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Simultaneous amperometric detection of ascorbic acid and antioxidant capacity in orange, blueberry and kiwi juice, by a telemetric system coupled with a fullerene- or nanotubes-modified ascorbate subtractive biosensor

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ABSTRACT

Four fullerenes- or nanotubes-modified graphite sensor–biosensor systems (SBs), coupled with a dual-channel telemetric device, based on an ascorbate oxidase (AOx) biosensor, were developed for on line simultaneous amperometric detection of ascorbic acid (AA) and antioxidant capacity in blueberry, kiwi and orange juice. Fullerene C_{60} (FC_{60}), fullerene C_{70} (FC_{70}), single-walled carbon nanotubes (SWCN) and multi-walled carbon nanotubes (MWCN) increased the sensitivity of graphite toward AA and phenols 1.2, 1.5, 5.1 and 5.1 times respectively. Fullerenes combined with AOx improved the selectivity toward AA more than nanotubes, being able to hold a higher number of AOx molecules on the biosensor surface.

The SBs work at an applied potential of +500 mV, in a concentration range between the LOD and 20 μM, with a response time of two minutes. The LOD is 0.10, 0.13, 0.20 and 0.22 μM for SBs modified with FC60, FC70, SWCN and MWCN respectively.

Biosensors register lower AA currents than the sensors due to the enzyme capability to oxidize AA before it reaches the transductor surface. Phenols currents registered by sensors and biosensors did not differ. Based on the difference between sensor and biosensor recorded currents a AA selectivity index was developed as an indicator of specificity toward AA and of the capacity to distinguish between AA and phenols contribution to the antioxidant capacity. This value is almost zero for fullerene-modified SBs, 0.13 and 0.22 for SWCN- and MWCN-modified SBs respectively.

The results of juices analysis performed with SBs were in accordance with reference methods.

1. Introduction

Electrochemical sensors and biosensors have been largely used for direct or indirect detection of ascorbic acid (AA) and antioxidants in food, since those molecules can be easily oxidized at bare electrodes (Buratti et al., 2008; Intarakamhang et al., 2011; Pisoschi et al., 2009). A great number of electrochemical studies with antioxidant capacity evaluation as a target were carried out with cyclic voltammetry (CV) (Piljac-Žegarac et al., 2010; Zielinska et al., 2008) and constant amperometric techniques (Buratti et al., 2008): these are based on the simple principle that the potential at which the oxidation starts enables the identification of the antioxidant involved, and that the oxidation peak potential is an indicator of the antioxidant capacity. The efficiency of antioxidant is determined by its redox potential, therefore the lower the potential, the easier the oxidation and the higher the antioxidant capacity (Barroso et al., 2011; Chevion et al., 2000; Karadag et al., 2009).

The use of enzymes, coupled in various ways with sensors of different materials, gives to biosensors a high selectivity towards specific bioactive compounds, allowing the direct detection of antioxidant substances without prior separation steps (Barroso et al., 2011; Mello and Kubota, 2007). Ascorbate oxidase was largely used for the development of biosensors devoted to AA

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detection in different matrices: in red and white wines (Campanella et al., 2004; Vig et al., 2010), in commercial fruit juices (Wang et al., 2008), in natural fruit juices and soft drinks (Pisoschi et al., 2010), in fruit juices and vitamin C tablets (Chauhan et al., 2010).

Fullerenes and carbon nanotubes were extensively studied for their distinctive properties and their potential applications (Zhang et al., 2013). Fullerenes C_{60} (FC_{60}) and C_{70} (FC_{70}), revealed a relatively high electron affinity, hydrophobic surface, high surface/volume ratio and good adsorption capacity towards organic molecules. A FC_{60}-modified gold electrode was employed for the determination of dopamine in the presence of an excess of AA (Goyal et al., 2008). Immobilized FC_{60}-glucose oxidase enzyme was applied to a C_{60}-coated piezoelectric quartz crystal glucose sensor to detect gluconic acid, and the interference of AA was investigated (Chuang and Shih, 2001). An electroreduced C_{60}-[dimethyl-(β-cyclodextrin)]$_2$ and Nafion-chemically-modified gold electrode showed high electrocatalytic activity towards AA oxidation allowing the separation of the electrochemical responses of AA and dopamine (Wei et al., 2002). Single-walled carbon nanotubes (SWCN) and multi-walled carbon nanotubes (MWCN), with a surface area which ranges from 150 to 1500 $m^2 g^{-1}$, have high adsorption capacity and rapid desorbability, being excellent candidates for sensing applications (Hussain and Mitra, 2011). They can be covalently or non-covalently functionalized with different organic molecules to provide a more selective interaction with analytes (Zhang et al., 2013), to promote electron-transfer reactions with enzymes and to fabricate biosensors with improved performances (Pérez-Lopez and Merkoçi, 2012; Scida et al., 2011; Hrapovic et al., 2003). The studies on the application of carbon nanotubes, alone or combined with different enzymes, as tools for the detection of analytes in biological samples, is quite limited. Investigations were carried out on their applicability to blood analysis (Tang et al., 2004), for measuring phenolic compounds (Lee et al., 2007), to detect hydrogen peroxide in milk (Xu et al., 2010) and, more recently, for the screening of total antioxidant capacity (Amatatongchai et al., 2012).

In our previous studies we detected AA in orange juice and in fresh-cut melon, kiwi and pineapple fruits, using a simple graphite working electrode at an applied potential of +120 mV (Barberis et al., 2010, 2012). That system was highly specific for AA due to the low applied potential but, in agreement with Buratti et al. (2008), applying a higher potential up to +500 mV or more, it could be used to detect the antioxidant capacity of the most represented phenolic compounds in fruit juices. In this paper we present four fullerenes- or nanotubes-modified carbon sensor–biosensor systems (SBs), coupled with a dual-channel telemetric device and based on an ascorbate oxidase biosensor. We coupled an enzyme highly specific towards AA with nanomaterials able to improve the sensitivity of carbon sensors towards AA and phenolic compounds. The system was developed for the on line simultaneous amperometric detection of AA and antioxidant capacity in orange, blueberry and kiwi juice, and to distinguish between the AA and phenols contribution to antioxidant activity.

2. Materials and methods

2.1. Reagents

The list of reagents used in this work and the methodologies for solutions' preparation are reported in Supplementary information (S-1).

2.2. Fruit samples, juice preparation and chemical composition analyses

Blueberry fruits (*Vaccinum corymbosum* L.) *cv* Brigitta blue, kiwi fruits (*Actinidia deliciosa* (A. Chev.) *cv* Hayward, and orange fruits (*Citrus sinensis* (L.) Osbek) *cv* Moro, were bought on the local market. Fruits were squeezed and the juices were filtered, centrifuged at 4629 g for 10 min at 4 °C (centrifuge A.L.C.-4227R, A.L.C. s.r.l. Milano, Italy), stored in ultra-freezer at −80 °C and lyophilized. All freeze-dried samples were fully rehydrated for chemical composition analyses, in vitro calibrations and antioxidant capacity evaluation. Tocopherols analysis and LC-MS determination of phenolic compounds in the juices are described in Supplementary data (S-2.1 and S-2.2). The AA analytical determination of all fruit juices was performed according to Schirra et al. (2007) (Supplementary data S-2.3).

2.3. Standard mixture preparation

In accordance with Luthria and Vinyard (2008), a standard mixture (MIX) containing multiple phenol phytochemicals was prepared as a AA-free reference material, to evaluate if and how phenols affect the SBs behavior. 2 μmole of malvidin 3-O glucoside (in methanol), 2 μmole of delphinidin-3-O-glucoside (in methanol), 2 μmole of hesperidin in N,N-dimethylformamide (DMF), 2 μmole of caffeic acid (in ethanol) and 2 μmole of chlorogenic acid (in ethanol) were mixed in order to have a total of 10 μmole in 1 mL of solvent (10 mM). All these chemicals can be oxidized at $E_{app} \leq +500$ mV (Table 1 and Supplementary data Fig. S-4).

2.4. Sensor–biosensor systems description and preparation

Four different SBs were assembled, two composed with fullerenes and two with nanotubes. Each SB assembly consists of four carbon rod (length=30 mm; Ø=300 μm; 2H Staedtler graphite

Table 1
Oxidation potential values of standards of α-tocopherol and phenols characterizing blueberry, kiwi and orange juices. Values were obtained by CV, performed by the sensors nanostructured with FC_{60}, FC_{70}, SWCN and MWCN, with a scanned potential (E_{app}) comprised between −200 mV and +1000 mV, at a scan rate of 100 mV/s, vs carbon pseudoreference, in the absence and in the presence of 1 mM chemicals. Cyclic voltammograms of all compounds are provided in Supplementary data (Fig. S-1).

Chemicals	Oxidation starts at (mV)			
	S-FC_{60}	S-FC_{70}	S-SWCN	S-MWCN
Tocopherols				
α-tocopherol	+400	+300	+200	+220
Hydroxycinnamic acids				
Caffeic acid	+200	+200	+230	+120
Chlorogenic acid	+270	+230	+200	+240
p-coumaric acid	+470	+470	+450	+480
Ferulic acid	+350	+330	+300	+350
Sinapic acid	+250	+250	+220	+240
Hydroxybenzoic acid				
Gallic acid	+210	+240	+170	+200
Flavanoids				
Quercetin	+160	+130	+130	+120
Rutin	+280	+250	+260	+250
Catechin	+300	+320	+250	+260
Hesperidin	+370	+370	+420	+460
Naringin	+520	+600	+560	+600
Naringenin	+560	+570	+510	+530
Anthocyanins				
Cyanidin 3-O glucoside (kuromanin)	+280	+300	+280	+250
Malvidin-3-O-glucoside (oenin)	+360	+320	+280	+300
Delphinidin-3-O-glucoside (myrtillin)	+220	+270	+220	+200

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