Food Chemistry 215 (2017) 501–507

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Influence of hydroxyl substitution on flavanone antioxidants properties

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ARTICLE INFO

Article history:
Received 31 March 2016
Received in revised form 28 July 2016
Accepted 29 July 2016
Available online 3 August 2016

Keywords:
Flavones
Electrochemical oxidation
UV–VIS
Antioxidant
ABTS
DPPH

ABSTRACT

The aim of our study was to determine the effect of the position of the hydroxyl group on the antioxidant properties of flavonoid derivatives. For this purpose, we performed electrochemical analysis and quantum-mechanical calculations to describe the mechanisms of electrochemical oxidation, and we selected the two methods of ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) and DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate), which allowed us to determine the ability to scavenge free radicals. On the basis of the research, we found that the derivatives of flavonoids, which have a hydroxyl group *substituted at the R-3 position* on the *C ring*, have outstanding antioxidant activity. Flavone, which had an OH group substituted at the *R*-6 and *R-7* position on the ring *A*, showed similar antioxidant activity to flavone without -OH groups in the structure and slightly higher activity than the di-substituted flavone on the ring A.

1. Introduction

Flavonoids occur in nature (in vegetables) and are a large group of phenolic secondary metabolites. Flavonoids are present in leaves and flowers, often as colorants, as well as in fruit, bark, wood and seeds. The flavonoids determine the colour, odour and flavour of fruits and flowers, with the result that the plants are recognizable to insects, birds and mammals carrying the pollen or seeds. Flavonoids play important functions in the interaction of plants with the external environment. There are natural repellents, that is, dissuasive factors to other organisms. Flavonoids are also toxic to pests and pathogenic fungi and bacteria and protect plants from infection. There are currently over 7000 different flavonoids, and this number is increasing due to the ongoing research in this area. Flavonoids have a beneficial biological effect in the body because they can prevent many diseases, such as cardiovascular diseases and cancer. Their biological activity is related to the nature and position of substituents on their ring system (Verma & Pratap, 2012). The main structure of flavonoids is two phenolic rings labelled A and B. Rings A and B are connected by a 3-C bridge, which is usually via an oxygen atom enclosed in a third ring called C (Güney, Yildiz, Capan, & Ozturk, 2010; Procházková, Boušová, & Wilhelmová, 2011). Due to the differences in the structural construction, flavones, isoflavones, flavonols, anthocyanidins and chalcones can perform many functions (Fig. 1 inset).

Flavones are a group of flavonoids that occurs more often in vegetables than fruit. Flavonols lack a hydroxyl group on the third carbon. The best-known flavones are luteolin and apigenin, which are present in red pepper, parsley, celery, millet, wheat, wild rose, mint, thyme and coltsfoot (Dobes et al., 2013; Huber, Hoffmann-Ribani, & Rodriguez-Amaya, 2009). The antioxidant properties of flavones determine their biological functions, such as antimutagenic action, anticancer and delaying the ageing process. For this reason, flavones have been used in the treatment of many diseases, including cardiovascular diseases and cancer. In recent years, there has been growing interest in a variety of flavonoids and flavone because they may be important for the agricultural, pharmaceutical and cosmetics industries. Significant research has been conducted to identify new compounds and to determine their physicochemical and pharmacological properties (Ziyatdinova & Budnikov, 2015a). Studies on the antioxidant properties of flavones are important to understand their biochemical properties. Several methods have been developed to evaluate the antioxidant capacity of flavonoids; these methods are mostly based on spectrophotometric measurements, such as UV–Vis spectrophotometry and fluorescence spectrophotometry, and the radical-trapping mechanism of antioxidants has been investigated. Most evaluation methods are categorized into one of three classes on the basis of the reaction of reagents with antioxidants: hydrogen-atom transfer reactions, electron-transfer reactions, and others. In particular, the ABTS and DPPH methods have been widely used to evaluate the antioxidant capacity of flavones. The ABTS and DPPH methods are related to the mechanism of free radical scavenging and are

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http://dx.doi.org/10.1016/j.foodchem.2016.07.183
0308-8146/

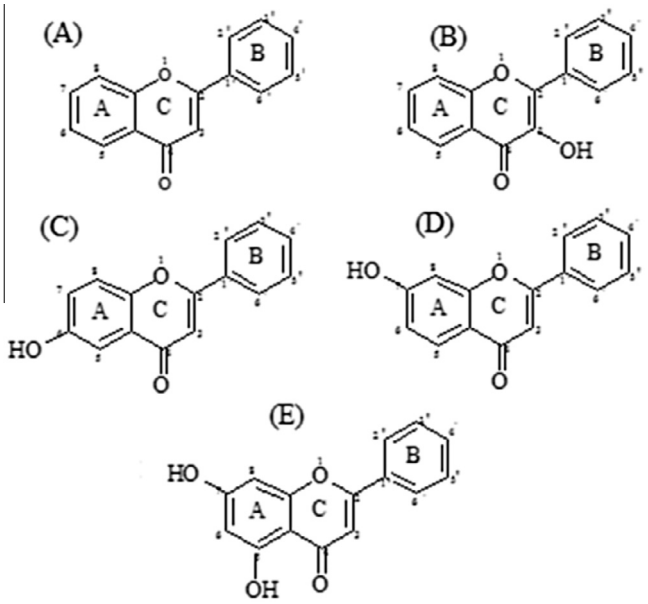

Fig. 1. Chemical structure of: (A) flavone, (B) 3-hydroxyflavone, (C) 6-hydroxyflavone, (D) 7-hydroxyflavone, (E) 5,7-dihydroxyflavone.

used to determine the antioxidant properties flavonoids (Ignat, Volf, & Popa, 2011).

Spectrophotometric and spectrofluorometric methods are also often used in quantitative determination of flavones (Buffa, Carturan, Quaranta, Maggioni, & Della Mea, 2012). These methods are characterized by simplicity of analysis and are less expensive and time-consuming in comparison with other analytical methods such as gas chromatography, liquid chromatography coupled with mass spectrometry (GC–MS, LC–MS) and high-performance liquid chromatography (HPLC). However, serious errors can arise if a coloured and/or muddy sample is evaluated, which presents a disadvantage. In contrast, electrochemical measurements do not require expensive instruments and are available for coloured and/or muddy samples, while the portability of the instrumentation is an advantage.

Electrochemical techniques are being developed and improved for the investigation and determination of phenolic compounds (Djeridane et al., 2006; Enujiugha, Talabi, Malomo, & Olagunju, 2012; Katalinic, Milos, Kulisic, & Jukic, 2006; Wojdyło, Oszmianski, & Czemerys, 2007; Ziyatdinova & Budnikov, 2015b). These techniques are low-cost, sensitive and enable rapid analysis of samples (Blasco, Crevillen, Gonzalez, & Escarpa, 2007). Electroanalytical techniques, more specifically voltammetric techniques, are especially well-suited to investigate the properties of polyphenols (Masek, Chrzescijanska, & Zaborski, 2014a). Applying this method, the oxidation potential of the substance, the number of transferred electrons and the rate of the electrode reaction can be determined. A simple electrochemical method based on the measurement of the half-wave potential ($E_{1/2}$) of the first oxidation wave for estimating the antioxidant activity of flavonoids has been developed (Güney et al., 2010). The antioxidant properties of phenolic compounds are related to their ability to donate electrons. The presence of voltammetric signals (anodic peaks) at low potentials correlates with the presence of polyphenols of high antioxidant activity, whereas those compounds with low antioxidant power have electrochemical activity at more positive potentials (Arribas, Martinez-Fernandez, & Chicharro, 2012; Chevion, Roberts, & Chevion, 2000; Medvidovic-Kosanovic, Samardzic, Malatesti, & Sak-Bosnar, 2011; Sánchez Arribas, Martínez-Fernández, & Chicharro, 2012).

Recently, quantum chemical computation data were used to supply quantitative predictions of the behaviour of flavone derivatives, such as their chemical reactivity or their physicochemical properties, and these methods are useful to explain the biological properties associated with this class of compounds. The highest-occupied molecular orbital (HOMO) energy may help in rationalizing the activity of the compounds. The orbital determines the way in which a molecule interacts with other species. Thus, HOMO is the orbital that acts as an electron donor and is most deeply concentrated on the benzene ring; this is where the electrophilic attack most likely occurs. In this context, the aim of this work was a qualitative, systematic and comparative study on the electrooxidation of flavone, 3-hydroxyflavone, 6-hydroxyflavone, 7-hydroxyflavone and 5,7-dihydroxyflavone (Fig. 1) by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) methods using a platinum electrode. Quantum-chemical calculations were also performed for the tested flavonoids. The DPPH and ABTS methods, which are related to the mechanism of free radical scavenging, are used to determine the antioxidant properties flavonoids (Goyal & Singh, 2006; Zhou, Kikandi, & Sadik, 2007; Zieja, Gadomska-Trzos, & Stojek, 2001).

2. Experimental

2.1. Chemicals

All chemicals were used of analytical grade supplied from Fluka and Sigma-Aldrich. Experiments were performed in non-aqueous media. The substrates solutions were prepared by dissolving in 0.1 mol L^{-1} ($(C_4H_9)_4NClO_4$ in acetonitrile. The concentration of the flavones was in the range of 1×10^{-3} mol L^{-1} to 5.0×10^{-3} mol L^{-1}.

The following flavones were tested: flavone (2-phenyl-4*H*-1-benzopyran-4-one, $C_{15}H_{10}O_2$), 3-hydroxyflavone (3-hydroxy-2-phenylchromen-4-one,$C_{15}H_{10}O_3$),6-hydroxyflavone(6-hydroxy-2-phenylchromen-4-one, $C_{15}H_{10}O_3$), 7-hydroxyflavone (7-hydroxy-2-phenylchromen-4-one, $C_{15}H_{10}O_3$), 5,7-dihydroxyflavone (5,7-dihydroxy-2-phenyl-4*H*-chromen-4-one, $C_{15}H_{10}O_4$).

Solutions were thoroughly deoxygenated by purging with purified argon gas (99.999%) for 15 min prior to the electrochemical experiments. Argon blanket was maintained over the solutions to supply an inert atmosphere during voltammetric measurements.

2.2. Measurement methods

2.2.1. Cyclic and differential pulse voltammetry

To assess the electrochemical oxidation mechanism and the kinetics for the flavones under investigation, cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were used with an Autolab analytical unit (EcoChemie, Holland). The analyzer was controlled using GPES program. A three-electrode system was used for the measurements consisted of a reference electrode, an auxiliary electrode (platinum wire) and a working electrode – platinum with geometric surface area of 0.5 cm^2. A potential of the working electrode was measured vs. ferricinium/ferrocene reference electrode (Fc^+/Fc) couple as recommended by IUPAC (Bard & Faulkner, 2001; Gritzner & Kuta, 1984). Reference electrode was made of platinum wire immersed in solution of ferrocene c = 1×10^{-3} mol L^{-1}in 0.1 mol L^{-1} ($(C_4H_9)_4NClO_4$ in acetonitrile placed in an glass tube with a very tiny hole (diameter w 0.2 mm) at the bottom. The tiny hole allows an electrochemical contact between the electrolyte in the reference electrode compartment and that in the reactor compartment. Next step was coulometric oxidation to obtain an equivalent of ferrocene ion concentration ferricinium (Fc^+) ($c_{Fc} = c_{Fc+}$).