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Short communication

Application of capillary electrophoresis to tea quality estimation

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Abstract

The qualitatively important components of green tea (theanine, caffeine, ascorbic acid, (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin gallate and (−)-epigallocatechin gallate) were analyzed simultaneously by using capillary electrophoresis. The running buffer used was borate buffer (80 m*M*, pH 8.4) containing 50 m*M* of sodium dodecylsulfate. The extracts of green tea, oolong tea and black tea were analyzed using this method. Green teas of different plucking dates and the leaf samples from different positions in one shoot were also measured using this method. These results showed that this method will considerably save time and labor for the analysis of these components, and is quite useful for the quality estimation of teas (particularly of green tea) and characterization of fresh tea leaves. © 1998 Elsevier Science B.V.

Keywords: Tea; Food analysis; Catechins; Theanine; Caffeine; Ascorbic acid

1. Introduction

Almost 10^8 kg of green tea are produced in Japan every year. The quality of green tea mostly depends on the components in fresh leaves. Usually the green teas plucked and manufactured in late April and early May are high quality and the quality declines in later harvests. The prices of green tea are quite variable depending on the quality, from 100 yen/100 g to several thousand yen/100 g at a market. It is therefore very important to precisely estimate the quality of green teas.

Chemical analysis is the most reliable method to estimate the quality of green tea. The relationship between the quality and chemical components in green tea has been studied, and has shown that free amino acids, catechins, caffeine and ascorbic acid are qualitatively important components [1,2]. Among free amino acids, theanine (1-glutamyl-γ-ethylamide) is the predominant amino acid in green tea, and high grade teas contain high amounts of this component. The major catechins in tea are (−)-epigallocatechin gallate (EGCg), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECg) and (−)-epicatechin (EC).

Our previous paper [3] reported the use of capillary zone electrophoresis (CZE) with a UV detector to analyze these components. Whereas these components can be separated well by this method, there still remains a possibility that the peak of caffeine might be affected by the organic solvent used for sample extraction. Moreover, it is not easy to analyze ascorbic acid with reasonable precision, because its absorbence is small at 200 nm, which is the wavelength selected in the previous report. To solve these problems, sodium dodecylsulfate was added to the running buffer, which facilitates the separation of caffeine with micellar electrokinetic chromatography (MEKC) mode [4], and a diode array detector was used instead of a UV detector, enabling multi-wavelength monitoring.

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2. Experimental

2.1. Reagents

Four major catechins, EGCg, EGC, ECg and EC, were purchased from Kurita Kogyo (Tokyo, Japan). Theanine was purchased from Tokyo Kasei (Tokyo, Japan). Other reagents used were analytical grade. Ultrapure water (Milli-Q, Millipore) was used throughout these experiments.

2.2. Instruments

A capillary electrophoresis system (P/ACE 5000) with a diode array detector (Beckman Instruments, Fellerton, CA, USA) was used. The silica capillary tubing employed for the analysis was uncoated, with an internal diameter of 75 μm and a length of 77 cm (70 cm from autosampler to detector).

2.3. Analytical conditions

The running buffer used was prepared by mixing 20 m*M* sodium tetraborate and 80 m*M* boric acid solution, both containing 50 m*M* of sodium dodecylsulfate, and adjusted to pH 8.4. The applied potential was 25 kV and detection was performed with a diode array detector at wavelengths of 194 and 270 nm. The temperature of the capillary was controlled at 30°C. Samples were injected into the capillary by N_2 pressure for 5 s. The capillary was rinsed sequentially with distilled water, 0.1 *M* HCl, distilled water, 0.1 *M* NaOH and the running buffer, between successive electrophoretic runs. The total time for rinsing was about 8 min.

2.4. Sample preparation

The standard mixture was prepared by dissolving the four catechins, theanine, caffeine and ascorbic acid in 0.1% metaphosphoric acid.

Oolong tea produced in China and black tea produced in India were purchased from a market. Green tea used to compare with these teas was produced in our institute.

To determine the difference in components among the teas plucked on different days, green tea samples, which were manufactured on the day of plucking, were collected from a tea factory during May 1996. These tea samples were milled and stored in a refrigerator before the analysis. Milled tea (250 mg) was extracted with 50 ml of extraction solution (acetonitrile–2% metaphosphoric acid (1:1, v/v)) for 30 min in an ultrasonic water bath, and the extract solution was diluted 10 times with water, before passing through the 0.45-μm membrane filter. The filtrate was used as a sample for capillary electrophoresis.

To measure the components of different leaf parts in one shoot, young tea shoots were collected from the tea field of our institute on 25 April 1997. The shoots were inactivated using a microwave oven for 1 min and dried at 80°C. Each leaf part was crushed well with a glass bar and extracted with the extraction solution (0.2 ml/mg dry leaf). After the extraction, further sample preparation was the same as in the case of milled tea.

p-Hydroxybenzoic acid was used as an internal standard at a concentration of 10 mg/l.

3. Results and discussion

3.1. Analytical conditions

Theanine is one of the most important components in the quality estimation of green tea, but it has no λ_{max} above 194 nm. We therefore chose 194 nm as one of the detection wavelengths. However, at this wavelength the absorbance of ascorbic acid is not large enough for measuring the levels of green tea samples. Recently, we have developed a method using HPLC to analyze catechins and caffeine simultaneously [5]. In this method 270 nm was selected for the detection of these components. This wavelength is also near the λ_{max} of ascorbic acid. Thus we chose 270 nm as the second detection wavelength. Using these two wavelengths, lower levels of ascorbic acid could be analyzed with the other components in a single run.

The pH of the running buffer was important for separation. When the pH was lower than 8.4, the buffering capacity of the running buffer was too low. However, when the pH was much higher, tailing of

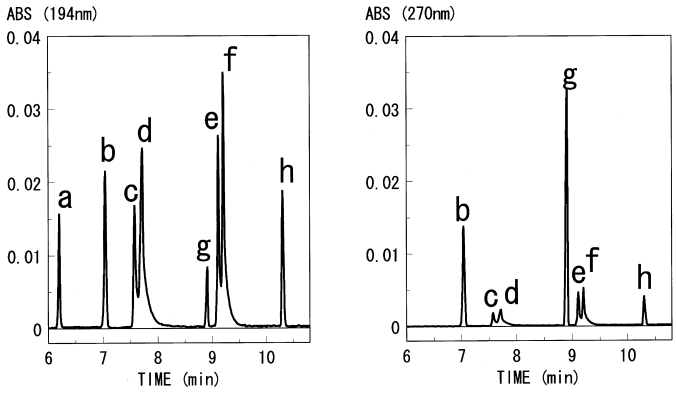

Fig. 1. Electropherograms of a standard mixture: (a) theanine; (b) caffeine; (c) EGC; (d) EC; (e) EGCg; (f) ECg; (g) ascorbic acid; (h) *p*-hydroxybenzoic acid (internal standard). (a–g) 100 mg/l; (h) 10 mg/l.

the peaks of catechins became significant. We therefore chose pH 8.4. The electropherogram of standards is shown in Fig. 1. A wavelength of 194 nm was used for the calibrations of theanine, caffeine and catechins, and of 270 nm for that of ascorbic acid.

3.2. Electropherograms of green tea, oolong tea and black tea

The electropherograms of three kinds of tea are shown in Fig. 2. For green tea (Fig. 2(1)), all the seven components separated clearly. In oolong tea (Fig. 2(2)), the peak areas of catechins were smaller than those of green tea, and the peaks of theanine and ascorbic acid were almost diminished. In black tea (Fig. 2(3)), EGC and EC were at trace levels, and theanine and ascorbic acid were undetectable. This method was intended for the analysis of qualitatively important components of green tea, and the results satisfy this purpose. In the case of oolong tea, high quality teas are reported to contain more amounts of theanine, EGCg and ECg than low quality ones [6], hence this method would be applicable to oolong tea. However, for black tea, theaflavins and thearubisins are also important components for its quality [7]. It is recommended to modify this method to determine these components together with catechins and caffeine.

3.3. Comparison of the components of green teas of different plucking date

Generally, the price and quality of green tea decline with later plucking. This method was applied to compare the components in green teas plucked on different days (Fig. 3). In our results, as date elapsed, the contents of theanine, caffeine, EGCg decreased, whereas those of EGC and EC increased. These results are consistent with earlier reports [1,8]. The components measured by this method are confirmed to be good indexes of green tea quality.

3.4. Components of different leaf parts in one leaf

Different leaf parts of a young shoot were analyzed using our method (Fig. 4). The results showed a wide variation of the components among the leaf parts. The bud contained much higher contents of theanine, EGCg, ECg and caffeine, and lower contents of EGC and EC, than the more mature leaves (second, fourth and over- wintered leaves). Miwa et al. [9] have already reported similar results from an analysis using paper chromatography, HPLC and semi-micro Kjeldahl methods. Many leaf samples were needed for the analysis of these components at that time. In our method, only a single leaf part is enough to determine these seven important com-

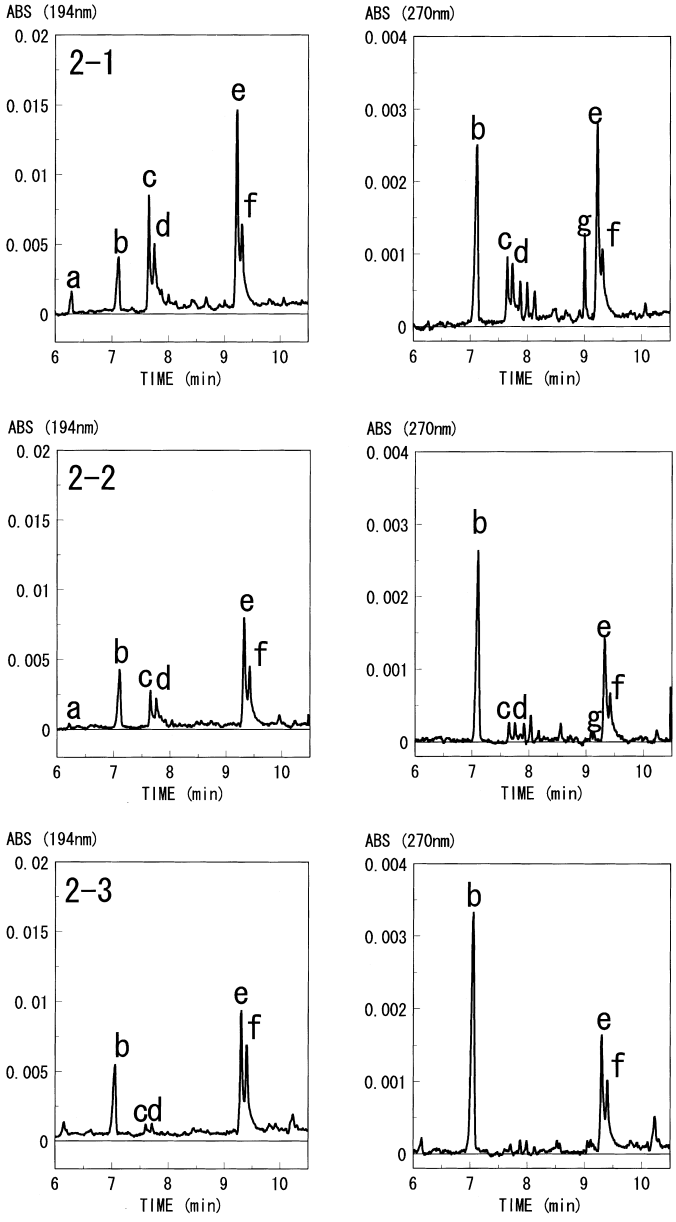

Fig. 2. Comparison of electropherograms of various teas (1) green tea; (2) oolong tea; (3) black tea. (a) Theanine; (b) caffeine; (c) EGC; (d) EC; (e) EGCg; (f) ECg; (g) ascorbic acid. No internal standard was added.

ponents. Further, our method needs much less time, labor, and reagents compared to their methods.

Since the information of the components in fresh leaves is important not only for green tea, but also oolong tea and black tea, this method is also applicable for the characterization of fresh leaves for oolong or black tea. Moreover, it will also become a useful tool in the physiological studies of tea plants.

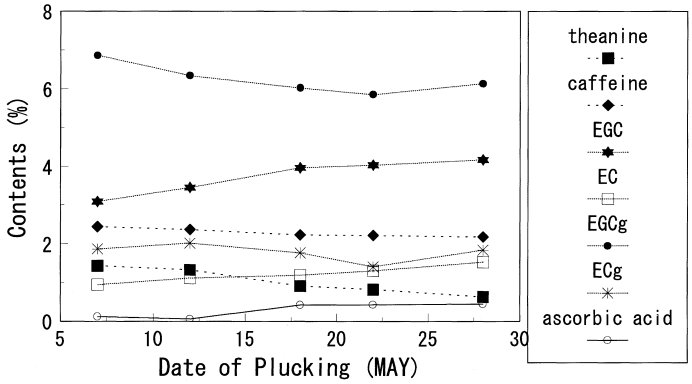

Fig. 3. Changes of tea components depending on the date of plucking.

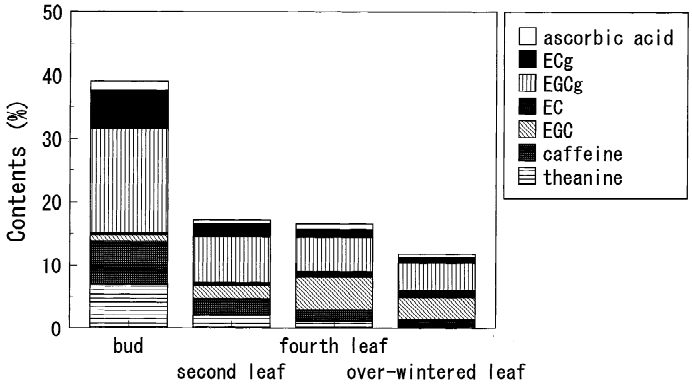

Fig. 4. The relationship between the leaf positions in one shoot and the contents of the components.

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References

[1] M. Nakagawa, JARQ 5(3) (1979) 43.
[2] T. Goto, Y. Yoshida, I. Amano, H. Horie, Foods Food Ingredients J. Jpn. 170 (1996) 46.
[3] H. Horie, T. Mukai, K. Kohata, J. Chromatogr. A 758 (1997) 332.
[4] S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya, T. Ando, Anal. Chem. 56 (1984) 111.
[5] H. Horie, Y. Namiki, T. Mukai, K. Kohata, Tea Res. J. 85 (1997) 9.
[6] H. Takayanagi, T. Anan, K. Ikegaya, T. Nakagawa, Tea Res. J. 60 (1984) 54.
[7] I. McDowell, S. Taylor, C. Gay, J. Sci. Food Agric. 69 (1995) 467.
[8] Y. Yoshida, M. Kiso, H. Nagashima, T. Goto, Tea Res. J. 83 (1996) 9.
[9] E. Miwa, H. Takayanagi, M. Nakagawa, Tea Res. J. 47 (1978) 48.