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Determination of Several Food Additives and Ingredients by Electrochemical Techniques

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The design, development and optimization of performant methods for food analysis represents a continuously increasing research interest and focus, as food safety is undeniably related to human health. Developing analytical techniques for food quality control involves the qualitative and quantitative determination of food additives and ingredients, as well as of contaminants, aiming at the identification of adulteration and/or contamination of foodstuffs [1,2].

Electrochemical methods are characterized by sensitivity and selectivity, fast response, as well as by simplicity of the analytical procedure, requiring no complicated sample pre-treatment and involving relatively low cost, when compared to traditional laborious instrumental techniques. That is why, they were applied to the qualitative and qualitative assessment of a series of food additives, ingredients and contaminants [3].

The aim of this Editorial is to review the main electrochemical methods applied to the analysis of frequently encountered food additives and ingredients, like sugars (e.g. glucose), antioxidant vitamins, such as ascorbic acid, as well as of other food and beverage components (e.g. ethanol). Potentiometric techniques are based on the potential/pH change measurement, which takes place as a result of an ion concentration change. In potentiometric biosensors, the biochemical reaction involves an ion concentration variation, which is consecutively sensed by the transducer. The most commonly used transducer in the construction of potentiometric enzyme sensors was the glass-pH electrode, as many enzyme reactions involving key analytes (glucose, urea, triglycerides, penicillin, pesticides) take place with either proton generation or consumption [4,5].

Glucose oxidase was immobilized in several semipermeable membranes such as cellophane, nitrocellulose or nylon (Biodyne), which were consecutively fixed on the surface of a glass pH electrode. Glucose oxidase catalyses glucose oxidation with gluconic acid generation and the pH diminution is sensed by the glass membrane of the pH electrode. The developed potentiometric enzyme sensors allowed rapid glucose determination in fruit juices, with specificity imparted by the biocatalytical element and sensitivity of the electrochemical transducer [6]. The analytical signal obtained with the nylon-based enzyme electrodes was 87.9% greater than the one obtained with the cellophane – based enzyme electrode [7]. The analytical signal obtained with the nitrocellulose-based enzyme electrode was 40.4% greater than the one obtained with the cellophane-based enzyme electrode, for the same analyte concentration, and when the same transducer was employed [8].

Metal, metal oxide electrodes or ion selective field effect transistors were also used as transducers in the development of potentiometric enzyme sensors [9]. Voltammetry is based on imposing a controled potential variation (in a triangular wave form, or as pulses superimposed on a continuously increasing potential) with the subsequent measurement of the current generated from oxidation or reduction reactions at the electrode surface. Voltammetric techniques have been considered as important methods for the identification and quantitative determination of many biological electroactive molecules, such as low molecular antioxidants (e.g. ascorbic acid) and for total antioxdant capacity of plasma or plant extracts assessment [10,11].

The performances of bare Pt and unmodified carbon paste electrodes in ascorbic acid determination were investigated in differential pulse voltammetry and cyclic voltammetry. The Limit of Detection (LOD) and the Limit of Quantification (LOQ) obtained by differential pulse voltammetry were 0.087 mM and 0.29 mM respectively, when a Pt electrode was used [12]. Lower LOD and LOQ values were obtained when a carbon paste electrode was employed as working electrode: the limit of detection and the limit of quantification (LOQ) obtained by differential pulse voltammetry were 0.02 mM and 0.068 mM, respectively. Nevertheless, the sensitivities given by the slopes of the calibration graph were lower than in cyclic voltammetry experiments: for a Pt working electrode, 65.42 μA/mmol was the sensitivity obtained in CV studies, whereas 21.839 μA/mmole was the sensitivity obtained in DPV experiments [12]. The ascorbic acid content ranged between 6.83 mg/100 mL sample for soft drinks (Fanta Madness) and 54.74 mg/100 mL sample for citrus (lemon) juices obtained by fruit squeezing [12].

The amperometric method implies the application of a constant potential to a working electrode, followed by the measurement of the current intensity. The emerging current is always the result of an electron transfer process, either the oxidation or reduction of an electroactive analyte [4,5,13]. Amperometric biosensors rely on the reduction or oxidation of an electroactive species, which is either product, or co-substrate of an enzyme reaction. Therefore, the role of the biocatalyst is to generate/consume an electroactive species, which can be stoichiometrically correlated to the analyte concentration. The biorecognition elements are represented by enzymes, nucleic acids, lectins or whole cells [4,5,9,13].

One of the simplest and easy to use amperometric transducer is the Clark type oxygen electrode. Ascorbate oxidase was immobilized on a nylon membrane subsequently fixed on an oxygen electrode [14]. The greater the analyte concentration, the greater the oxygen consumption in the ascorbate oxidase catalysed reaction. Hence, the available molecular oxygen amount that undergoes reduction at the Pt cathode of the oxygen transducer, decreases, which determines the variation of the registered current intensity. The optimum biosensor response was obtained for an ascorbate oxidase amount of 100 U. The linear range of analytical response was comprised between 0.10 and 0.55 mM, and

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the amperometric ascorbate oxidase sensor was successfully applied to fruit juices analysis [14].

Another amperometric method was developed, for ethanol determination in some alcoholic beverages, by using an alcohol dehydrogenase/peroxidase-based amperometric enzyme electrode [15]. The enzymes alcohol dehydrogenase and horseradish peroxidase, as well as the coenzyme nicotinamide adenine dinucleotide (NAD+) were immobilized on a nylon (Biodyne A) membrane. Manganese cation was the peroxidase cofactor. The enzyme-membrane was consecutively attached to the polyethylene membrane of a Clark oxygen electrode. Ethanol is oxidized by NAD+ in the presence of alcohol dehydrogenase, generating the reduced coenzyme; in a second enzyme reaction, NADH is aerobically oxidized by horseradish peroxidase. The molecular oxygen consumption, which is directly proportional to ethanol concentration present in the sample, is amperometrically monitored with the transducer. The obtained calibration graph was linear within the range 10-80 mM. Ethanol was successfully determined in various alcoholic beverage samples with the bienzyme sensor [15].

Recently, amperometric enzyme sensors based on carbon nanotube composites were constructed. It was demonstrated that ferritin protein bound to single wall carbon nanotubes enhances the oxidation reaction of ascorbic acid over 11-fold in the amperometric determination of this antioxidant vitamin [16].

A biamperometric method was developed, based on the measurement of the current flowing between two identical Pt working electrodes, at a small potential difference, in a solution containing a reversible redox couple [17]. The redox pair chosen in this study was DPPH•/DPPH. Antioxidants react with DPPH• (radical form) generating DPPH (reduced form), the intensity of the resulted current being proportional to the concentration of DPPH•, remaining after its reaction with the analyte (antioxidant). Thus, each antioxidant addition diminishes the concentration of the oxidized radical form and increases the concentration of the reduced one, generating a current that can be correlated to the analyte concentration. Working conditions were chosen for the DPPH• concentration to be smaller than DPPH concentration [17,18]. Hence, the biamperometric detector response is linear with respect to that constituent of the redox couple which is present in smaller concentration. In this case the current is limited by the lower concentration of DPPH• radical found in the indicating mixture [17,18]. The calibration graph obtained for Trolox presents linearity between 5 and 30 μM. The R.S.D. value for the biamperometric method was 1.29% [17]. The developed method was applied to total antioxidant activity determination in natural juices and soft drinks and the results were consistent with the ones obtained by the spectrophotometric technique with DPPH•. The greatest values of the total antioxidant capacity determined biamperometrically, resulted for fresh fruit juices obtained by fruit squeezing, namely 9.07 mmoles Trolox per liter of orange juice and 6.25 mmoles Trolox per liter of lemon juice [17].

The developed electrochemical techniques allowed the determination of several additives and ingredients, as well as other components, with good performances, in food and beverage samples.

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