Evaluation of the Antioxidative Activity of Tea by an Oxygen Electrode Method

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The antioxidative activity was studied for 25 kinds of tea and catechins by a new evaluation method using an oxygen electrode. The concentration of catechins in 6 types of green tea was analyzed by HPLC. The result indicates that the antioxidative activity of green tea depends on some extent on the amount of catechins present.

Key words: antioxidative activity; oxygen electrode; catechin; tea; HPLC

Various kinds of tea have appeared on the market in recent years due to trends toward natural foods and greater health awareness, and many physiological functions of tea have been found. The antioxidative effect has been paid particular interest for the prevention of aging, disease, and oxidation of lipids in food. There are many methods for evaluating antioxidative activity, one being the thiobarbituric acid (TBA) method. However, these methods need a long experimental time and lack accuracy. We have established a new method for evaluating antioxidative activity with an oxygen electrode that only needs a short time. This method was applied to examine the antioxidative activity of four catechins ([(-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), and (-)-epigallocatechin gallate (EGCG)] (Fig. 1) and 25 kinds of tea.

The antioxidative effect of tea is generally believed to be due to its polyphenol content, and especially catechins in green tea. However, there have been few studies on the relationship between catechins and antioxidative activity for tea. We therefore investigated in this study the effect of catechins on the antioxidative activity.

It has been reported that catechins were isomerized to their corresponding epimers by heating in a green tea infusion. To prevent the isomerization of catechins in this study, extraction with 80% (v/v) aqueous methanol was performed at room temperature. Two hundred mg of a ground tea was weighed and put into a 10 ml volume flask. Two ml of 80% (v/v) aqueous methanol was added to each sample, which was ultrasonically extracted for one hour. The flask was then topped up with water and passed through a hydrophilic membrane filter of 0.45 μm.

The general procedure for evaluating the antioxidative activity by an oxygen electrode involved measuring the peroxidation of linoleic acid, which was induced by a hydrophobic radical initiator, by a biological dissolved-oxygen meter (Oxygraph 8, Sentoraru Kagaku Co., Tokyo, Japan). A 50 mM linoleic acid micellar solution (0.5 M SDS/0.05 M Tris-HCl buffer solution at pH 7.4; 1.2 ml) was placed in the reaction cell, which was then saturated with oxygen in air by using a magnetic stirrer for about 10 minutes. The reaction cell was maintained at 37°C by circulating water. Twenty μl of a 1 M 2, 2'-azobis(2, 4-dimethylvaleronitrile) (AMVN) methanol solution as the hydrophobic radical initiator was injected into the reaction cell, and the amount of oxygen consumed was then measured until no oxygen remained. Before the AMVN injection, a known amount of an antioxidant solution was added to the system. All experiments were conducted in a dark room.

Figure 2 shows a plot of the concentration of dissolved oxygen against the reaction time. The dissolved oxygen was decreased at a constant rate by AMVN without any added antioxidant (a). On the other hand, when 5 μl of a Kamairi-cha extract was injected (b), the oxidation rate was retarded. Data obtained by the oxygen electrode method generally have an induction period in the presence of the antioxidant; however, we noticed that measurements for tea and catechins had no induction period, and that the oxidation rate was linear. Therefore, the antioxidative activity can be evaluated as the ratio of the rate (R_\text{c}/R_p) in the absence (R_p) and presence (R_\text{c}) of an antioxidant. This implies that the lower R_\text{c}/R_p is, the higher the antioxidative activity is.
When 10 μl of a catechin (EC, EGC, ECG or EGCG; 1 mM) was added, the antioxidative activity was 0.857±0.031, 0.566±0.018, 0.568±0.023 and 0.459±0.023, respectively. The values for tert-butylhydroxyanisole (BHA; 1 mM) and 2,6-di-tert-butyl-4-methylphenol (BHT; 1 mM) were 0.442±0.012 and 0.445±0.002 in comparison. The order for the antioxidative activity of the catechins was EC<EGC<EGC<EGCG. EGCG showed particularly high antioxidative activity compared with BHA and BHT. This order agrees with that measured with the active oxygen method (AOM) by Matsuzaki et al. The result suggests that the antioxidative activity increases as the number of hydroxyl groups increases, and when a hydroxyl group at the 5’ position exists in the chemical structure. It has proposed that the antioxidative activity of catechins is due to the effect of radical scavenging. This effect prevents linoleic acid from peroxidation by the radical formation of phenolic hydroxyl groups, i.e., by the oxidation of catechins.

The antioxidative activity is summarized in Table I for 5 μl of various tea extracts. Most of the tea extracts examined showed antioxidative activity. The identification of the substance showing antioxidative activity in different tea is difficult, but is progressing. For example, the effective substance in Rooibos tea, which had the highest antioxidative activity of all the tea samples, is considered to be a flavonoid glycoside with a molecular weight of about 800 (M. Kuwahara and M. Nakano, unpublished result).

In order to determined the concentration of catechins in green tea, an HPLC analysis (Mitsui Norin Co., unpublished method) was performed with a Waters 600E instrument (Waters Co., Tokyo, Japan), using a Capcellpak C18 AG120 column (4.6×250 mm; Shiseido Co., Tokyo, Japan), and Waters 490E UV detector. The tea samples used were Ban-cha (Yame, Fukuoka, Japan), Gyokuro (Yame, Fukuoka, Japan), Kamairi-cha (Ureshino, Saga, Japan), Mat-cha (Uji, Kyoto, Japan), and Sen-cha (two types of high (H) and low (L) grade; Yame, Fukuoka, Japan). The eluting solvent was a 0.05% phosphoric acid aqueous solution, acetanilide and ethyl acetate in the proportions of 90:12:0.6 v/v. The flow rate, injection volume, temperature of the column and absorption wavelength were set at 1.0 ml/min, 10 μl, 40°C and 280 nm, respectively.

Calibration curves for standard solutions of catechins were obtained as good straight lines for 0.5-10×10⁻⁴ M (r=0.9996-1.000), the concentrations of catechins in each type of tea based on these calibration curves being summarized in Table II. The experimental uncertainty of three repetitive measurements was less than 4% for all samples. The HPLC determination of the catechins in green tea gave a molar ratio order of ECG<EC<EGCG<EGC for every sample. The result for Sen-cha (high and low grades) is similar to that described by other workers, but there is little data in the literature for the other green tea. By the kind of tea, the order of the total amount of catechins was Mat-cha<Gyokuro<Sen-cha (H)<Kamairi-cha<Sen-cha (L).
Ban-cha. This result is supported by the fact that the more tea is exposed to the sun, the greater is the formation of catechins.\(^{15}\)

It was expected that the amount of catechins in green tea would be related to the antioxidative activity of the respective catechins. The concentration of catechins in tea corrected by the antioxidative activity ([C], calculated value) is defined by the following expression:

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[C] = 4\left(\frac{R'_p}{R_p}\right)_{EC} + \left(\frac{R'_p}{R_p}\right)_{EGC} + \left(\frac{R'_p}{R_p}\right)_{EGG} + \left(\frac{R'_p}{R_p}\right)_{ECC} \times \left(\frac{[EC]}{R_p} + \frac{[EGC]}{R_p} + \frac{[EGG]}{R_p}\right)
\]

where [EC], [EGC], [EGG] and [ECC] are the concentrations of the respective catechins, and \(\left(\frac{R'_p}{R_p}\right)_{EC}, \left(\frac{R'_p}{R_p}\right)_{EGC}, \left(\frac{R'_p}{R_p}\right)_{EGG}\) and \(\left(\frac{R'_p}{R_p}\right)_{ECC}\) are the antioxidative activity of the respective catechins. Figure 3 shows the \(\left(\frac{R'_p}{R_p}\right)^{-1}\) vs. [C] plot, where \(\left(\frac{R'_p}{R_p}\right)^{-1}\) is the reciprocal of the antioxidative activity of the tea itself (observed value). The solid regression line was obtained by least-squares fitting, the correlation coefficient \(r\) being 0.6402. The antioxidative activity of green tea thus depends to a significant degree on the amount of catechins present. However, the intercept of the regression line of 1.7821 (larger than 1) shows that the antioxidative activity isn’t only related to the amount of catechins. It is considered that substances other than the four catechins in green tea, like other types of polyphenol and antioxidative vitamins, would have an effect on the antioxidative activity of tea. For example, the synergistic interaction of vitamin C with vitamin E for inhibiting oxidation in a methyl linolate solution has been reported.\(^{16}\) It is considered that vitamin C would support catechins, making the antioxidative activity of green tea high.

In conclusion, the antioxidative activity of catechin and tea samples was rapidly and accurately measured by an oxygen electrode. We also studied the relationship between the antioxidative activity of green tea and amount of catechins present. The result indicates that the antioxidative activity of green tea was significantly dependent on the amount of catechins present, although there would be other substances contributing to the antioxidative activity.

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References