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Peroxidase-based biosensor as a tool for a fast evaluation of antioxidant capacity of tea

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Abstract

Phenol compounds are significant constituents in vegetables and can be correlated with antioxidant capacity of plants. Thus, the relationship between total antioxidant activity (TAA) and total phenol content was evaluated by using a horseradish peroxidase-based biosensor. Antioxidant activities of tea were investigated by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging method. The anti-radical activity determined was based on IC_{50} values (IC_{50} represents the antioxidant concentration needed to reduce 50% of the initial amount of DPPH$^{\cdot}$). Among the tested samples, black tea showed the best antioxidant effect. TAAs of the investigated tea samples were well correlated with phenol content, the correlation coefficients R being >0.9. According to these observations a simple reading of the biosensor is possible to find the TAA of tea samples, using this correlation.

Keywords: Antioxidant activity; Polyphenols; Biosensor; DPPH

1. Introduction

Tea is one of the most widely consumed beverages in the world. The growing popularity of this drink may be based on evidence of a relationship between consumption and prevention of certain human diseases (Ho, Ferraro, Chen, Rosen, & Huang, 1994; Stavric, 1994; Yen & Chen, 1995). It is well known that phenolic compounds are the main antioxidants in tea. Polyphenols are a class of phytochemical found in high concentration and they have been associated with the slight adstringent and bitter taste of this drink (Ferrara, Montensano, & Senatore, 2001). Despite many other components being present, such as alkaloids, amino acids, minerals and vitamins, polyphenols are perhaps the most relevant constituents of all, in terms of both occurrence and concentration (Finger, Kurth, & Engelhardt, 1992).

Several laboratory studies have provided evidence that phenolic compounds in tea leaves protect against cardiovascular disease and some forms of cancer in animals (Yang, 1997). Other studies have shown that these compounds are antioxidants in vitro and their activity as radical-scavengers has been of primary interest, since oxygen free radical species are well known to be involved in conditions such as autoimmune and neurodegenerative diseases, artherosclerosis, arthritis, chronic inflammation and diabetes (Aruoma, 1994; Halliwell, 1994; Tapiero, Tew, Nguyen Ba, & Mathé, 2002).

The chemical structure of these compounds is suitable for free radical-scavenging activities. They are excellent hydrogen- or electron-donors and the radicals formed are relatively stable due to delocalization through resonance and to lack of suitable sites for attack by molecular oxygen (Silva et al., 2000).

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Therefore, it is of great interest to evaluate the antioxidant potential of teas in relation to their phenolic constituents. Methods have been proposed for the detection of antioxidants in vivo and in vitro. Methods for characterization of antioxidants in vitro, such as photometric (Cao, Allesio, & Cutler, 1993), fluorometric (Naguib, 2000), chromatographic (Winstin, Regoli, Dugas, Fong, & Blanchard, 1998) and electrochemical (Chevion, Roberts, & Chevion, 2000) techniques, have been developed. The photometric methods are based on the detection of light absorption by radical-scavengers, such as cytochrome *c* or synthetic radicals. One of the most common methods, based on a free radical, is that using the stable radical DPPH (1,1-diphenyl-2-picrylhydrazyl) (Blois, 1958; Sánchez-Moreno, Larrauri, & Saura-Calixto, 1998). The antioxidant activity is compared with that of trolox as the standard. This method is widely used to evaluate antioxidant capacity in a relatively short time (Blois, 1958).

Several methods for detection of polyphenols in plants have been described in the literature, e.g., high-performance liquid chromatography (HPLC) combined with UV–Vis detection, chemiluminescence detection, fluorescence detection (Dalluge & Nelson, 2000) and electrochemical detection (Escarpa & Gonzalez, 2001). In spite of their existence, the Folin–Ciocalteu method is the most used (Singleton & Rossi, 1964/1965) and, although the method is well established for this purpose, simpler and faster methods are required for quantification purposes.

Modified electrodes that combine redox enzyme reactions with electrochemical detection have been proposed as alternative devices for the detection of polyphenols in plant extracts (Ghindilis, Gavrilova, & Yaropolov, 1992; Horie, Murai, Goto, Kawanaka, & Shimohara, 1992; Imabayashi, Kong, & Watanabe, 2001). In many cases, it is more important to measure the total content of polyphenol compounds than to determine each of them individually.

The amperometric biosensors measure the current produced for the chemical reaction of an electroactive species at an applied potential, which is related to the concentration of the species in solution. The main advantage in the use of redox enzyme is the low applied potential in relation to the reference electrode, which measures the generated catalytic current for the reductions or oxidations of species on the electrode surface, that in a general way occur between −0.2 and 0 vs. SCE. In this potential range, the interference is minimized. Biosensors for polyphenol detection have been developed on the basis of enzymes such as tyrosinase, laccase and peroxidase using different electrode materials, flow systems and sample pretreatment techniques with shorter times for analysis and simple instrumental procedure (Mello & Kubota, 2002).

The aim of the present work was the evaluation of the correlation between the content of total polyphenols present in tea infusions, determined initially by an electrochemical peroxidase-based biosensor, and the antioxidant capacity of these extracts.

2. Materials and methods

2.1. Samples

Samples of tea were obtained from local supermarkets. Amounts of the tea samples were dissolved in hot water in triplicate. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), was purchased from Sigma (St. Louis, USA); 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox) was supplied by Aldrich (Milwaukee, USA). All aqueous solutions were prepared using water purified with a Mili-*Q* system (Millipore, System).

The absorbance in all assays was measured in a quartz cuvette (10 mm path length), using a spectrophotometer UV/VIS from Pharmacia Biotech® Ultraspec 2000 connected to a PC (software Wavescan®).

2.2. Determination of polyphenol content using the biosensor method

The biosensor was based on a carbon paste electrode and was prepared by immobilization of 0.1 mg of DNA additive and 200 μl of a solution of 1 mg ml^{-1} of horseradish peroxidase on 25 mg silica–titanium by adsorption and cross-linking using 5 μl of glutaraldehyde 5% (w/v). The preparation of silica–titanium involved modifying the silica gel surface with titanium oxide according to the procedure described in previous paper (Kubota, Gushikem, Castro, & Moreira, 1991; Rosatto, Kubota, & Neto, 1999). The mixture was dried at room temperature and, after mixed with 25 mg of graphite powder, dropping in 35 μl of mineral oil, to get a homogeneous paste. This paste was put into a cavity, 1 mm deep, consisting of a platinum disk sealed into the extremity of a glass tube (4 mm i.d.) and pressed to smooth the surface. The DNA additive was used to improve the stability and to maintain the sensitivity of the biosensor. The optimization of the conditions for the biosensor was studied according to Mello, Sotomayor, and Kubota (2003).

The biosensor was used to measure the total polyphenol content of tea extracts without pretreatment. Chlorogenic acid was used as the reference compound.

2.3. Evaluation of free radical-scavenging capacity

In order to evaluate the efficiency of the vegetable extracts as radical-scavengers, they were allowed to react with commercially available free radical DPPH

(Blois, 1958). The colorimetric test for free radicals relies on the reaction of a specific antioxidant (AH) with DPPH ($DPPH^{\cdot} + AH \rightarrow DPPH\text{-}H + A^{\cdot}$) and was adapted from Ohnishi et al. (1994). In the radical form, $DPPH^{\cdot}$ presents a maximum absorption at 517 nm but, upon reduction by an antioxidant, the absorption disappears and the pale-yellow non-radical form is produced. Aliquots of the tea samples were added to 0.1 mM DPPH ethanol purple blue solution. Reduction of $DPPH^{\cdot}$ was followed by monitoring the decrease of the absorbance after 10 min at 517 nm. The radical-scavenging effect was expressed as % of the absorbance of the DPPH control solution without antioxidant and was compared on the basis of IC_{50}. All assays were carried out in triplicate and trolox was used as the reference compound.

3. Results and discussion

3.1. Free radical-scavenging capacity

One way to evaluate the effect of antioxidants is through their antiradical activity. The decrease of $DPPH^{\cdot}$ concentration is an index to estimate radical-scavenger capacity (RSC) of tea extracts. The remaining $DPPH^{\cdot}$ concentration in the reaction medium was calculated from the following calibration curve determined experimentally.

$$A_{517\ nm} = -0.01(\pm 0.01) + 0.0561(\pm 0.0002)\ [\mathrm{DPPH}].$$

The concentration of phenol required to decrease $DPPH^{\cdot}$ to 50% of the initial radical concentration (IC_{50}) in 10 min was also calculated. This parameter is widely used to measure antioxidant power. A low IC_{50} indicates that strongly antioxidant compounds are present in vegetables (Schinella, Troiani, D'avilla, Buschiazzo, & Tournier, 2000). Black tea is a better antioxidant ($IC_{50} = 0.44\ \mu mol\ l^{-1} \pm 0.09$) than mate tea ($IC_{50} = 12.0\ \mu mol\ l^{-1} \pm 0.3$). This difference can be attributed to the difference in composition of the two plants. The types and amounts of phenolic compounds present in tea will differ, depending on the variety of leaf, growing environment, processing, manufacturing, particle size of ground tea leaves and infusion preparation (Astill, Birch, Dacombe, Humphrey, & Martin, 2001). Black teas are *Camellia sinensis* fermented extracts and mate teas are *Ilex paraguariensis* unfermented extracts. Unfermented tea leaves contain more of the simple phenols and flavonoids, while the oxidation that the green tea leaves undergo to make black tea converts these simple compounds to the more complex components called theaflavins and thearubigins (Astill et al., 2001).

The antioxidant capactity of tea has been compared to that of fruits or vegetables in some studies. Polyphenols in tea, mainly catechins, have shown greater antioxidant protection than vitamin C or E (Cao, Sofic, & Prior, 1996; Du Toit, Volsteedt, & Apostolides, 2001).

3.2. Correlation between polyphenol content and antioxidant capacity

In order to evaluate whether there is a correlation between antioxidant capacity and polyphenol content determined by HRP-based biosensor, five samples of each tea were analyzed. The content of total phenols obtained for mate tea ranged from 2.3 to 11.2 mmol l^{-1} and, for black tea, from 0.018 to 0.15 mmol l^{-1} (values were calculated as means of triplicates). The used parameter was total antioxidant activity (TAA) (Simonetti, Pietta, & Testolin, 1997), calculated by

$$\mathrm{TAA} = \frac{\mathrm{Abs_{standard}}}{\mathrm{Abs_{sample}} - \mathrm{Abs_{standard}}},$$

where $Abs_{standard}$ is the absorbance of the $DPPH^{\cdot}$ solution in the presence of trolox and Abs_{sample} is the absorbance of the $DPPH^{\cdot}$ solution in the presence of the tea sample.

A linear model expressed the TAA against polyphenol concentration determined by peroxidase-based biosensor in tea samples. The results are shown in Fig. 1 and Fig. 2 for the mate and black tea, respectively. This means that the antioxidant activity may be obtained directly from following relationships:

$$\mathrm{TAA\ of\ mate\ tea} = 0.8(\pm 0.1)\ [\mathrm{total\ phenol}] + 2.8(\pm 0.6)\quad (R = 0.986),$$

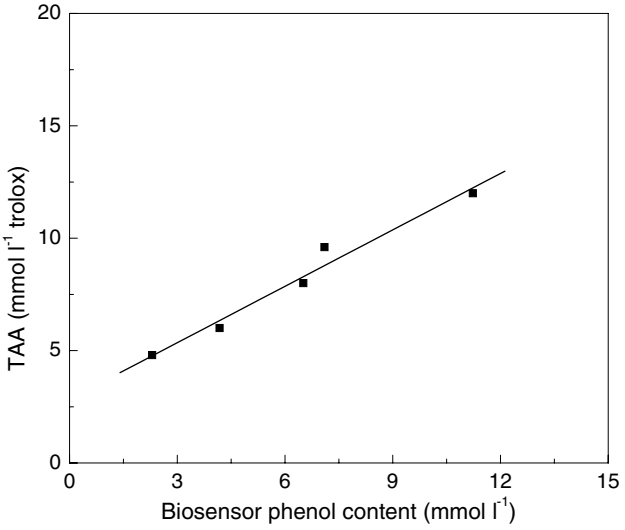

Fig. 1. Correlation between total phenol content present in mate tea samples and total antioxidant activity (TAA).

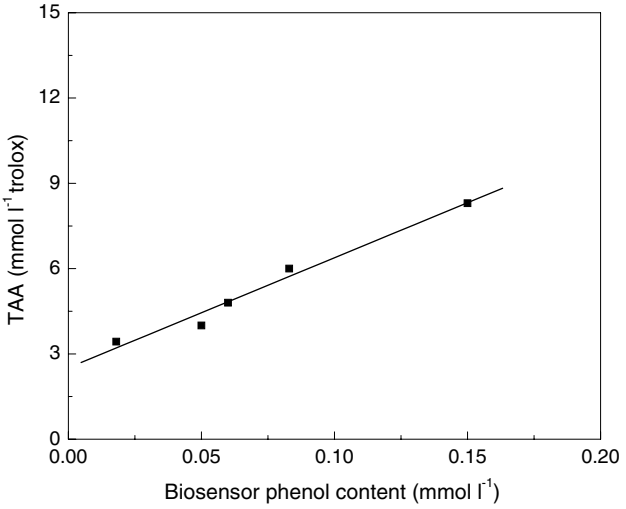

Fig. 2. Correlation between total phenol content present in black tea samples and total antioxidant activity (TAA).

$$\text{TAA of black tea} = 39(\pm 3)\ [\text{total phenol}] + 2.5(\pm 0.3) \quad (R = 0.989).$$

The TAAs of the investigated extracts correlated well with total phenol contents. The correlation coefficients were $R > 0.9$ in both cases and, as can be seen, the correlation depends on the kind of tea. It is important to emphasize that each kind of tea has a correlation between TAA and total phenol concentration but, for the same kind of tea, the correlation is very good. This implies that a sample reading with the biosensor allows the TAA of the tea to be estimated. However, for a different kind of tea, a new correlation needs to be established.

Compounds, such as ascorbate or carbohydrates, did not interfere with the biosensor response (Mello et al., 2003), leaving the phenol content as the main source of the antioxidants in the tea. It is also important to emphasize that no study has been made on application of oxi-reductase-based biosensors for the evaluation of antioxidant capacity of natural extracts, even though several biosensors for phenol compounds have recently-been described in the literature.

4. Conclusion

The determination of the total phenol content by the biosensor was representative in terms of antioxidants compounds. In the assay, of the antioxidant potential of tea aqueous infusions and its correlation with the phenol content, determined by the biosensor, was demonstrated. A good correlation was obtained between the antioxidant properties of the extracts and total phenol content. According to the results it can be considered that simple reading of the biosensor is sufficient to find the TAA of teas. The use of a biosensor in this case provides some important advantages, such as easy manipulation, selective response and fast evaluation of antioxidant capacity of plant extracts.

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