant effect and other chemical structural aspects of their redox behavior (Hendrickson et al., 1994; Cao et al., 1997; Simic et al., 2007; Reis et al., 2009; Abdel-Hamid & Newair, 2011).

On the other hand, the electroanalytical techniques have advantages over other analytical methods, such as rapid response, higher sensitivity and low detection limits, as well the possibility to improve the selectivity by using suitable electrode conditions (Vestergaard et al., 2005; Reis et al., 2009; Zielinska & Zielinski, 2010; Mülazımoğlu et al., 2011).

Among the electrochemical methods, coulometry (Peyrat-Maillard et al., 2000) and voltammetry (Adam et al., 2007; Escarpa et al., 2007; Bara et al., 2008; Rene et al., 2010, Santos et al., 2013) are the most representative examples of genuinely electroanalytical approaches, but coupled techniques, in which electrochemical detectors are employed in chromatographic (Aguilar-Sanchez et al., 2005; Novak et al., 2008; Kilinc, 2009) or FIA systems (Volikakis & Efstathiou, 2005), as well electrochemical biosensors have been intensively applied on flavonoid analysis (Korbut et al., 2003; Labuda et al., 2003).

Accordingly, for a representative number of compounds and also for the great biological importance of flavonoids, many reviews of treasured value have been published. However, their biological properties have been often discussed by means of electron donor ability and no review has focused their redox behavior and electrochemical profile (Cook & Samman, 1996; Rice-Evans et al., 1996; Cao et al., 1997; Harborne & Williams, 2000; Narayana et al., 2001; Nijveldt et al., 2001; Erlund, 2004; Montoro et al., 2005; Tsao, 2010). Nevertheless, the aim of this review is to present the state of the art regarding the main applications of electroanalysis on the flavonoid research. The approaches on redox behavior characterization, leading to a better understanding of structure antioxidant activity relationships are highlighted.

Redox behavior of flavonoids

The electrochemical profile of flavonoids, akin to their redox behavior is mainly driven by the stability of electro generated phenoxyl radicals, which as a consequence determines the overall electrode reactions (Hendrickson et al., 1994; Jovanovic et al., 1996; Jorgensen et al., 1999; Volikakis & Efstathiou, 2000; Labuda et al., 2003; Yamamura, 2003; Nasr et al., 2005; Vestergaard et al., 2005; Ferreira et al., 2006; Simic et al., 2007; Dueñas et al., 2010; Zielinska & Zielinski, 2010; Abdel-Hamid & Newair, 2011; Enache & Oliveira-Brett, 2011). Furthermore, as it occurs for other organic compounds, the redox behavior is characterized by proton-electron transfer mechanisms in a wide range of pH, whose extension is defined by chemico-structural properties, such as pka and Ks (Jovanovic et al., 1996; Jorgensen et al., 1999; Volikakis & Efstathiou, 2000; Yamamura, 2003; Nasr et al., 2005; Ferreira et al., 2006; Dueñas et al., 2010; Enache & Oliveira-Brett, 2011).

Therefore, the experimental conditions and chemical properties have an interdependent role on the stability of radical intermediates as well as on the course of electrochemical reactions. Indeed, such aspects lay on structural relationships, especially the number and position of hydroxyl substituent (Hendrickson et al., 1994; Jovanovic et al., 1996; Jorgensen et al., 1999; Volikakis & Efstathiou, 2000; Nasr et al., 2005; Simic et al., 2007; Dueñas et al., 2010; Enache & Oliveira-Brett, 2011).

Phenol pattern and electrochemical profile

Since there is an unambiguous connection between biological activity and redox behavior of flavonoids, a large number of papers, in which electroanalysis were employed, have been published (Jovanovic et al., 1996; Cao et al., 1997; Nijveldt et al., 2001; Labuda et al., 2003; Volikakis & Efstathiou, 2005;

Adam et al., 2007; Escarpa et al., 2007; Reis et al., 2009; Dueñas et al., 2010; Masek et al., 2011; Diculescu et al., 2012; Gil et al., 2012). Therefore, it is already possible to postulate the structural aspects that have major influence on the electrochemical profile of flavonoids. In turn, the attempts to establish structural relationships begin with the correct numbering. The numbering of rings A and C begin with the heteroatom (*O*) in the ring C and continues clockwise around these fused rings. Whereas, in the ring B, it starts in the bridge carbon and follows the usual rule of small numbers, by using superscript coma 1´, 2´ to 6´(Erlund, 2004; Tsao, 2010; Mülazımoğlu et al., 2011) (**1**).

Table 1 show some examples of flavonoids, where the electroanalytical assays have been applied. The first observation, from the undertaken analysis of literature data is that while ring C is the basis for flavonoid classification, its influence on their redox behavior is reduced (Hendrickson et al., 1994; Cao et al., 1997; Romani et al., 2000; Escarpa et al., 2007; Simic et al., 2007; Reis et al., 2009). In fact, the electroactivity of flavonoids regards on rings B and A, in which phenolic groups dictates the electrochemical profile (Hendrickson et al., 1994; Rice-Evans et al., 1996; Pannala et al., 2001; Tsimogiannis & Oreopoulou, 2004).

Whilst, other type of substituents, methoxyl, acetylesters, glucosides, and keto have secondary influence on the redox behaviour and formation of oxidation products (Hendrickson et al., 1994; Jovanovic et al., 1996; Jorgensen et al., 1999; Yamamura, 2003; Nasr et al., 2005; Ferreira et al., 2006; Simic et al., 2007; Dueñas et al., 2010; Abdel-Hamid & Newair, 2011; Enache & Oliveira-Brett, 2011). So that, the phenolic groups of rings A and especially the ring B, have great influence not only on the electrochemical profile but also on the electron/proton donor ability and resulting antioxidant power (Slabbert, 1977; Hendrickson et al., 1994; Pannala et al., 2001; Yang et al., 2001; Tsimogiannis & Oreopoulou, 2004; Simic et al., 2007; Gomez-Pineda et al., 2009).

Another assumption is that the phenol pattern drives the electron delocalization, affecting the stability of radical intermediates, which can even lead to different electrode reaction pathways and electrochemical profile (Jovanovic et al., 1996; Zoulis & Efstathiou, 1996; Jorgensen et al., 1999; Volikakis & Efstathiou, 2000; Labuda et al., 2003; Oliveira-Brett & Ghica, 2003; Yamamura, 2003; Nasr et al., 2005; Ferreira et al., 2006; Wu et al., 2007; Dueñas et al., 2010; Medvidovic-Kosanovic et al., 2010; Enache & Oliveira-Brett, 2011). For example, the electrochemical oxidation of phenol and resorcinol is an irreversible process, occurring in one step, though the catechol and hydroquinone oxidation is two electron-proton reversible mechanisms (Zoulis & Efstathiou, 1996; Jorgensen et al., 1998; Volikakis & Efstathiou, 2000; Oliveira-Brett & Ghica, 2003; Ghica & Oliveira-Brett, 2005; Nasr et al., 2005; Wu et al., 2007; Sokolova et al., 2008; Medvidovic-Kosanovic et al., 2010; Enache & Oliveira-Brett, 2011; Masek et al., 2011; Mulazimoglu et al., 2012).

Electrooxidation of mono-phenol and resorcinol moieties

Phenol is oxidized in an one-electron and one proton step, to a phenoxy radical which is thermodynamically unstable and coexists in three isomeric forms. The highest spin density of this radical is in the *ortho*- and *para*-positions, whereas the *meta* position is not favoured for any kind of chemical reaction (Jovanovic et al., 1996; Jorgensen et al., 1999; Yamamura, 2003; Nasr et al., 2005; Enache & Oliveira-Brett, 2011). Therefore, the stabilisation of the phenoxyl radical is followed by

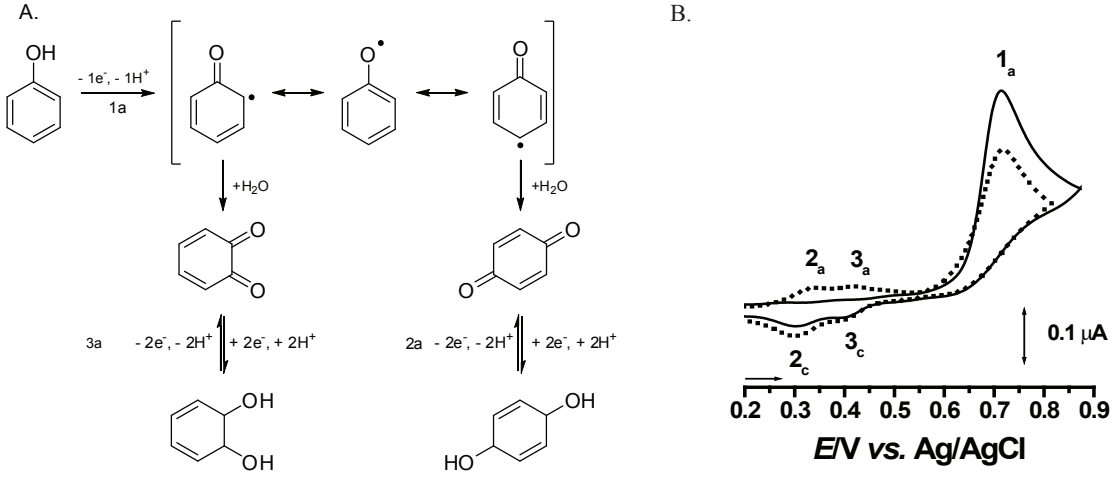

Figure 1. The oxidation mechanism of phenol (A) and First (straight line) and second (dotted Line) scan obtained for 30 μM phenol solution in pH 7.0 0.1 M phosphate buffer, $\nu = 50$ mV.s^{-1} (B).

Table 1. Structural pattern of electroanalyzed flavonoids.

Compound	Subclass	3	5	7	8/2′/5′	3′	4′	References
myricetin	flavonol	OH	OH	OH	5′-OH	OH	OH	Zoulis & Efstathiou, 1996; Volikakis & Efstathiou, 2000; Wu et al., 2007
quercetin	flavonol	OH	OH	OH	H	OH	OH	Zoulis & Efstathiou, 1996; Jorgensen et al., 1998; Volikakis & Efstathiou, 2000; Oliveira-Brett & Ghica, 2003; Sokolova et al., 2008; Dueñas et al., 2010; Medvidovic-Kosanovic et al., 2010; Mülazımoğlu et al., 2012
rutin	flavonol	*O*-rham-glu	OH	OH	H	OH	OH	Zoulis & Efstathiou, 1996; Volikakis & Efstathiou, 2000; Ghica & Brett, 2005; Medvidovic-Kosanovic et al., 2010; Masek et al., 2011; Mülazımoğlu et al., 2012
quercetrin	flavonol	*O*-rham	OH	OH	H	OH	OH	Volikakis & Efstathiou, 2000; Adam et al., 2007
fisetin	flavonol	OH	H	OH	H	OH	OH	Volikakis & Efstathiou, 2000; Markovic et al., 2009; Brondani et al., 2010
rhamnetin	flavonol	OH	OH	M	H	OH	OH	Zoulis & Efstathiou, 1996; Volikakis & Efstathiou, 2000
isorhamnetin	flavonol	OH	OH	M	H	OH	OH	Liu et al., 2008
morin	flavonol	OH	OH	OH	2′-OH	H	OH	Janeiro & Oliveira-Brett, 2005a; Xiao et al., 2006; Masek et al., 2011; Temerk et al., 2011; Mülazımoğlu et al., 2012
kampferol	flavonol	OH	OH	OH	H	H	OH	Zoulis & Efstathiou, 1996; Jorgensen et al., 1998; Volikakis & Efstathiou, 2005
galangin	flavonol	OH	OH	OH	H	H	H	Rapta et al., 1995; Volikakis & Efstathiou, 2000; Masek et al., 2011;
robinin	flavonol	*O*-gal-rham	OH	*O*-rham	H	OH	H	Gil et al., 2012
luteolin	flavone	H	OH	OH	H	OH	OH	Liu et al., 2008; Dongming et al., 2011
orientin	flavone	H	OH	OH	8-glu	OH	OH	Gil et al., 2012
apigenin	flavone	H	OH	OH	H	H	OH	Xing et al., 2009; Masek et al., 2011; Mülazımoğlu et al., 2011
diosmin	flavone	H	OH	*O*-rham-glu	H	OH	M	El-Shahaw, 2006; Diculescu et al., 2012
diosmetin	flavone	H	OH	OH	H	OH	M	Diculescu et al., 2012
isorhoifolin	flavone	H	OH	*O*-rham-glu	H	H	OH	Diculescu et al., 2012
linarin	flavone	H	OH	*O*-rham-glu	H	H	M	Diculescu et al., 2012
chrisin	flavone	H	OH	OH	H	H	H	Janeiro et al., 2005b; Masek et al., 2011
taxifolin	flavanonol	OH	OH	OH	H	OH	OH	Janeiro et al., 2005b
eriodictyol	flavanone	H	OH	OH	H	OH	OH	Gil et al., 2012
hesperitin	flavone	H	OH	OH	H	OH	M	Zhang et al., 2011
hesperidin	flavanone	H	OH	*O*-rham-glu	H	OH	M	Volikakis & Efstathiou, 2000; Temerk et al., 2009
naringenin	flavanone	H	OH	OH	H	H	OH	He et al., 2009; Mülazımoğlu et al., 2011; Zhang et al., 2011
naringin	naringin	H	OH	*O*-rham-glu	H	H	OH	Reichart & Obendorf, 1998; Volikakis & Efstathiou, 2000
catechin	flavanol	OH	OH	OH	H	OH	OH	Martinez et al., 2005; Dueñas et al., 2010; Medvidovic-Kosanovic et al., 2010; Zhang et al., 2011;
epicatechin	flavanol	OH	OH	OH	H	OH	OH	Dueñas et al., 2010; Zhang et al., 2011
epicatechin gallate	flavanol	Gall	OH	OH	H	OH	OH	Novak et al., 2009
epigallocatechin gallate	flavanol	Gall	OH	OH	H	OH	OH	Novak et al., 2009
myrtilin	anthocyanin	*O*-glu	OH	OH	5′-OH	OH	OH	Janeiro & Oliveira-Brett, 2007

delphinidin	anthocyanin	OH	OH	OH	5´-OH	OH	OH	Lima et al., 2007
cyanidin	anthocyanin	OH	OH	OH	H	OH	OH	Lima et al., 2007
kuromanin	anthocyanin	*O*-glu	OH	OH	H	OH	OH	Janeiro & Oliveira-Brett, 2007; Lima et al., 2007
cyanin	anthocyanin	*O*-glu	*O*-glu	OH	H	OH	OH	Janeiro & Oliveira-Brett, 2007
oenin	anthocyanin	*O*-glu	OH	OH	5´-M	M	OH	Janeiro & Oliveira-Brett, 2007
peonidin	anthocyanin	OH	OH	OH	H	M	OH	Lima et al., 2007
petunidin	anthocyanin	OH	OH	OH	5´-M	M	OH	Janeiro & Oliveira-Brett, 2007
malvin	anthocyanin	*O*-glu	*O*-glu	OH	5´-M	M	OH	Janeiro & Oliveira-Brett, 2007
pelargonidin	anthocyanin	OH	OH	OH	H	H	OH	Lima et al., 2007
callistephin	anthocyanin	*O*-glu	OH	OH	H	H	OH	Lima et al., 2007
genistein	isoflavone	-	OH	OH	H	H	OH	Wu et al., 1997; Volikakis & Efstathiou, 2000; Han et al., 2009; Fogliatto et al., 2010
biochanin a	isoflavone	-	OH	OH	H	H	M	Fogliatto, Barbosa, & Ferreira, 2010
daidzein	isoflavone	-	H	OH	H	H	OH	Liang et al., 2008; Han et al., 2009; Fernandes et al., 2010
equol (flavan)	isoflavone	-	H	OH	H	H	OH	Han et al., 2009
puerarin	isoflavone	-	H	OH	8-*O*-glu	H	OH	Han et al., 2009

Galactose (gal); glucose (glu); rhamnose (rham); OCH_3 (M); gallate residue (Gall).

hydrolysis at a high potential mainly at *ortho*- and *para*-positions (Figure 1A).

The oxidation of mono-phenols is always irreversible on the whole pH range (Jorgensen et al., 1999; Yamamura, 2003; Enache & Oliveira-Brett, 2011). It is often followed by electrodeposition of oxidation products, hydroquinone and catechol, that undergoes reversible oxidation at lower potentials (Jovanovic et al., 1996; Jorgensen et al., 1999; Nasr et al., 2005; Enache & Oliveira-Brett, 2011). Figure 1B shows a typical voltammetric profile of mono phenol like compounds. The irreversible anodic peak 1a, at E_{pa}>0.7 (neutral pH) is followed on the reverse scan by two reversible redox pairs at low potentials corresponding to the oxidation products (Figure 1A).

The mono phenol pattern can occur on ring B, where there are higher number of examples, *i.e*. kampferol (Zoulis & Efstathiou, 1996; Jorgensen et al., 1998; Volikakis & Efstathiou, 2005), naringenin (Oliveira-Brett & Ghica, 2003; He et al., 2009; Sims et al., 2009; Mülazımoğlu et al., 2011), apigenin (Xing et al., 2009; Masek et al., 2011; Mülazımoğlu et al., 2011), genistein (Wu et al., 1997; Volikakis & Efstathiou, 2000; Han et al., 2009; Fogliatto et al., 2010), pelargonidin (Lima et al., 2007), on ring A, exemplified by fisetin (Volikakis & Efstathiou, 2000; Markovic et al., 2009; Brondani et al., 2010), as well on both aromatic rings, *i.e.* naringin (Reichart & Obendorf, 1998; Volikakis & Efstathiou, 2000), diosmin (El-Shahaw, 2006; Adam et al., 2007; Diculescu et al., 2012), daidzen (Volikakis & Efstathiou, 2000; Liang et al., 2008; Han et al., 2009; Fernandes et al., 2010) (Table 1).

Perhaps, the resorcinol like pattern is the most frequent one in flavonoid compounds. Indeed, this pattern is almost ubiquitous in A ring unit, meanwhile its occurrence in B ring is rare, accordingly represented on Table 1 by the flavonoid morin (Janeiro & Oliveira-Brett, 2005; Xiao et al., 2006; Temerk et al., 2011). The *meta* pattern of substitution does not allow stabilization of intermediates, thus the resorcinol group is oxidized at higher positive potentials (>0.7 V, neutral pH) (Jovanovic et al., 1996; Jorgensen et al., 1999; Yamamura, 2003; Janeiro & Oliveira-Brett, 2005; Nasr et al., 2005; Enache & Oliveira-Brett, 2011; Mulazimoglu et al., 2012). As in mono phenol moiety, such oxidation mechanism occurs in EC mechanism involving one electron-proton transfer and the formation of an electroactive product. Although the first step is irreversible in all pH range, the electroactive product undergoes reversible process in non-alkaline medium (Jovanovic et al., 1996; Jorgensen et al., 1999; Yamamura, 2003; Nasr et al., 2005; Enache & Oliveira-Brett, 2011).

The reversibility of each process is easily demonstrated by Square Wave Voltammetry (SWV) experiments (Enache & Oliveira-Brett, 2011) (Figure 2). The SW voltammogram obtained at a clean electrode surface shows an irreversible process, peak 1a (Figure 2A) which leads to adsorption of oxidation products. On the second SW voltammogram recorded immediately after, without cleaning the electrode surface, peak 2a corresponding to oxidation product appears and its reversibility is evidenced by two symmetrical peaks (dotted lines), thus corresponding to equal oxidation and reduction process (Janeiro & Oliveira-Brett, 2005; Enache & Oliveira-Brett, 2011) (Figure 2B).

And so, the electrochemical profile of flavonoids presenting only resorcinol or mono-phenol groups is closely similar. Actually, morin, a 5,7,2´,4´-tetra-hydroxy flavonoid, and other representative examples of non-cathecol containing aglycones have showed similar

electrochemical profile (Rapta et al., 1995; Wu et al., 1997; Volikakis & Efstathiou, 2000; Janeiro & Oliveira-Brett 2005; Janeiro et al., 2005; Xiao et al., 2006; Liang et al., 2008; Han et al., 2009; Fernandes et al., 2010; Fogliatto et al., 2010; Temerk et al., 2011; Zhang et al., 2011; Diculescu et al., 2012; Gil et al., 2012; Mulazimoglu et al., 2012).

Then, all these derivatives undergoes an irreversible anodic process at potentials higher than 0.8 V in mild acid pH, while on successive scans the reversible redox pair corresponding to catechol like products may appear at lower peak potentials (Wu et al., 1997; Volikakis & Efstathiou, 2000; Janeiro & Oliveira-Brett 2005; Nasr et al., 2005; Xiao et al., 2006; Liang et al., 2008; Han et al., 2009; Fernandes et al., 2010; Fogliatto et al., 2010; Enache & Oliveira-Brett, 2011; Temerk et al., 2011; Mulazimoglu et al., 2012).

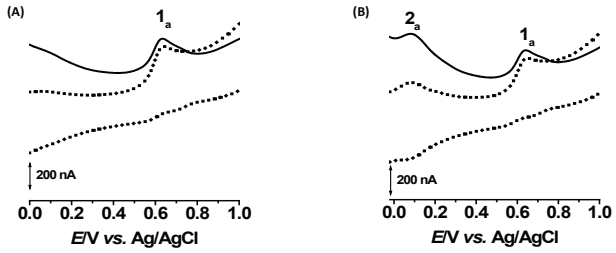

Figure 2. SW voltammograms obtained in pH 7.0 0.1 M phosphate buffer with a GCE in 25 μM resorcinol (A) first and (B) second scan; f = 25 Hz, ΔE_s = 2 mV, v_{eff} = 50 mV s^{-1}, pulse amplitude 50 mV; I_t: total current; I_f: forward current, I_b: backward current.

Furthermore, the difference between required potential for mono-phenol and resorcinol oxidation are usually very narrow, and can increase or even be annulled upon other structural aspects (Janeiro & Oliveira-Brett 2005; Xiao et al., 2006; Liang et al., 2008; Han et al., 2009; Fernandes et al., 2010; Fogliatto et al., 2010; Temerk et al., 2011). For instance, phenolic groups in B-ring undergo electrochemical oxidation at lower potential than more acidic A-ring ones (Janeiro & Oliveira-Brett 2005; Fogliatto et al., 2010; Zhang et al., 2011). Likewise, owing to specific solvation effect, isoflavones and flavanones are usually less acidic than isomeric flavones, thus leading to lower peak potentials. Meanwhile, the higher hydrosolubility of 3-OH derivatives may also reduce the bond dissociation enthalpy for phenolic groups in B-ring (Liang et al., 2008; Han et al., 2009; Zhang et al., 2011).

Electrooxidation of catechol and hydroquinone moieties

As a rule, *para*- and *orto*- benzenediols position suffer prior oxidation than *m*-ones or mono phenols (Hendrickson et al., 1994; Jorgensen et al., 1999; Oliveira-Brett & Ghica, 2003; Enache & Oliveira-Brett, 2011). Moreover, unlike phenol and resorcinol moieties, catechol and hydroquinone undergo a two electron two proton reversible oxidation process (Jovanovic et al., 1996; Jorgensen et al., 1999; Yamamura, 2003; Nasr et al., 2005; Sims et al., 2009; Enache & Oliveira-Brett, 2011; Zhang et al., 2011).

The reversibility is explained by means of electron delocalization resonance in *para* and *orto* positions, which leads to the stabilization of phenoxy radicals and reduces the required overpotential (Jovanovic et al., 1996; Jorgensen et al., 1999; Volikakis & Efstathiou, 2000; Nasr et al., 2005; Dueñas et al., 2010; Enache & Oliveira-Brett, 2011). Thus, the oxidation of such phenolic pattern groups undergoes at very low positive potentials, E_{pa} ~0.2 V (pH 7.0), for catechol, and 0.15 V for hydroquinone.

The deconvolution of total currents in SWV obtained for catechol and hydroquinone like compounds may lead to symmetrical forwards and backwards peaks. Although, in alkaline media (pH >9.0) the deprotonation of OH groups leads to lower peak potentials, the reversibility and pH dependence is higher in acid medium (Zoulis & Efstathiou, 1996; Oliveira-Brett & Ghica, 2003; Janeiro & Oliveira-Brett, 2007; Xing et al., 2009; Medvidovic-Kosanovic et al., 2010). Indeed, in higher pH the phenol oxidation undergoes a non-proton electron transfer mechanism, which often includes irreversible bond breaking followed by chemical reactions, i.e. dimerizations and fall of peak currents (Rapta et al., 1995; Yamamura, 2003; Ferreira et al., 2006; Fernandes et al., 2010; Zhang et al., 2011).

Though to the best of our knowledge no hydroquinone and also no A-ring catechol like flavonoid have been described, both benzenediol patterns might be possible in their oxidation products (Sims et al., 2009; Enache & Oliveira-Brett, 2011). Yet, the B ring catechol moiety is present in the strongest antioxidants (Hendrickson et al., 1994; Zoulis & Efstathiou, 1996; Wu et al., 1997; Abdel-Hamid & Newair, 2011; Zhang et al., 2011). Indeed, the inherent redox reversibility and low anodic potentials of 3´,4´-di-hydroxyl is in accordance with the higher electron-donating properties of catechol B ring compounds (Hendrickson et al., 1994; Jovanovic et al., 1996; Zoulis & Efstathiou, 1996; Jorgensen et al., 1998; Jorgensen v, 1999; Volikakis & Efstathiou, 2000; Pannala et al., 2001; Oliveira-Brett & Ghica, 2003; Nasr et al., 2005; Wu et al., 2007; Sokolova et al., 2008; Dueñas et al., 2010; Medvidovic-Kosanovic et al., 2010; Mulazimoglu et al., 2012).

Electrooxidation of other hydroxyl groups

Besides the aforementioned mono and di-phenol patterns, it is also possible to find compounds with a "gallocatechol like" pattern (Table 1). Such phenol pattern is characterized by the presence of a third oxidizable

hydroxyl group adjacent to catechol moiety, *i.e*. myricetin, delphinidin, gallocatechins and catechin gallates.

The gallocatechol moiety akin to catechol, is reversibly oxidized at low potential, thus conferring high electron donor ability, but minor effect on the voltammetric profile (Volikakis & Efstathiou, 2000; Martinez et al., 2005; Sims et al., 2009; Medvidovic-Kosanovic et al., 2010).

In order, the non-phenolic hydroxyl group, 3-OH, of flavonols can be electrochemically oxidized at potential close to that one observed for A ring phenolic groups (Volikakis & Efstathiou, 2000; Janeiro et al., 2005). Meanwhile, in flavanonols and catechins, the absence of conjugated double bond hampers this oxidation process, shifting the expected peak potential to very positive values (Janeiro et al., 2005; Sims et al., 2009; Zhang et al., 2011).

Due to the narrow differences between oxidation processes observed for catechol and gallacatechol or flavonol 3-OH and A ring phenolic groups, the anodic waves might overlap. Nevertheless, upon certain experimental conditions, the appearance of shoulder and/or additional peaks can be observed, thus producing changes on the voltammetric profile (Zoulis & Efstathiou, 1996; Wu et al., 2007). For instance, it has been described for quercetin two, three or even four anodic peaks, depending on pH solution and electrodic material (Sokolova et al., 2008; Dueñas et al., 2010; Medvidovic-Kosanovic et al., 2010; Mulazimoglu et al., 2012). In fact, as the acidity of each hydroxyl group is different, also will be the deprotonation and extension of potential shift upon different experimental conditions (Slabbert, 1977; Sokolova et al., 2008; Gomez-Pineda et al., 2009; Medvidovic-Kosanovic et al., 2010; Mulazimoglu et al., 2012).

Electrochemical parameters and antioxidant activity

On the biological point of view, the electron/proton donor ability of flavonoids is one of their most relevant chemical properties (Cook & Samman, 1996; Rice-Evans et al., 1996; Harborne & Williams, 2000; Tsao, 2010). Indeed, by H-atom or electron transfer process, flavonoids can scavenge radicals, thus terminating chain reactions and then exerting their biological actions (Montoro et al., 2005; Reis et al., 2009).

Flavonoids as antioxidants have been reviewed several times including outlines of many claims to their beneficial health effects. Due to their complex structures and different classes (eight thousand different compounds are known (Reis et al., 2009; Hidalgo et al., 2010; Tsao, 2010; Mülazımoğlu et al., 2011), researchers often resorted to qualitative screening methods to evaluate their antioxidant potentials in mixed aqueous/lipid phases. For example, the concentration of Trolox (a water-soluble derivative of vitamin E) with 'equivalent antioxidant activity' of a 1 mM concentration of the substrate is frequently used in heterogeneous systems. Unfortunately, this can be an unreliable measure of the activity of the substance, especially if initiation is also carried out in the aqueous phase. Nevertheless, there have been some efforts made to evaluate antioxidant activities of specific flavonoids using more quantitative methods in heterogeneous systems in order to mimic natural environments (Aaby et al., 2004; Reis et al., 2009; Zielinska & Zielinski, 2010; Mishra et al., 2012).

In turn, the radical scavenging activity, as well as the reductor power is intrinsically connected to the redox behavior, which is dictated by electroactive groups (Bara et al., 2008; Zielinska & Zielinski, 2010; Abdel-Hamid & Newair, 2011). Hence, the antioxidant activity driven by its electron donor ability is feasibly traduced by peak potentials, E_{pa} and also peak currents, I_{pa}. In simple words, the lower the E_{pa} value, the higher the electron donor ability, whilst the higher the I_{pa}, the higher may be the rate and/or number of transferred electrons (Yang et al., 2001; Amic et al., 2003; Blasco et al., 2004; Huang et al., 2004; Escarpa et al., 2007; Simic et al., 2007; Yakovleva et al., 2007; Gomez-Pineda et al., 2009; Zielinska & Zielinski, 2010). Indeed, good correlations between such electroanalytical and spectrophotometric assays have been reached *i.e.* E_{pa} value *versus* radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DDPH) (Aaby et al., 2004; Zhang et al., 2011; Mishra et al., 2012). A few selected examples from different groups to illustrate some of the relationships between their detailed structures and related antioxidant properties are enrolled on Table 2, and the basic flavonoid structure was aforementioned in Table 1.

In order to achieve comparable values, Table 2 was constructed taking into account the influence of experimental conditions, thus since cyclic voltammetry carried out at glassy carbon electrode in mild acid medium have been mostly used, it was chosen as criterion.

However, it is a 'risky' business to attempt an evaluation of literature reports on antioxidant properties of flavonoids, since it is not surprising that these polyphenols give different results from different experimental methods, some DPPH values relatively to quercetin activity are also presented in order to offer tentative comparisons (Aaby et al., 2004; Zhang et al., 2011; Mishra et al., 2012).

Therefore, flavonoids with lower E_{pa} values, *i.e*. myricetin, quercetin, rutin, luteolin, taxifolin, myrtillin and catechin (Table 2) will be better antioxidant defense agents than derivatives lacking catechol moiety on ring B, where the expected E_{pa} values are higher (Nasr et al., 2005; Yakovleva et al., 2007; Fernandes et al., 2010).

For instance, 3′,4′-dihydroxyflavonoids with peak potentials ranging around 0.35 V would be thermodynamically able to scavenge peroxyl radicals (E_{pa} ~0.8 V), whereas non-catecholic derivatives (E_{pa} >0.8 V) may not (Rapta et al., 1995; Nasr et al., 2005; Rene et al.,

Table 2. Anodic peak potentials and DPPH values* for different flavonoids.

Compound	E_{pa1} V	E_{pa2} V	pH	Phenol Pattern		DPPH %Q	References
				A ring	B ring		
myricetin	0.30	nd	3.6	R	G	84*,78**	Wu et al., 2007;
quercetin	0.33	1.03	4.0	R	C	100	Zoulis & Efstathiou, 1996; Jorgensen et al., 1998; Oliveira-Brett & Ghica, 2003; Medvidovic-Kosanovic et al., 2010;
rutin	0.40	1.06	4.0	P	C	87**	Jorgensen et al., 1998; Oliveira-Brett & Ghica, 2003; Sokolova et al., 2008; Medvidovic-Kosanovic, et al., 2010; Mülazımoğlu et al., 2012
fisetin	0.39	1.00	4.0	P	C	nd	Volikakis & Efstathiou, 2000; Markovic et al., 2009; Brondani et al., 2010
isorhamnetin	0.36	1.15	4.0	P	C	62*	Liu et al., 2008
morin	0.44	0.98	4.0	R	R	nd	Janeiro & Oliveira-Brett, 2005a; Xiao et al., 2006; Temerk et al., 2011
kampferol	0.45	nd	3.6	R	P	37*,32**	Zoulis & Efstathiou, 1996; Jorgensen et al., 1998
luteolin	0.40	1.05	4.0	R	C	73*,32**	Liu et al., 2008; Dongming et al., 2011
diosmin	0.70	1.08	4.0	P	P	nd	El-Shahaw, 2006; Diculescu et al., 2012
diosmetin	0.68	1.06	4.0	R	P	nd	Diculescu et al., 2012
isorhoifolin	0.65	1.10	4.0	P	P	nd	Diculescu et al., 2012
chrisin	-	1.05	4.0	R	-	nd	Janeiro et al., 2005b
taxifolin	0.39	1.03	4.0	R	C	60*	Janeiro et al., 2005b
eriodictyol	0.35	1.00	4.0	R	C	nd	Gil et al., 2012
hesperidin	0.40	1.09	4.0	P	P	nd	Volikakis & Efstathiou, 2000; Temerk et al., 2011; Zhang et al., 2011
naringenin	0.45	nd	3.6	R	P	12*	He et al., 2009; Mülazımoğlu et al., 2011; Zhang et al., 2011
catechin	0.33	0.74	4.0	R	C	79*,92**	Zoulis & Efstathiou, 1996; Martinez et al., 2005; Medvidovic-Kosanovic et al., 2010
epicatechin	0.33	0.74	4.0	R	C	73*,89**	Dueñas et al., 2010; Zhang et al., 2011
myrtillin	0.36	0.85	4.0	R	G	50**	Janeiro & Oliveira-Brett, 2007
kuromanin	0.49	0.83	4.0	R	C	74**	Janeiro & Oliveira-Brett, 2007; Lima et al., 2007
cyanin	0.42	0.91	4.0	P	C	nd	Janeiro & Oliveira-Brett, 2007
oenin	0.49	0.83	4.0	R	P	42**	Janeiro & Oliveira-Brett, 2007
malvin	0.49	0.91	4.0	P	P	nd	Janeiro & Oliveira-Brett, 2007
genistein	0.72	1.03	4.0	R	P	0*	Wu et al., 1997; Volikakis & Efstathiou, 2000; Han et al., 2009; Fogliatto et al., 2010
daidzein	0.68	0.90	4.0	P	P	0*	Volikakis & Efstathiou, 2000; Liang et al., 2008; Han et al., 2009

Electroactive Groups: Gallic (G), Catechol (C), Resorcinol (R), Phenol (P); Data calculated from *Zhang et al., 2011 and **Aaby et al., 2004 and expressed by means of quercetin activity; non determined (nd).

2010).

Furthermore, the catechol B ring derivatives, *i.e.* quercetin (~0.4 V), catechin (~0.4 V), epigalocatechin gallate (~0.3 V) can also exert their antioxidant activity by restoring some low molecular weight bioantioxidants, *i.e.* tocopherol (~0.5 V). In turn, guaicolic (3´-OH, 4´-OCH3) derivatives, *i.e.* hesperidin, presenting intermediate reduction potentials (~0.7 V), can still donate an electron to free radical but slower. Meanwhile, in this specific case, the antioxidant function of flavonoid would be limited to radical scavenging activity (Rapta et al., 1995; Nasr et al., 2005).

Effect of substituent on electron donor ability

Several investigations on how the substitution pattern on flavonoid core reflects the electron-hydrogen donating ability have been published (Montoro et al., 2005; Reis et al., 2009; Tsao, 2010). It has been well established that the B ring chemistry has far great influence on the electron donor properties of flavonoids, whilst the number and position of hydroxyl groups are unambiguously pivotal for the highest antioxidant activity, whereas the catechol containing derivatives are the stronger reducing agents (Hendrickson et al., 1994; Pannala et al., 2001; Tsimogiannis & Oreopoulou, 2004).

However, such redox behavior is directly related to the phenol pattern, the effect of other substituents may not be neglected. The influence of non-electroactive substituent on peak potentials and overall electrode reactions is expressed by electronic and steric effects, which might also have substantial effect on the solubility and diffusion properties (Rice-Evans et al., 1996; Cao et al., 1997; Nijveldt et al., 2001; Blasco et al., 2004; Huang et al., 2004; Montoro et al., 2005; Gomez-Pineda et al., 2009). In turn, such properties not only can alter the redox behavior, but also the antioxidant activity in physiological conditions (Amic et al., 2003; Montoro et al., 2005; Tsao, 2010).

Electronic effects

The electrochemical oxidation of phenolic groups shifted to more positive values, when the substituent present higher Hammett´s constants. In simple words, electron withdrawing groups hamper electron loss, while electron-donor substituent may reduce the expected peak potentials (Rice-Evans et al., 1996; Cao et al., 1997; Simic et al., 2007; Han et al., 2009).

Likewise, the presence of C_2=C_3 double bond, and also the C_3-OH group on C unity enhance the electron delocalization stabilizing the phenoxyl radical stabilization, and then lowering the expected potentials (Tsimogiannis & Oreopoulou, 2004; Simic et al., 2007; Han et al., 2009). Indeed, the relatively low E_{pa1} values (Table 2) observed for flavonoids, morin and kaempferol, both lacking catechol moiety on ring B, can be attributed to the presence of 3-OH and C_2=C_3 double bond (Pannala et al., 2001; Tsimogiannis & Oreopoulou, 2004; Han et al., 2009; Markovic et al., 2009). On the other hand, the slight shift to higher potential observed for the flavanonol, taxifolin, when compared to the flavonol, quercetin reinforces that the double bond has at least a minor importance on the electron donor ability (Rice-Evans et al., 1996; Tsimogiannis & Oreopoulou, 2004; Janeiro et al., 2005).

By contrast, catechin has E_{pa1} values and closer to quercetin, which might be associated to the absence of carbonyl group on C unity (Zoulis & Efstathiou, 1996; Martinez et al., 2005; Markovic et al., 2009). In fact, carbonyl group exerts a dual effect on antioxidant activity, while it enhances the acidity of phenolic groups, increasing their peak potentials, when coupled to C_5-OH or C_3-OH, such group offers a strong site for metal quelation, thus improving the indirect antioxidant mechanism (Yakovleva et al., 2007; Gomez-Pineda et al., 2009).

In order, the peak potentials are positively shifted if the hydroxyl hydration is decreased. So, hydrophilic substituents enhance the proton donor ability and antioxidant activity in aqueous media. Therefore, owing to the presence of 3-OH group, flavonols generally oxidize at lower potentials than flavones (Rice-Evans et al., 1996; Zoulis & Efstathiou, 1996; Tsimogiannis & Oreopoulou, 2004; Montoro et al., 2005).

Steric effects

As mentioned before, according to electron donor and hydrophilic contribution, non-oxidizable substituent can push down the required overpotentials. The comparison of peak potentials between apigenin and its isomer genistein (Han et al., 2009), obtained in similar conditions have pointed to the importance of structural symmetry on the hydration and electron transfer mechanism. Thus in aqueous medium the isoflavonoid showed to undergo oxidation at lower peak potential (Liang et al., 2008; Han et al., 2009; Fogliatto et al., 2010). In turn, the diastereomization have very small effect on the redox behavior of flavonoids. Therefore, catechin and epicatechin present quite similar antioxidant activity (Martinez et al., 2005; Medvidovic-Kosanovic et al., 2010; Zhang et al., 2011; Mishra et al., 2012).

On the other hand, the inherent steric effects of such substituent may not be discarded. In fact, bulky groups might sterically hinder the electrode reactions (Ferreira et al., 2006; Dueñas et al., 2010). For instance, anthocyanidins have presented higher antioxidant activity and slightly lower peak potentials than anthocyanins, which agrees with the steric hindrance effect of sugar moieties (Sokolova et al., 2008; Markovic et al., 2009; Brondani et al., 2010; Medvidovic-Kosanovic et al., 2010; Masek et al., 2011; Temerk et al., 2011; Gil et al., 2012; Mulazimoglu et al., 2012).

On the other hand, the steric hindrance effect on electron transfer is also expressed by the fall of peak currents in experiments carried out in equimolar concentrations. As an example, voltammograms obtained for quercetin have showed higher peak currents than its derived glycosides as rutin and quercetrin (Adam et al., 2007; Sims et al., 2009). Thus, although in such cases the number of transferred electrons should be the same, the rate of electrode reaction may not (Yakovleva et al., 2007).

Furthermore, the secondary chemical reactions between phenoxyls radicals can be driven by non-oxidizable substituent (Nasr et al., 2005; Ferreira et al., 2006; Diculescu et al., 2012). It was found that the electrochemical oxidation of methyl substituted derivatives results in more linear eletropolymerized films, thus producing faster blockade of electrode surface (Ferreira et al., 2006).

Influence of experimental conditions on electroanalytical assays

Despite the obvious applicability of electroanalysis on the evaluation of electron/proton donor ability, the robustness of electrochemical approaches

depends greatly on experimental conditions (Arslan et al., 2005; Reis et al., 2009). In the case of organic compounds, in which redox reactions often involve proton/electron transfer processes, it is mainly defined by pH of electrolyte solution (Slabbert, 1977; Arslan et al., 2005; Gomez-Pineda et al., 2009).

As a rule, owing to the easier deprotonation, peak potentials decrease as the pH increases, while the reaction is pH dependent at least in non-alkaline medium (Liang et al., 2008; Han et al., 2009; Brondani et al., 2010; Fogliatto et al., 2010; Medvidovic-Kosanovic et al., 2010; Masek et al., 2011).

The plot, E_{pa} *versus* pH, commonly obtained for flavonoids results in a straight line up to their pKa, and a typical slope of around 59 mV is consistent with electrode reactions involving a 1:1 ratio of electrons/protons (Liang et al., 2008; Han et al., 2009; Brondani et al., 2010; Fogliatto et al., 2010; Medvidovic-Kosanovic et al., 2010; Masek et al., 2011; Gil et al., 2012).

For instance, the anodic peak, E_{pa1} observed for daidzen shift from 0.73 V to 0.38 V, as the pH change from 2.0 to 8.0 (Liang et al., 2008; Fernandes et al., 2010). Thus, the influence of pH on peak shifts is far greater than the usually observed for different flavonoids presenting the same phenol pattern, Tables 1 and 2.

In turn, higher E_{pa} values are usually found when low ionic strength electrolyte solution or mixed solvent systems, *i.e*. ethanol:water, acetonitrile:water are employed. Such fact is in agreement with the lower strength of electrolyte solution, which leads to overpotential drop (Xing et al., 2009; Masek et al., 2011; Mülazımoğlu et al., 2011).

Therefore, the correlation between E_{pa} and antioxidant power must always consider the electrolyte conditions. Furthermore, the electrode material, concentration of electroactive species and also the electrochemical technique might also produce great peak shifts, whereas temperature and pressure have minor impact (Arslan et al., 2005; Reis et al., 2009; Abdel-Hamid & Newair, 2011; Masek et al., 2011).

The common cell used in electrochemical investigation of flavonoids is a three electrode cell. In this configuration, the potential of the working electrode is monitored relative to the reference potential (Ag/AgCl or SCE); however, the current passes between the working electrode and a counter electrode (platinum electrode). The tip of the reference electrode should be placed as close as possible to the working electrode in order to minimize solution resistance. Generally, the experiments were performed at the room temperature in aqueous supported electrolytes (citrate, HCl+KCl, acetate, phosphate, ammonia etc.) (Reis et al., 2009; Zielinska & Zielinski, 2010; Abdel-Hamid & Newair, 2011), but organic solvents (Sokolova et al., 2008; Han et al., 2009) were also used as a media buffer.

Although exist a high variety of working electrode materials, the must involved in the electrochemical studies of flavonoids are carbon electrodes, especially glassy carbon electrode (GCE) (Medvidovic-Kosanovic et al., 2010; Temerk et al., 2011; Zhang et al., 2011; Diculescu et al., 2012; Gil et al., 2012; Mulazimoglu et al., 2012). One explication for this is the oxidation potential of phenol derivatives which fit in the windows working potential of GCE (- 1.0 V to + 1.4 V). Nevertheless, other electric and mechanical properties such as extreme chemical inertness, highly resistant to acid attack, impermeability to gases and reproducible performance are considered.

Since the flavonoids, with some exception, are hardly soluble or even insoluble in water, the stock solutions were prepared in ethanol, methanol, NaOH or mixture of these in different proportions, and the sample solutions are diluted in the supporting electrolyte at the desired concentrations. However, due to the high sensitivity of voltammetric techniques, the electrochemical experiments request a small amount of stock solution, a concentration of sample of 5 to 25 μM being sufficient.

On the other hand, the solid-state electrochemistry, a methodology based on the mechanical immobilization of solid particles at the electrode surface, widens the investigation possibilities due to its broad applicability (Mulazimoglu et al., 2012).

The main involved techniques in the electrochemical studies and analysis of flavonoids are cyclic, differential pulse and square wave voltammetry in stationary state, although exist many reports of the flow analysis (Volikakis & Efstathiou, 2000; Volikakis & Efstathiou, 2005).

Quality control purposes

Besides the well-established use of the electrochemical parameters, E_p and I_p, on the evaluation of redox behavior and antioxidant capacity, they also have promising applications on the quantitative and qualitative analysis of electroactive antioxidants (Romani et al., 2000; Escarpa et al., 2007; Simic et al., 2007; Reis et al., 2009). Therefore, considering the almost ubiquitous presence of flavonoids in natural products, the electrochemical techniques may be very helpful for their quality control purposes (Bara et al., 2008; Novak et al., 2008; Kilinc, 2009; Rene et al., 2010; Zielinska & Zielinski, 2010).

Quantitative assays

The quantitative determination of flavonoid is based on Faraday Law, and good linear correlations have been obtained between concentration and peak currents for various flavonoids (Adam et al., 2007;

Liu et al., 2008a; Liu et al., 2008b; Xing et al., 2009; Dongming et al., 2011; Temerk et al., 2011; Macikova et al., 2012). In spite of remarkable advantages, *i.e. l*ow cost of apparatus, low reagent consuming, good sensitivity and suitable selectivity, regardless the low reproducibility, the use of electroanalysis in quantitative analysis of phytopharmaceuticals still remains neglected (Reis et al., 2009; Zielinska & Zielinski, 2010). Indeed, the reproducibility of electrochemical reactions requires the same experimental conditions, which is hard to afford after the renewal of electrode surface prior to each measurement (Huang et al., 2004; Ferreira et al., 2006; Adam et al., 2007; Mülazımoğlu et al., 2011; Mülazımoğlu et al., 2012).

Another obstacle is concerned to the interfering effect of other polyphenolic compounds in real samples, which may difficult the direct analysis of one specific flavonoid (Reichart & Obendorf, 1998; Adam et al., 2007; Novak et al., 2008; Reis et al., 2009). Therefore, the analysis of flavonoids in complex matrices, *i.e.* teas, beverages, crude extracts and phytopharmaceuticals often requires methods of separation, where owing to the inherent electroactivity, electrochemical detectors is always suitable. Thus, it has driven the development of High Performance Liquid Chromatography coupled to electrochemical detection (HPLC-ED) methods (Romani et al., 2000; Aaby et al., 2004; Kilinc, 2009).

Other efforts to overcome the effect of interferences, enabling the direct quantitative analysis is the development of selective electrodes (Vestergaard et al., 2005; Reis et al., 2009; Brondani et al., 2010; Mülazımoğlu et al., 2011), as well as the use of other coupled systems FIA (Volikakis & Efstathiou, 2000; Volikakis & Efstathiou, 2005), and electrophoresis (Moreno et al., 2011).

Table 3 shows some few examples of electroanalytical approaches, where it was used different modified electrodes on direct detection of the phytopharmaceutical, rutin in pharmaceutical samples.

Qualitative assays

The characterization of redox behavior can be a useful tool for the tentative identification of electroactive species (Aaby et al., 2004; Bara et al., 2008; Reis et al., 2009; Abdel-Hamid & Newair, 2011; Diculescu et al., 2012). In the case of flavonoids, the voltammetric profile is archetypal, accordingly to phenol pattern. Nevertheless, additional peaks and shoulders can appear upon determined experimental conditions in flavonoids containing diverse number of substituents, whilst voltammograms with no defined peaks are associated to the absence of free phenolic groups (Vestergaard et al., 2005; Fernandes et al., 2010; Fogliatto et al., 2010; Zhang et al., 2011; Diculescu et al., 2012).

The main electroanalytical techniques employed on the electrochemical characterization are cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV) (Adam et al., 2007; Novak et al., 2009a; 2009b; Fernandes et al., 2010; Temerk et al., 2011). The reversibility of each electron transfer process can be evaluated by means of CV and SWV (Ghica & Oliveira-Brett, 2005; Adam et al., 2007; Fernandes et al., 2010; Abdel-Hamid & Newair, 2011).

On the other hand, CV assays carried out at different scan rates can provide information about the coefficient of diffusion of the electroactive species, while the plot I_p *versus* ν or $\nu^{1/2}$, indicates if the process is controlled by adsorption or diffusion (Janeiro et al., 2005; El-Shahaw, 2006; Liu et al., 2008a; Liu et al., 2008b; Xing et al., 2009; Abdel-Hamid & Newair, 2011; Dongming et al., 2011; Diculescu et al., 2012; Gil et al., 2012).

In turn, the number of electrons involved can

Table 3. Modified electrodes and related limit of detection (LoD) on the direct electroanalytical detection of rutin in pharmaceutical samples.

Electrode	Technique	LoD (nM)	References
LF-GCE	SWV	0.25	Tyszczuk, 2009
ss-HGCE	SWV	1	Wu et al., 2008
DNA-IL-CPE	DPV	1.3	Wang et al., 2010
IL-CPE	DPV	5	Macikova & Skopalova, 2010
Copper(II)-resin-CPE	CV	26.5	Freitas et al., 2009
AuNPs/En/MWNTs/GCE	DPV	32	Oliveira & Mascaro, 2011
CNT-CPE	DPV	33.9	Yang et al., 2010
MWCNT-CPE	SWV	50	Behzad et al., 2011
PABSA-GC	SWV	100	Chen et al., 2010
Biomimetic Sensor	SWV	175	Ziyatdinova, et al., 2011
MWNT-GCE	CV	710	Franzoi et al., 2009

Carbon paste electrode (CPE); Multiwall carbon nanotube (MWCNT); Carbon nanotubes (CNT); Glassy carbon electrode (GCE); Ionic Liquid (IL); gold nanoparticles (AuNPs); ethylenediamine (En); poly(*p*-aminobenzene sulfonic acid (PABSA/GC); Lead film (LF); Single-sided heated graphite cylindrical electrode (ss-HGCE).

be stated by means of CV, DPV or coulometry (Peyrat-Maillard et al., 2000; Oliveira-Brett & Ghica, 2003; Reis et al., 2009; Abdel-Hamid & Newair, 2011). From CV assays, the calculated values, ($|E_{pa} - E_{pa/2}|$) of about 29 mV and 57 mV points to the involvement of two and one electrons, respectively. Whereas, from DPV assays, bi and mono-electron transfer process is theoretically determined by the half width, $W_{1/2}$ of 45 mV and 90 mV, respectively (Janeiro & Oliveira-Brett, 2005; Janeiro et al., 2005; El-Shahaw, 2006; Abdel-Hamid & Newair, 2011; Enache & Oliveira-Brett, 2011; Diculescu et al., 2012).

The establishment of an electrochemical index (Blasco et al., 2004; Escarpa et al., 2007) and the analysis of such voltammetric parameters have been proposed as attempts for qualitative analysis of such natural products (Kilmartin et al., 2002; Volikakis & Efstathiou, 2005; Bara et al., 2008; Kilinc, 2009; Reis et al., 2009).

Conclusions

The electroactivity of flavonoids resides on the phenolic groups and the electron donor ability is mainly governed by B-ring chemistry. The redox behavior varies according to the number and position of chemical substituent, and the potential required for oxidation is reduced as the stability of radicals is improved. Consequently, catechol like patterns, as well as electron donor substituent leads to compounds with higher electron donor ability, which may be capable to neutralize deleterious free radicals and also to restore physiological low molecular weight antioxidant agents.

So that, in most cases, the potential peak determination truly expresses the antioxidant power of each flavonoid. Furthermore, owing to the variable mechanisms of antioxidant action presented by flavonoids, the electroanalytical methods are the most suitable techniques for the evaluation of antioxidant capacity, as well as to characterize electroactive species. Furthermore, the electrochemical approaches have showed good correlations with spectrometric methods, and have also been applied to evaluate metal quelation properties.

Nevertheless, the mechanism of oxidation of each phenolic group often involves the transfer of one electron and one proton, being easier for hydroxyl groups with low acid character, and might be lower in non-acidic medium. Therefore, the association between electrochemical parameters and antioxidant activity may be always correlated to well-defined experimental conditions.

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Authors´ contributions

ESG, study guidance and development; ROC, editing and critical reading of the manuscript.

References

Aaby K, Hvattum E, Skrede G 2004. Analysis of flavonoids and other phenolic compounds using High-Performance Liquid Chromatography with coulometric array detection: relationship to antioxidant activity. *J Agric Food Chem 52*: 4595-4603.

Abdel-Hamid R, Newair EF 2011. Electrochemical behaviour of antioxidants: I. Mechanistic study on electrochemical oxidation of gallic acid in aqueous solutions at glassy carbon electrode. *J Electroanal Chem 657*: 107-112.

Adam V, Mikelova R, Hubalek J, Hanustiak P, Beklova M, Hodek P, Horna A, Trnkova L, Stiborova M, Zeman L, Kizek R 2007. Utilizing of square wave voltammetry to detect flavonoids in the presence of human urine. *Sensors 7*: 2402-2418.

Aguilar-Sanchez R, Ahuatl-Garcia F, Davila-Jimenez MM, Elizalde-Gonzalez MP, Guevara-Villa MRG 2005. Chromatographic and electrochemical determination of quercetin and kaempferol in phytopharmaceuticals. *J Pharmaceut Biomed 38*: 239-249.

Amic D, Davidovic-Amic D, Beslo D, Trinajstic N 2003. Structure-radical scavenging activity relationships of flavonoids. *Croatica Chim Acta 76*: 55-61.

Arslan G, Yazici B, Erbil M 2005. The effect of pH, temperature and concentration on electrooxidation of phenol. *J Hazard Mater B124*: 37-43.

Bara MTF, Serrano SHP, Asquieri ER, Lúcio TC, Gil ES 2008. Medida del potencial anódico en estado sólido: Una herramienta para la determinación del potencial antioxidante de fitoterápicos. *Lat Am J Pharm 27*: 89-92.

Behzad R, Najmeh M, Ali AE, Hassan KM 2011. Molecularly imprinted-multiwall carbon nanotube paste electrode as a biosensor for voltammetric detection of rutin. *Anal Method 3*: 2510-2516.

Blasco AJ, Gonzalez MC, Escarpa A 2004. Electrochemical approach for discriminating and measuring predominant flavonoids and phenolic acids using differential pulse voltammetry: towards an electrochemical index of natural antioxidants. *Anal Chim Acta 511*: 71-81.

Brondani D, Vieira IC, Piovezan C, Silva JMR, Neves A, Dupont J, Scheeren CW 2010. Sensor for fisetin based on gold nanoparticles in ionic liquid and binuclear nickel complex immobilized in silica. *Analyst 135*: 1015-1022.

Cao G, Sofic E, Prior RL 1997. Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. *Free Rad Bio Med. 22*: 749-760.

Chaves DS, Frattani FS, Assafim M, de Almeida AP, de Zingali RB, Costa SS 2011. Phenolic chemical composition

of *Petroselinum crispum* extract and its effect on haemostasis. *Nat Prod Commun 6*: 961-964.

Chen X, Wang Z, Zhang F, Zhu L, Li Y, Xia Y 2010. Determination of rutin on the poly(*p*-aminobenzene sulfonic acid) modified glassy carbon electrode. *Chem Pharm Bull 58*: 475-478.

Cook NC, Samman S 1996. Flavonoids-chemistry, metabolism, cardioprotective effects, and dietary sources. *J Nutr Biochem 7*: 66-76.

Diculescu V, Santana HE, Gil ES, Oliveira-Brett AM 2012. Methoxylation and glycosylation effect on the redox mechanism of citroflavones. *Electroanal 24*: 1019-1026.

Dongming Z, Xiuhua Z, Lijun F, Qi Q, Wang S 2011. Sensitive electrochemical determination of luteolin in peanut hulls using multi-walled carbon nanotubes modified electrode. *Food Chem 127*: 694-698.

Dueñas M, González-Manzano S, González-Paramás A, Santos-Buelga C 2010. Antioxidant evaluation of *O*-methylated metabolites of catechin, epicatechin and quercetin. *J Pharmaceut Biomed 51*: 443-449.

El-Shahaw MS 2006. Determination of trace levels of diosmin in a pharmaceutical preparation by adsorptive stripping voltammetry at a glassy carbon electrode. *Anal Sci 22*: 1351-1354.

Enache TA, Oliveira-Brett AM 2011. Phenol and para-substituted phenols electrochemical oxidation pathways. *J Electroanal Chem 655*: 9-16.

Erlund I 2004. Review of the flavonoids quercetin, hesperetin and naringenin. Dietary sources, bioactivities, bioavailability, and epidemiology. *Nutr Res 24*: 851-874.

Escarpa A, González MC, Blasco AJ, Rogerio MC, Hervás M 2007. Evaluation of accuracy of electrochemical isoflavonoid index for the determination of total isoflavones in soy samples. *Electroanal 19*: 952-957.

Fernandes IPG, Oliveira SCB, Ghalkhani M, Shahrokhian S, Oliveira-Brett AM 2010. Antioxidants daidzein and 7-hydroxy-4-chromone electrochemical oxidation mechanisms. *Anal Chim Acta 22*: 1-9.

Ferreira M, Varela H, Torresi RM, Tremiliosi-Filho G 2006. Electrode passivation caused by polymerization of different phenolic compounds. *Electrochim Acta 52*: 434-442.

Fogliatto DK, Barbosa AMJ, Ferreira VS 2010. Voltammetric determination of the phytoestrogen genistein in soy flours and soy based supplements using cationic surfactant cetyltrimetylammonium bromide. *Colloid Surf B: Biointerfaces 78*: 243-249.

Franzoi AC, Peralta RA, Neves A, Vieira IC 2009. Biomimetic sensor based on Mn(III)Mn(II) complex as manganese peroxidase mimetic for determination of rutin. *Talanta 78(1)*: 221-226.

Freitas KHG, Medeiros RA, Fatibello-Filho O 2009. Voltammetric determination of rutin using a carbon composite electrode modified with Copper(II)-resin. *Anal Lett 42*: 881-897.

Ghica ME, Oliveira-Brett AM 2005. Electrochemical oxidation of rutin. *Electroanalysis 17*: 313-318.

Gil ES, Enache AT, Oliveira-Brett AM 2012. Anodic behaviour of flavonoids orientin, eriodictyol and robinin at a glassy carbon electrode. *Electroanal 24*: 1576-1583.

Gomez-Pineda LE, Oropeza-Guzman MT, Pina-Luis GE 2009. Identification of flavonoid oxidation potentials as a function of pH. *ECS Transactions 20*: 141-149.

Han RM, Tian YX, Liu Y, Chen CH, Ai XC, Zhang JP, Skibsted LH 2009. Comparison of flavonoids and isoflavonoids as antioxidants. *J Agric Food Chem 57*: 3780-3785.

Harborne JB, Williams CA 2000. Advances in flavonoid research since 1992. *Phytochemistry 55*: 481-504.

He JB, Yuan SJ, Du JQ, Hu XR, Wang Y 2009. Voltammetric and spectral characterization of two flavonols for assay-dependent antioxidant capacity. *Bioelectrochem 75*: 110-116.

Hendrickson HP, Kaufman AD, Lunte CE 1994. Electrochemistry of catechol-containing flavonoids. *J Pharmaceut Biomed 12*: 325-334.

Hidalgo M, Sánches-Moreno C, Pascual-Teresa S 2010. Flavonoid-flavonoid interaction and its effect on their antioxidante activity. *Food Chem 121*: 691-696.

Huang T, Gao P, Hageman MJ 2004. Rapid screening of antioxidants in pharmaceutical formulation development using cyclic voltammetry - potential and limitations. *Curr Drug Disc Tech 1*: 173-179.

Janeiro P, Oliveira-Brett AM 2005. Solid state electrochemical oxidation mechanism of morin in aqueous media. *Electroanal 17*: 733-738.

Janeiro P, Corduneanu O, Oliveira-Brett AM 2005. Chrysin and (±)taxifolin electrochemical oxidation mechanisms. *Electroanal 17*: 1059-1064.

Janeiro P, Oliveira-Brett AM 2007. Redox Behavior of Anthocyanins present in *Vitis vinifera* L. *Electroanal 19*: 1779-1786.

Jorgensen LV, Cornett C, Justesen U, Skibsted LH, Dragsted LO 1998. Two-electron electrochemical oxidation of quercetin and kaempferol changes only the flavonoid C-ring. *Free Rad Res 29*: 339-350.

Jorgensen LV, Madsen HL, Thomsen MK, Dragsted LO, Skibsted LH 1999. Regeneration of phenolic antioxidants from phenoxyl radicals: an ESR and electrochemical study of antioxidant hierarchy. *Free Rad Res 30*: 207-220.

Jovanovic SV, Steenken S, Hara Y, Simic MG 1996. Reduction potentials of flavonoid and model phenoxyl radicals. Which ring in flavonoids is resp*onsible for antioxidant activity?* J Chem Soc Perk T 2 11: 2497-2504.

Kilinc E 2009. Significance of chromatographic and voltammetric data for the classification of green teas in Türkiye: a principle component analysis approach. *J Liq Chromatogr R T 32*: 221-241.

Kilmartin PA, Zou H, Waterhouse AL 2002. Correlation of wine phenolic composition *versus* cyclic voltammetry

response. *Am J Enol Viticult 53*: 294-302.

Korbut O, Buková M, Labuda J, Gründler P 2003. Voltammetric detection of antioxidative properties of flavonoids using electrically heated DNA modified carbon paste electrode. *Sensors 3*: 1-10.

Labuda J, Buková M, Heilerová L, Šilhár S, Štepánek I 2003. Evaluation of the redox properties and anti/pro-oxidant effects of selected flavonoids by means of a DNA-based electrochemical biosensor. *Anal Bioanal Chem 376*: 168-173.

Liang J, Tian YX, Fu LM, Wang TH, Li HJ, Wang P, Han RM, Zhang JP, Skibsted LH 2008. Daidzein as an antioxidant of lipid: effects of microenvironment in relation to chemical structure. *J Agric Food Chem 56*: 10376-10383.

Lima AA, Sussuchi EM, de Giovani WF 2007. Electrochemical and antioxidant properties of anthocyanins and anthocyanidins. *Croatica Chem Acta 80*: 29-34.

Liu AL, Zhang SB, Chen W, Huang LY, Lin XH, Xia XH 2008a. Study of the electrochemical behavior of isorhamnetin on a glassy carbon electrode and its application. *Talanta 77*: 314-318.

Liu A, Zhang S, Huang L, Cao Y, Yao H, Chen W, Lin X 2008b. Electrochemical oxidation of luteolin at a glassy carbon electrode and its application in pharmaceutical analysis. *Chem Pharm Bull 56*: 745-747.

Macikova, P; Halouzka, V; Hrbac, J; Bartak, P; Skopalova J 2012. Electrochemical Behavior and Determination of Rutin on Modified Carbon Paste Electrodes. *ScientificWorldJournal*. 2012, Article ID 394756, 9 pages.

Markovic ZS, Mentus SV, Dimitric MJM 2009. Electrochemical and density functional theory study on the reactivity of fisetin and its radicals: implications on *in vitro* antioxidant activity. *J Phy Chem A 113*: 14170-14179.

Martinez S, Valek L, Petrovic Z, Metikos-Hukovic M, Piljac J 2005. Catechin antioxidant action at various pH studied by cyclic voltammetry and PM3 semi-empirical calculations. *J Electroanal Chem 584*: 92-99.

Masek A, Zaborski M, Chrzescijanska E 2011. Electrooxidation of flavonoids at platinum electrode studied by cyclic voltammetry. *Food Chem 127*: 699-704.

Medvidovic-Kosanovic M, Šeruga M, Jakobek L, Novak I 2010. Electrochemical and antioxidant properties of (+)-catechin, quercetin and rutin. *Croatica Chem Acta 83*: 197-207.

Mishra K, Ojha H, Chaudhury NK 2012. Estimation of antiradical properties of antioxidants using DPPH Assay. A critical review and results. *Food Chem 130*: 1036-1043.

Montoro P, Braca A, Pizza C, De Tommasi N 2005. Structure-antioxidant activity relationships of flavonoids isolated from different plant species. *Food Chem 92*: 349-355.

Moreno M, Arribas AS, Bermejo E, Zapardiel A, Chicharro M 2011. Analysis of polyphenols in wine by CZE with amperometric detection using carbon nanotube-modified electrodes. *Electrophoresis 32*: 877-883.

Mülazımoğlu IE, Özkan E, Solak AO 2011. Covalently grafted on to the glassy carbon electrode in non-aqueous media of apigenin and naringenin as different flavonoid derivatives. *Anal Bioanal Electrochem 3*: 102-118.

Mülazımoğlu IE, Özkan E, Solak AO 2012. High energetic body source and strong antioxidant quercetin, morin and rutin: Their covalent grafting onto the glassy carbon electrode surfaces and investigation of surface properties. *Energy Educ Sci Tech A 28*: 957-968.

Narayana KR, Reddy MS, Chaluvadi MR, Krishna DR 2001. Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. *Indian J Pharmacol 33*: 2-16.

Nasr B, Abdellatif G, Cañizares P, Sáez C, Lobato J, Rodrigo MA 2005. Electrochemical oxidation of hydroquinone, resorcinol, and catechol on boron-doped diamond anodes. *Environ Sci Technol 39*: 7234-7239.

Nijveldt RJ, Van Nood E, Van Hoorn DEC, Boelens PG, Van Norren K, Van Leeuwen PAM 2001. Flavonoids: a review of probable mechanisms of action and potential applications. *Am J Clin Nutr 74*: 418-425.

Novak I, Janeiro P, Seruga M, Oliveira-Brett AM 2008. Ultrasound extracted flavonoids from four varieties of Portuguese red grape skins determined by reverse-phase high-performance liquid chromatography with electrochemical detection. *Anal Chim Acta 630(2)*: 107-115.

Novak I, Seruga M, Komorsky-Lovric S 2009a. Square-wave and cyclic voltammetry of epicatechin gallate on glassy carbon electrode. *J Electroanal Chem 631*: 71-75.

Novak I, Seruga M, Komorsky-Lovric S 2009b. Electrochemical characterization of epigallocatechin gallate using square-wave voltammetry. *Electroanal 21*: 1019-1025.

Oliveira AC, Mascaro LH 2011. Characterization of carbon nanotubes paste electrode and its application as rutin sensor. *Curr Anal Chem 7*: 101-109.

Oliveira-Brett AM, Ghica ME 2003. Electrochemical oxidation of quercetin. *Electroanal 15*: 1745-1750.

Pannala AS, Chan TS, O´Brien PJ, Rice-Evans CA 2001. Flavonoid B-ring chemistry and antioxidant activity: fast reaction kinetics. *Biochem Bioph Res Co 282*: 1161-1168.

Peyrat-Maillard MN, Bonnely S, Berset C 2000. Determination of the antioxidant activity of phenolic compounds by coulometric detection. *Talanta 51*: 709-716.

Rapta P, Misik V, Stasko A, Vrabel I 1995. Redox intermediates of flavonoids and caffeic acid esters from propolis: an EPR spectroscopy and cyclic voltammetry study. *Free Rad Bio Med 18*: 901-908.

Reichart E, Obendorf D 1998. Determination of naringin in grapefruit juice by cathodic stripping differential pulse voltammetry at the hanging mercury drop electrode. *Anal Chim Acta 360*: 179-187.

Reis NS, Serrano SHP, Meneghatti R, Gil ES 2009. Métodos

eletroquímicos usados para avaliação da atividade antioxidante de produtos naturais. *Lat Am J Pharm 28*: 949-53.

Rene A, Abasq ML, Hauchard D, Hapiot P 2010. How do phenolic compounds react toward superoxide ion? A simple electrochemical method for evaluating antioxidant capacity. *Anal Chem 82*: 8703-8710.

Rice-Evans CA, Miller NJ, Paganga G 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Rad Bio Med, 20*: 933-956.

Romani A, Minunni M, Mulinacci N, Pinelli P, Vincieri FF, Del Carlo M, Mascini M 2000. Comparison among differential pulse voltammetry, amperometric biosensor, and HPLC/DAD analysis for polyphenol determination. *J Agric Food Chem 48*: 1197-1203.

Santos CMM, Garcia MBQ, Silva AMS, Santus R, Morlière P, Fernandes E 2013. Electrochemical characterization of bioactives hydroxyxanthones by cyclic voltammetry. *Tetrahedron Lett 54*: 85-90.

Slabbert NP 1977. Ionization of some flavonols and dihydroflavonoids. *Tetrahedron 33*: 821-824.

Simic A, Manojlovi D, Šegan D, Todorovi M 2007. Electrochemical behavior and antioxidant and prooxidant activity of natural phenolics. *Molecules 12*: 2327-2340.

Sims MJ, Li Q, Kachoosangi RT, Wildgoose GG, Compton RG 2009. Using multiwalled carbon nanotube modified electrodes for the adsorptive striping voltammetric determination of hesperidin. *Electrochim Acta 54*: 5030-5034.

Sokolova R, Degano I, Ramesova S, Bulıckova J, Hromadova M, Gal M, Fiedler J, Valášek M 2008. The oxidation mechanism of the antioxidant quercetin in nonaqueous media. *Electrochim Acta 56*: 7421-7427.

Taylor LP, Grotewold E 2005. Flavonoids as developmental regulators. *Curr Opin Plant Biol 8*: 317-323.

Temerk YM, Ibrahim MS, Kotb M 2011. Square-wave cathodic adsorptive stripping voltammetric determination of 3-hydroxyflavone, morin and hesperidin in bulk form and biological fluids in absence and presence of Cu(II). *J Braz Chem Soc 22*: 2056-2064.

Tsao R 2010. Chemistry and biochemistry of dietary polyphenols. *Nutrients 2*: 1231-1246.

Tsimogiannis DI, Oreopoulou V 2004. Free radical scavenging and antioxidant activity of 5,7,3´4´-hydroxy-substituted flavonoids. *Innov Food Sci Emerg 5*: 523-528.

Tyszczuk K 2009. Sensitive voltammetric determination of rutin at an in situ plated lead film electrode. *J Pharmaceut Biomed 49*: 558-561.

Vestergaard M, Kerman K, Tamiya E 2005. An electrochemical approach for detecting copper-chelating properties of flavonoids using disposable pencil graphite electrodes: possible implications in copper-mediated illnesses. *Anal Chim Acta 538*: 273-281.

Volikakis GJ, Efstathiou CE 2000. Determination of rutin and other flavonoids by flow-injection/adsorptive stripping voltammetry using nujol-graphite and diphenylether-graphite paste electrodes. *Talanta 51*: 775-785.

Volikakis GJ, Efstathiou CE 2005. Fast screening of total flavonols in wines, tea-infusions and tomato juice by flow injection/adsorptive stripping voltammetry. *Anal Chim Acta 551*: 124-131.

Xiao P, Zhou Q, Xiao F, Zhao F, Zeng B 2006. Sensitive voltammetric determination of morin on a multi-walled carbon nanotubes-paraffin oil paste electrode. *Int J Electrochem Sci 1*: 228-237.

Xing TL, Wang F, Mao YY, Wang LP, Ye BX 2009. Electrochemical behavior of apigenin at a glassy carbon electrode and its analytical application. *J Chin Chem Soc 56*: 303-309.

Wang Y, Xiong H, Zhang X, Wang S 2010. Detection of rutin at DNA modified carbon paste electrode based on a mixture of ionic liquid and paraffin oil as a binder. *Microchim Acta 170*: 27-32.

Wu L, Laughlin-Jr JB, Dewald HD 1997. Voltammetry and LCEC of isoflavones. *Electroanal 9*: 796-799.

Wu DM, Liu S, Gao HF, Li JL, Liu HY, Liang QC 2007. Investigation on the Electrochemical and Spectra Properties of Myricetin. *J Electrochem 13*: 29-34.

Wu SH, Sun JJ, Zhang DF, Lin ZB, Nie FH, Qiu HY, Chen GN 2008. Nanomolar detection of rutin based on adsorptive stripping analysis at single-sided heated graphite cylindrical electrodes with direct current heating. *Electrochim Acta 53*: 6596-6601.

Yakovleva KE, Kurzeev SA, Stepanova EV, Federova TV, Kuznetsov BA, Koroleva OV 2007. Characterization of plant phenolic compounds by cyclic voltammetry. *Appl Biochem Micro+ 43*: 661-668.

Yamamura S 2003. Oxidation of phenols. In: Rappoport Z, *The chemistry of phenols*, John Wiley & Sons Ltd, Chichester, p. 1154-1156.

Yang B, Kotani A, Arai K, Kusu F 2001. Estimation of the antioxidant activities of flavonoids from their oxidation potentials. *Anal Sci 17*: 599-604.

Yang S, Qu L, Li G, Liu C 2010. Gold nanoparticles/ethylenediamine/carbon nanotube modified glassy carbon electrode as the voltammetric sensor for selective determination of rutin in the presence of ascorbic acid. *J Electroanal Chem 645*: 115-122.

Zhang D, Chu L, Liu Y, Wang A, Ji B, Wu W, Zhou F, Wei Y, Cheng Q, Cai S, Xie L, Jia G 2011. Analysis of the antioxidant activity capacities of flavonoids under different spectrophotometric assays using cyclic voltammetry and density functional theory. *J Agr Food Chem 59*: 10277-10285.

Zielinska D, Zielinski H 2010. Antioxidant activity of flavone C-glucosides determined by updated analytical strategies. *Food Chem 124*: 672-678.

Ziyatdinova G, Aytuganova I, Nizamova A, Morozov M, Budnikov H 2011. Cyclic voltammetry of natural

flavonoids on MWNT-modified electrode and their determination in pharmaceuticals. *Collect Czech Chem C 76*: 1619-1631.

Zoulis NE, Efstathiou CE 1996. Preconcentration at a carbon-paste electrode and determination by adsorptive-stripping voltammetry of rutin and other flavonoids. *Anal Chim Acta 320*: 255-261.

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