

AMPEROMETRIC BIOSENSOR FOR DETERMINATION OF TOTAL TEA-CATECHINS CONTENT IN COMMERCIAL KOREAN AND JAPANESE TEAS

Young Tae Kong

Department of Science Education, Chinju National University of Education, Jinju 678-800, Korea

Abstract

The reductive detection of H_2O_2 with a membrane-covered horseradish peroxidase (HRP)-entrapped, ferrocene (Fc)-embedded carbon paste (CP) electrode is applied to determine the total amount of polyphenols in commercial teas. The steady-state reduction current of H_2O_2 decreases with the addition of polyphenol into a buffer solution containing H_2O_2 and HRP. The magnitude of the decreased current is proportional to the amount of the added polyphenol and the sensitivity of the detection limit depends on the electron-donating properties of polyphenol. For (+)-catechin, the HRP-Fc-CP electrode allows the detection limit to come down to $0.3 \mu M$ with a linear relation going up to $150 \mu M$ and without changing of the response over one month. The total amounts of polyphenols in several teas detected by the present method are well correlated to those determined by the photometric determination.

Key words: Amperometric Biosensor, HRP, Carbon paste electrode, Commercial teas, Catechins

1. INTRODUCTION

Many analytic methods using a high-performance liquid chromatography (HPLC) combined with several kinds of detection methods (Bronner 1998, Price 1993) have been used to measure polyphenol in teas. While HPLC methods separately detect polyphenolic compounds, simpler techniques which allow us to estimate total polyphenol contents are desired for certain purposes, e.g., assessment of the antioxidant activity or real-time monitoring of the polyphenol level in the tea-making process (Singleton 1999).

Recently, biosensors modified with the enzyme (polyphenol oxidase or horseradish peroxidase) have been proposed for the rapid detection of the total amount of phenolic components (Lindgren 1997, Ruzgas 1996, Rosatto 1995). These methods have some advantages, i.e., without pretreatment, there is a shorter time for taking a measurement, more specificity, and less instrumental demand in terms of its size and portability, in particular. However, Ruzgas and Wang et al. (Ruzgas 1995, Wang 1998) pointed out that the inefficient reduction of phenoxy radicals readily polymerize in the aqueous solution and the polymerized products hamper the direct electrochemical detection, leading to the deterioration of the biosensor properties.

In the present paper, we propose a peroxidase-based amperometric biosensor which is free from the above disadvantages for determination of total polyphenolic content in commercial teas. We adopt the indirect-detection method using a membrane-covered peroxidase and ferrocene-embedded carbon-paste (HRP-Fc-CP) electrode. We make use of HRP-Fc-CP electrode to detect the decrease of H_2O_2 concentration in a sample solution.

Another purpose of this work is to monitor the degradation over time of the polyphenol content in the sample. It is known that the phenolic content included in green tea is influenced by the cultivars, the type of tea extraction procedure, and the storage conditions. Although the classic spectrophotometric analysis of the polyphenol content with the Folin-Ciocalteu reagent is widely used, it is not specific and detects only the final products of these degradation reactions (Cpannesi 2000, Campanella 1999).

2. EXPERIMENTAL

2.1 Chemicals

Peroxidase from horseradish (HRP: EC 1.11.1.7, 180 U mg^{-1}) was purchased from Toyobo (Japan) and used as received. (-)-Epigallocatechin gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), (+)-catechin (CA), and (-)-epicatechin (EC) were purchased from Sigma and used without further purification. All

other chemicals were of analytic grade. A 0.1 M sodium phosphate buffer (pH 6.9) containing polyphenol and H_2O_2 was prepared daily using water purified by the Milli-Q system. Canned teas (20 samples) used for real sample were purchased in Korean and Japanese markets. Powder type green teas (10 samples) were purchased from Japanese markets and then extracted by pouring the 100 ml boiling water over 2 g of tea leaves for 3 min. The extract was filtered through a filter cartridge and diluted five-fold with water before electrochemical analysis.

2.2 Fabrication of HRP-Fc-CP Electrodes

Weighed amounts of HRP, Fc, mineral oil, and graphite powder in a weight ratio of 5:5:25:45 were thoroughly mixed and packed into the well of the CP electrode purchased from Bioanalytical Systems (BAS: 11-2210, inner diameter = 3.0 mm), resulting in the HRP-Fc-CP electrode. The HRP-Fc-CP electrode surface was first covered with a cellulose ester dialysis membrane (cut-off molecular weight 100) and the whole system was fixed on the Nylon net by means of a Teflon O-ring. The electrode thus prepared was stored at 4°C in 0.1 M phosphate buffer (pH 6.9) when it was not in use.

2.3 Electrochemical Measurements

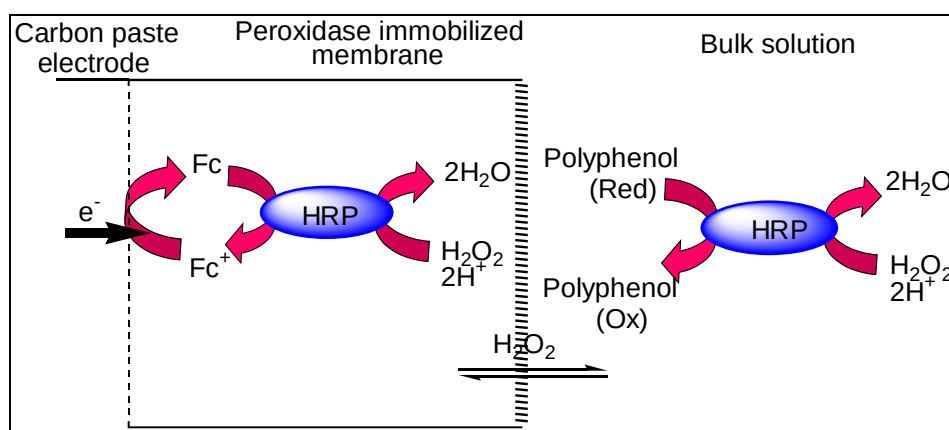
A conventional single compartment Pyrex cell that houses three electrodes was used for voltammetric measurements. The HRP-Fc-CP electrode was used as a working electrode. An Ag/AgCl (in saturated SCE) electrode was used as a reference electrode and a platinum wire was used as the auxiliary electrode, respectively. Voltammetric experiments were carried out with Bioanalytical Systems, Inc. Model CV-50 W, which was coupled with with a clone of computer. All amperometric measurements were performed with an applied potential + 0.1 V vs. SCE under moderate stirring at 35°C unless otherwise stated.

3. RESULTS AND DISCUSSION

3.1 Detection of polyphenol compounds using the membrane-covered HRP-Fc-CP electrode

The principle concept for the detection of polyphenol assay with the membrane-covered HRP-Fc-CP electrode is illustrated in scheme 1. This indirect-detection method uses the well-known fact that polyphenols can also be used as electron donating compounds in the reaction of HRP with H_2O_2 . This method detects the change in the H_2O_2 concentration, which correlates to the content of polyphenol.

A typical time-current signal response displayed by the membrane-covered HRP-Fc-CP electrode in a series of increments to EGCG concentrations is presented in Figure 1. The electrode was held at +0.1V under the nitrogen atmosphere. As it can be seen in Fig. 1-(a), the current reached a constant limit level in 1 min after the addition of 0.2 mM H_2O_2 . This current is due to the electrochemical reduction of Fc^+ ion generated by the HRP-catalyzed reduction of H_2O_2 into H_2O in the inner-layer of the membrane. Figure 1-(b) shows a change in the limiting current with the incremental addition of aliquots of 20 μM EGCG solution into a 0.1 M phosphate buffer containing 0.2 mM H_2O_2 . The response time, which is defined as the time taken to reach a 95% level of the steady-state current, was 4 min and 5 min when the concentration of EGCG was 150 μM and 20 μM , respectively.



Scheme 1. Reaction pathway for the detection of polyphenols using membrane-covered HRP-Fc-CP electrode.

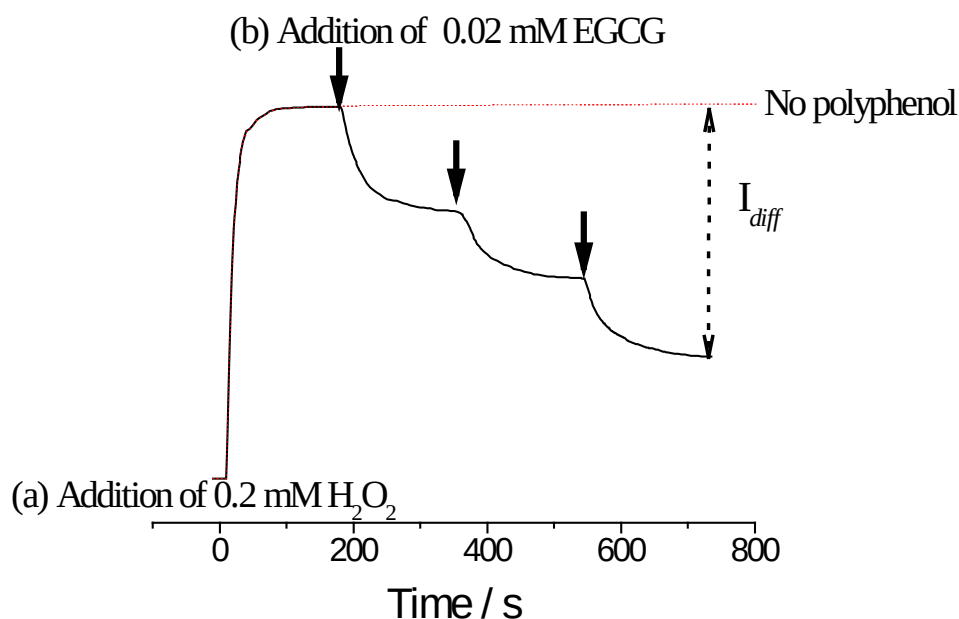


Fig. 1. Steady-state current response (a) with the addition of H_2O_2 and (b) with the incremental addition of EGCG solution with HRP at +0.1 V in 0.1 M phosphate buffer at pH 6.5 under stirring.

The difference between the limiting current with and without EGCG indicated by I_{diff} in Fig. 1 corresponds to the decreased H_2O_2 concentration. No alteration in the reduction current was observed in the absence of HRP or polyphenol in the bulk buffer, indicating that the decrease in the H_2O_2 concentration was caused by the HRP cycle reaction in which the polyphenol acted as an electron donor.

In order to examine the effect of I_{diff} on the applied voltage, we investigated the change of I_{diff} during the change of the electrode potential from 0.0 V to +0.25 V. In the range of this potential window, only the reduction of Fc^+ was occurring without the interfering of the reduction of a small size of phenol and impurities contained within the buffer solution. As a result, I_{diff} values were practically independent of the electrode potential with the potential window 0.0 to 0.25 V at pH 6.9

3.2 Calibration curve

Figure 2 shows the change of I_{diff} as a function of the concentration of several polyphenols. A strictly linear calibration was obtained from 2 to 130 μM at the pH 6.5. The least square analysis from the data of Fig. 6 provided the equation $I \text{ (nA)} = 3.27 C - 6.24$ and the correlation coefficient of 0.999. As shown in Fig. 2, it deviated downward from the linear relationship at the higher concentration due to the HRP reaction kinetics. The lowest detection limit was 0.1 μM at a signal-to-noise ratio of three.

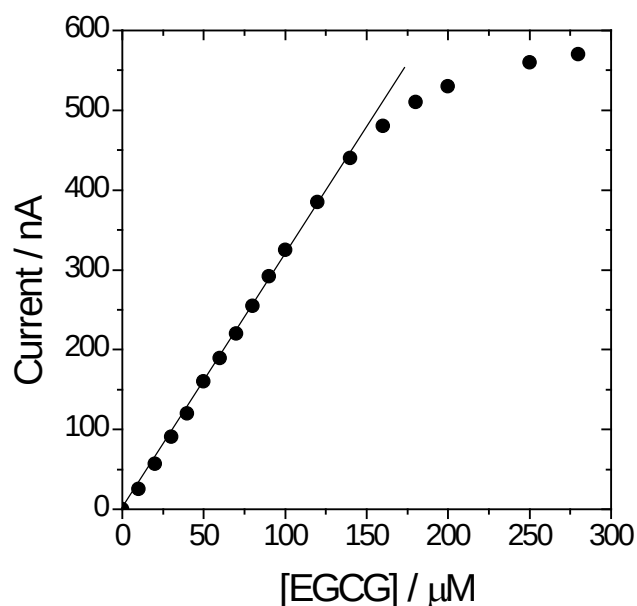


Fig. 2. Magnitude of change in the steady-state reduction current, I_{diff} , as a function of the concentration of EGCG.

We also examined the reactivity of several tea-catechins contained in green tea using the HRP-Fc-CP electrode. For all tea-catechins, c.f. EGC, ECG, EC, C, the linear relationship between I_{diff} and their concentration was obtained up to 150 μM . The sensitivity of the polyphenol detection, which corresponds to the slope of the I_{diff} vs. polyphenol concentration plot, was similar for (-)-epicatechin and caffeic acid. In previous work, the relative activity for different phenol and polyphenol compounds for the HRP cycle reaction was related to the difference in the electron donating ability. The similar oxidation potential measured on a glassy carbon electrode in a phosphate buffer at pH 6.5 revealed the same degree of sensitivity (Iwai 2000). Tab. 1 shows the relationships between the analyte concentration and their peak current.

Tab 1. Relationships between analyte concentrations and their peak current, $y = Ax + B$

Compound	A	B	r	SD
EGCG	7.45	-0.92	0.999	4.2
ECG	7.79	-1.82	0.999	2.8
EGC	11.29	-3.51	0.997	4.7
EC	12.01	-2.57	0.996	4.7
C	12.03	-2.52	0.996	4.5

3.3 The application of electrode for determining the polyphenol concentration in teas

Green tea also contains other tea-catechins besides the above mention compounds, whose sensitivity in the present method was examined. However, the containing ratio of the five kinds of polyphenol contained in green tea and their leaves is over 90 %. Although it is easily predicted that the sensitivity of the present method is not equal for all polyphenol, the total amount of polyphenol in the tea was evaluated by assuming that the I_{diff} vs. EGCG concentration relationship can be applied for all other polyphenols. We applied the HRP-Fc-CP electrode to measure the total polyphenol contents of several canned teas and several powdered tea made by Korea and

Japan (listed in fig. 3).

The total polyphenol content detected by the present method ranged from 1.4 to 3.4 mM for canned green tea and from 0.2 to 0.8 mM for canned blended teas. Specifically, the powder type of teas had more than the canned teas 5-10 times more polyphenol. These values are a little smaller than the reported values determined by other methods (Wang 2000). However, the detection limit of the present method is sufficiently large for determining the content of polyphenol in tea samples.

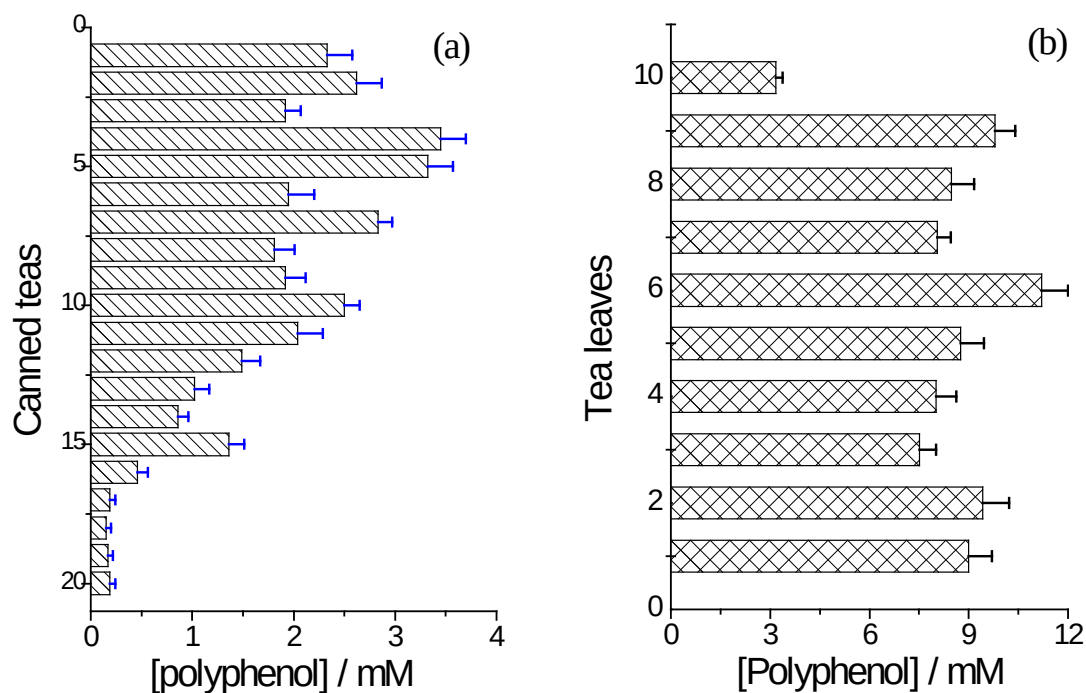


Fig. 3. Total polyphenol concentration in different tea samples: (a) canned teas and (b) powder typed teas.

In order to confirm the evaluated value of amount of the polyphenol determined by the electrochemical method, we compared it to that evaluated by the photometric method. Fig. 4 compares the present method with the Folin-Ciocalteu method for the determination of the total polyphenol content of several tea samples using EGCG as the standard. The difference between the contents evaluated by the two methods was fairly small, but the linear relationship between the values by the two methods was seen. This indicates that the present electrochemical method is useful for detecting the total contents of polyphenols in real samples.

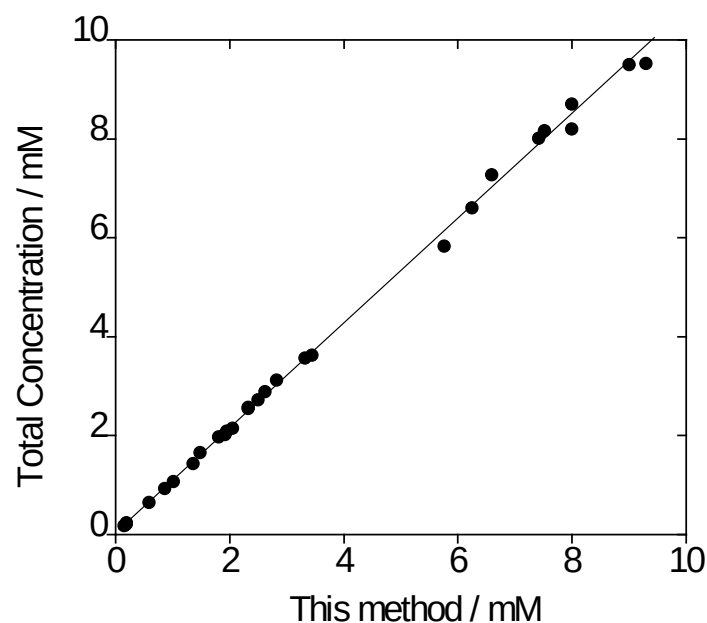


Fig. 4. Correlation between the present method and the Folin-Ciocalteu method for the determination of the total polyphenol content in tea samples.

4. CONCLUSION

The reductive detection of H_2O_2 with a membrane-covered horseradish peroxidase (HRP)-entrapped, ferrocene (Fc)-embedded carbon paste (CP) electrode has been applied to determine the total amount of polyphenols in commercial teas. The steady-state reduction current of H_2O_2 decreases with the addition of polyphenol into a buffer solution containing H_2O_2 and HRP. The magnitude of the decreased current is proportional to the amount of the added polyphenol and the sensitivity of the detection depends on the electron-donating properties of the polyphenol. For (+)-catechin, the HRP-Fc-CP electrode allows the detection down to $0.1 \mu\text{M}$ with a linear relation up to $130 \mu\text{M}$ and without a change in the response at least for one month. The total amounts of polyphenols in several teas detected by the present method are well correlated to those determined by the photometric determination.

5. ACKNOWLEDGMENTS

This work was supported by the National Research Foundation of Korea Grant funded by the Korean Government (NRF-2013R1A1A2004851).

REFERENCES

- Bronner, WE & Beecher, GR 1998, 'Method for determining the content of catechins in tea infusions by high-performance liquid chromatography', *Journal of Chromatography. A*, vol 805, pp. 137-142.
- Price, WE & Spitzer, JC 1993, 'Variations in the amounts of individual flavonols in a range of green tea'. *Food Chemistry*, vol. 47, pp. 271-276.
- Singleton, VL Orthofer, R Lamuela-Raventos, RM 1999, *Methods in Enzymology*, vol. 299, pp. 152-178.
- Lindgren, A Emneus, J Ruzgas, T Gordon, L Marko-Varga, G 1997, 'Amperometric detection of phenols using peroxidase-modified graphite electrodes', *Analytica Chimica Acta*, vol. 347, pp. 51-62.
- Ruzgas, T Csoregi, E Emneus, J Gorton, L Marko-Varga, G 1996, 'Peroxidase modified electrodes: Fundamentals and application', *Analytica Chimica. Acta*, vol. 330, pp 123-138.

Rosatto, SS Kubota, LT Neto, GO 1995, 'Biosensor for phenol based on the direct electron transfer blocking of peroxidase immobilizing on silica-titanium', *Analytica Chimica Acta*, vol. 390, pp. 65-72.

Ruzgas, T Gorton, L Marko-Varga, G 1995, 'The development of a peroxidase biosensor for monitoring phenol and related aromatic compounds', *Analytica Chimica Acta*, vol. 311, pp. 245-253.

Wang, J Jiang, M Lu, F 1998, 'Electrochemical quartz crystal microbalance investigation of surface fouling due to phenol oxidation', *Journal of Electroanalytical Chemistry*, vol. 444, pp. 127-132.

Capannesi, C Palchetti, I Mascini, M Parenti, A 2000, 'Electrochemical sensor and biosensor for polyphenol detection in olive oils', *Food Chemistry*, vol. 71, pp. 553-562.

Campanella, L Favero, G Psatorino, M Tomassetti, M 1999, 'Monitoring the rancidification process in olive oils using a biosensor operating in organic solvents', *Biosensor Bioelectronics*, vol. 14, pp. 179-186.

Iwai, K Sato, M Matue, H 2000, 'Relationship between antioxidative activity estimated by XYZ-dish method and catechin concentration in commercially available tea drinks', *Nippon Shokuhin Kagaku Kogaku Kaishi*, vol. 47, pp. 120-129.

Wang, H Helliwell, K You, X 2000, 'Isocratic elution system for the determination of catechins, caffeine and gallic acid in green tea using HPLC', *Food Chemistry*, vol. 68, pp. 115-121.