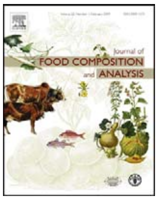
Journal of Food Composition and Analysis 22 (2009) 709–713

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

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



Original Article

Tyrosinase immobilized reactor as a fast tool for polyphenolic index of tea

Anna Maria Girelli *, Enrico Mattei, Domenica Papaleo

Dipartimento di Chimica – Sapienza Università di Roma, P.le A. Moro 5 - 00185 Roma, Italy

A R T I C L E I N F O

Article history:
Received 26 May 2008
Received in revised form 16 March 2009
Accepted 5 April 2009

Keywords:
Immobilized tyrosinase
Enzymatic bioreactor assay
Tea
Polyphenolic index
Flavonoids
Antioxidant capacity of green tea
Food analysis
Food composition

A B S T R A C T

A new approach for the determination of polyphenolic index of black and green teas has been developed employing a bioreactor with mushroom tyrosinase immobilized on aminopropyl-controlled pore glass (AMP-CPG). Initially a linear correlation ($r^2 = 0.997$) of catechin content between the Folin-Ciocalteu (FC) and the enzymatic assays is obtained. Our method appears to be more specific and rapid. Successively the inhibition of the enzymatic oxidation of catechin by some tea components like gallic acid (GA), epigallocatechin (EGC), epigallocatechingallate (EGCG) and epicatechin (EC) is investigated. Finally when tea samples are analyzed by using the new method it appears that green tea is the strongest inhibitor followed by black tea and flavored tea. The level of polyphenols, which is correlated to the extent of inhibition, is reported as epigallocatechin equivalents and the results are compared with those obtained using the FC assay and the vanillin index. The differences in phenol content found by applying the three methods are discussed in terms of the different specificities of the analytical basis.

1. Introduction

Recently the recognition of the antioxidant properties of polyphenols, greatly abundant in our diet, has reached a sparked interest since their probable role in the prevention of various diseases associated with oxidative stress, such as cancer, cardiovascular and neurodegenerative diseases. In particular it has been demonstrated that considerable antioxidant properties in vivo and in vitro have been found in wine, coffee and tea, the most important beverages in the world (Gardner et al., 2007; Manach et al., 2004; Rechner et al., 2002; Richelle et al., 2001). The total phenolic content of these commodities is therefore an important parameter for the evaluation of their antioxidant properties and quality.

The major phenolics present in the teas are comprised of flavonoids, in green tea ranging from flavan-3-ols and flavonols to their complex oxidation products, in black tea mainly theaflavins and thearubigins. The main flavan-3-ols are epicatechin and its gallate derivatives, while the main flavonols in tea are conjugates of quercetin and kaempferol with lower levels of myricetin and the conjugating moiety varying from mono- to di- and tri-glycosides. However, the composition of tea varies, besides its form (green, oolong or black), with crop season, the age of leaf, climate and horticultural practices.

Conventional methods for the determination of total polyphenols content are based on either their oxidation or reduction properties. More recently analytical techniques are used to isolate, identify and determine individual polyphenolic compounds by HPLC (Friedman et al., 2006; Neilson et al., 2006; Saito et al., 2006; Yang et al., 2007), but spectrophotometric methods are still the most widely used, due to their simplicity for the determination of total phenolic compounds. The leading spectrophotometric method is Folin-Ciocalteu (FC) assay which is based on a non specific phenol oxidation reaction by the two strong inorganic oxidant (phosphotungstic and phosphomolibdic acids) (Lakenbrink et al., 2000; Stevanato et al., 2004) Another important spectrophotometric method is based by the colorimetric reaction with vanillin on the estimate of free C_6 and C_8 atoms of flavanols.

The goal of the present study is to determine the whole profile of phenolic compounds in some traditional commercial teas by the evaluation of inhibition of tea components on the catechin oxidation to catechinquinone catalyzed by a tyrosinase immobilized bioreactor. This work may also be a contribution to the few existing data on the polyphenol content of tea infusions in ordinary hot water (Arts et al., 2000; Chandra and De Mejia, 2004; Del Rio et al., 2004; Liang et al., 2003; Liang and Xu, 2003), which can be suitable for further use in epidemiological studies.

2. Materials and methods

2.1. Apparatus and chemicals

Catechin, epigallocatechin (EGC), epigallocatechingallate (EGCG), gallic acid, vanillin, Folin-Ciocalteu phenol reagent and ethanol were supplied by Sigma (Milan, Italy). For buffers

---

* Corresponding author. Fax: +39 06490631.
E-mail address: annamaria.girelli@uniroma1.it (A.M. Girelli).

0889-1575/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.jfca.2009.04.006

solutions, phosphoric acid (Carlo Erba, Milan, Italy), potassium dihydrogenphosphate and dipotassium hydrogenphosphate eptahydrate (Sigma, Milan, Italy) of analytical grade were used. The water was purified by Milli-Q Ultra-pure system (Millipore, Bedford, MA, USA).

Chromatographic analysis was performed with a Kontron system (Milan, Italy), consisting of a model 422 pump and a UV–visible 432 detector, complete with a Rheodyne 7125 injector with a 20 μL sample loop (Rheodyne, Berkeley, CA, USA). The absorbance values of the effluent monitored at 440 nm (corresponding to catechinquinone absorption), were registered by an integrator system constituting a PC equipped with an Intel processor, Pentium III 800 MHz CPU, and Agilent ChemStation software for LC version A.08.03(847) running under MS Windows NT 4.00.31 OS.

Spectra of peaks were obtained with a Thermo Quest Spectra Series HPLC (Thermoquest Inc., San Josè, CA, USA) equipped with a Spectra Series UV 6000 photodiode array detector (Thermoquest Inc.) using the Chrom Quest software.

Spectrophotometric determinations were performed on a Kontron Uvikon 920 UV–vis spectrophotometer with cuvettes of 1 cm length.

2.2. Sample collection and preparation

2.2.1. Tea

Three green teas from *Camelia sinensis* (Chinese, bioagricoltural, green tea mixture), three fruit-flavored green teas (apple flavored, rooibos–elder flavored and an infusion of mate containing green teas), six classic blends of black tea from different brands (English Breakfast, Ceylon, Prince of Wales, Earl Grey, blackcurrant flavored, red fruit flavored), all in teabag form, were purchased from a local supermarket. To prepare the tea, 0.5 g of tea was infused for 5 min with 100 mL of boiled water. The infusion was successively filtered on Acrodisk 0.45 μm cartridge (Millipore, Bedford, Mass., USA) and successively several aliquots of the filtrate were frozen for further use.

2.3. Immobilized tyrosinase bioreactor

Tyrosinase was immobilized on aminopropyl-controlled pore glass with the in situ technique as previously described (Girelli et al., 2007a). Briefly a stainless steel column (50 mm × 2.1 mm ID) was dry-packed with 70 mg of AMP-CPG (505 Å mean pore diameter) and tyrosinase was immobilized by covalent bond through glutaraldehyde as cross-linking agent. Reduction of the Shiff's base formed between enzyme amino groups and glutaraldehyde was made using a solution of cyanoborohydride according to our previous study (Girelli et al., 2007a).

2.4. Assay of teas by tyrosinase immobilized bioreactor

The polyhenolic index of the tea samples was determined by their inhibition power on the catechin oxidation to catechinquinone, and the results were expressed as epigallocatechin equivalents. The extent of tea inhibition was determined by subtracting from the peak area of catechinquinone, enzymatically formed by the injection of 20 μL of a catechin ethanolic solution (84 mg/L), the peak area of the same enzymatically formed product obtained from a mixture of catechin and tea infusion. This latter mixture was prepared by adding 50 μL of tea infusion diluted fivefold at pH 3 (to prevent autoxidation) to 100 μL of catechin (210 mg/L) (Mochizuki et al., 2002; Roginsky and Alegria, 2005) and a volume of ethanol to reach total volume of 250 μL (final catechin concentration was 84 mg/L).

Data reported were obtained by triplicate analysis. For the calibration curve, the mixture was prepared employing epigallocatechin ranging between 32 and 500 mg/L instead of tea infusion and plotting on the *y*-axis the response as

$$\text{Response} = \text{Area}_0 - \text{Area}_{\text{st}}$$

where Area_0 was relative to the catechinquinone formed by the oxidation of the catechin (84 mg/L) alone and Area_{st} was relative to the catechinquinone formed by the oxidation of the catechin (84 mg/L) in presence of EGC. The calibration curve equation was

$$y = 140.3x + 6833 \quad (r^2 = 0.994).$$

2.5. Vanillin index determination

The concentration of polyphenols reactive to vanillin in a highly acidic environmental was determined according to the method described by Vacca et al. (2003): 5 mL of tea infusion and 3 mL of vanillin 4% (w/v) in methanol were placed in an amber flask maintained in a cold water bath for 3 min. Then 1.5 mL of H_2SO_4 were added and the mixture was incubated for 15 min. Finally absorbance at 500 nm was spectrophotometrically read against a blank prepared in the same way of the sample but containing water instead of tea infusion. Each sample analysis was repeated twice.

2.6. Folin-Ciocalteu assay

The level of total phenols was determined using Folin-Ciocalteu reagent (Folin-Ciocalteu, 1992; Singleton et al., 1999) at 784 nm, and the results were expressed as catechin equivalents. Standard solutions of catechin in the concentration range between 0.05 and 2 g/L were used to prepare the calibration curve. Each sample analysis was repeated twice.

3. Results

Preliminary measurements were performed in order to select the reference substrate for tyrosinase bioreactor. The chromatograms obtained by the injection of three flavanols commonly present in teas: catechin, epigallocatechin (EGC) and epigallocatechingallate (EGCG) on the immobilized enzyme reactor (IMER), show that the quinones of catechin and of EGCG were fully separated by their substrate whereas that of EGC was eluted with the void volume together with the not reacted substrate (Fig. 1). The individuation of the species, confirmed by the spectra registered with photodiode array detector, showed that the retention times for catechinquinone and epigallocatechingallate quinone were 1.8 min and ~10 min, respectively. In addition taking into account also that catechin is the flavanol present in the teas at the lowest percentage (2 and 5% for green and black teas, respectively) (Khokhar and Magnusdottir, 2002), this compound was chosen as reference substrate. In this way, if the sample can be diluted, the eventual contribution of tea catechin component to the catechinquinone area may be considered negligible.

Successively the response of the IMER to the oxidation of catechin was performed by injecting a different amount of catechin in the chromatographic system and determining the catechinquinone peak areas at 440 nm. The area values were compared, with the absorbance values obtained with the same solutions by Folin-Ciocalteu assay (Fig. 2). The good linearity (correlation coefficient of 0.997) indicated that the bioreactor was sensitive to the catechin amount.

In order to ascertain the inhibition of the enzymatic oxidation of catechin by flavanols present in the teas, stock solutions of gallic acid, EGC and EGCG at the amount generally present in green teas,