Capillary electrophoresis- A new tool for quality evaluation.

Hideki Horie, Tomomi Ujihara and Katsunori Kohata

National Institute of Vegetable and Tea Science
2769 Kanaya, Shizuoka 428-8501 JAPAN

Summary

We have developed a method to determine theanine, caffeine and major catechins simultaneously in tea extracts using a capillary electrophoresis (CE). The time and the sample volume required for the analysis is only 15 min and less than 50 μl. This method was successfully applied to the analysis of the components of each one leaf of new shoots.

We have developed CE methods to measure sodium ion and to analyze glutamic acid and aspartic acid in tea extracts. The concentration of sodium ions and the rate of glutamic acid / aspartic acid were useful indicators to discriminate flavored (sodium-glutamate added) teas.

Keywords

green tea, adulteration, theanine, catechins, glutamate

Introduction

The study of the relationship between the quality of green teas and their chemical components has been continued more than a half century in Japan. It was discovered that the teas that are sold at higher prices contain more amount of total nitrogen. After that, it has been shown that the teas of higher grade, such teas show stronger UMAMI taste, contains higher amount of amino acids1). Other components in tea are also important for its quality and taste, caffeine is a stimulator and catechins taste astringent and bitter. Ascorbic acid is a good indicator for the freshness of green teas.

Hundreds of reports have been written on the analysis of tea components2,3). Recently the most popular methods for the analysis of tea components are those using high performance liquid chromatography (HPLC). No HPLC method has ever been reported to determine the simultaneous analysis of amino acids and catechins, although many reports have been published on the analysis of catechins using HPLC. For the quality evaluation of green tea, it is beneficial to analyze amino acids simultaneously with other qualitatively important compounds. We applied capillary zone electrophoresis for this purpose, and could be separated theanine, which is the amino acid contained most abundantly in tea leaves, caffeine, ascorbic acid and catechins4). Recently the method have been improved to analyze caffeine more precisely5,6) . While several other reports using a capillary electrophoresis (CE) have been published on the analysis of catechins in tea7), no reports analyze amino acids simultaneously with catechins except for ours.

In this report, we measured the qualitatively important major components (theanine, caffeine and catechins) of each leaf and bud of one new shoot to show the superiority of our method.

For the marketing, it is also important to find adulterated teas. While flavoring is popular in black and oolong tea, flavored Japanese green tea is not common. The teas that are added with artificial UMAMI seasoning are sold only a small amount in Japan. Sometimes troubles happen since such flavored teas are distributed to the market without notifying the addition of seasonings. Some dealers have begun to examine their teas by chemical methods.

We have developed methods to discriminate such teas using a sodium ion selective electrode8,9). As the major compound in the UMAMI seasonings is sodium glutamate, the concentration of sodium ion in tea extracts is a good and sensitive indicator of such flavoring. In our research we found most of the unflavored teas showed low concentrations of sodium ion, however the concentrations of the ion in some unflavored teas were as high as those in flavored teas10). Therefore, another indicator is required to correctly discriminate between flavored and unflavored teas.

In our previous reports we have shown that the contents of glutamic acid and aspartic acid are almost the same in unflavored teas10), and we have reported that these amino acids can be analyzed simultaneously with other organic acids using CE11). In this report, the CE method was modified to get the sharper peaks of these two amino acids and its application for the discrimination of flavored teas was discussed.

Material and Methods

Instruments

Capillary electrophoresis system P/ACE 5000 with UV detector (Beckman Instruments, Fullerton, CA, USA) with an uncoated silica capillary (internal diameter 75 μm * length 57 cm) was used throughout the experiments. Samples were introduced into the capillary by high pressure injection for 5 s, and the capillary temperature was set at 20°C.

Analysis of major tea components

New shoots of the same tea tree was collected on May 5th, 2001 in the field of NIVTS (Kanaya, Shizuoka, Japan) and blanched with microwave oven for 1 min. After each shoot was dried in a drying oven for 1 day at 60°C, and was divided into 6 parts, bud, first leaf, second leaf, third leaf, fourth leaf and stem. Each part was weighed in a test tube and crushed with a glass bar, and the extraction solution (1% metaphosphoric acid : ethanol = 1 : 1 (V/V)) was added to each part as the rate of 0.2 ml/mg of dry matter. The test tube was mixed well and stayed at room temperature for 1 hour. The extract was diluted 20 times with water and passed through a membrane filter. The filtrate was used as the sample for the CE analysis.

The electrolyte used was 50 mM boric acid, 10 mM disodium hydrogen phosphate, 50 mM sodium dodecyl sulfate and the pH was adjusted to 8.26). The applied potential was 30 kV (instead of 25 kV) for more rapid analysis and the detection wavelength was 200 nm. The capillary was rinsed sequentially with water, 0.1 M HCl, water, 0.1M NaOH, water and the electrolyte between the analysis.

Extraction and analysis of sodium ion

Three groups of green teas were collected. Group 1 was very common unflavored teas, group 2 was the unflavored teas that shows high concentrations of sodium ion, and group 3 was teas sold as flavored tea.

Five grams of green tea leaves were extracted with 100 ml of distilled water for 10 min8). The extract was passed through the membrane filter and the filtrate was used as the sample for the CE analysis of sodium ion or amino acids.

Sodium ion was measured according to our report12). The electrolyte was 10 mM imidazole, 20 mM 18-crown 6-ether. The pH was adjusted to 4.3 using 0.1 M sulfuric acid. Applied potential was 25 kV and the detection wavelength was 214 nm (indirect).

Analysis of aspartic acid and glutamic acid

The method of Soga and Ross13) was modified for our purpose. The electrolyte used was 10 mM dipicolinic acid and 0.5mM cetyltrimethylammonium bromide. The pH was adjusted to 11.5 on the day of the analysis. Applied potential was –25 kV, and the detection wavelength was 254 nm (indirect). The conditions of rinsing were the same as those of major tea components. This method was applied to the sample solutions used for the analysis of sodium ion.

Results and Discussion

Analysis of major components of tea

In our previous paper[6], major tea components (theanine, caffeine, ascorbic acid and four catechins) were monitored at both 200 nm and 270 nm using diode array detector. In this report we monitored at a single wavelength of 200 nm, since we did not concern about ascorbic acid, which has high absorbance at 270 nm. The potential was increased from 25 kV[6] to 30 kV, which saved 3 min of analytical time, and the total time for the analysis of one sample became 15 min.

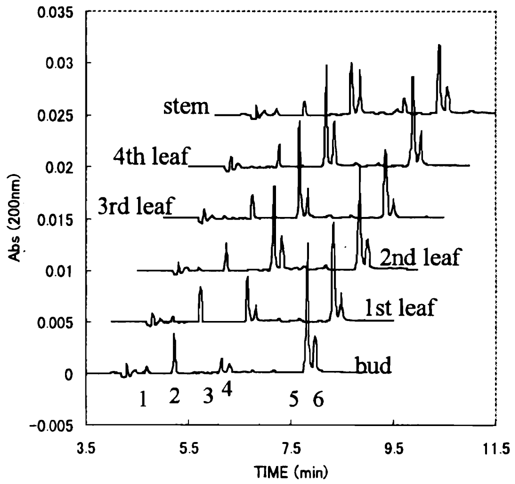

Fig. 1 Pherograms of the major components in tea leaves
1: theanine, 2: caffeine, 3: EGC, 4: EC, 5: EGCg, 6: ECg.

This method can be applied to the analysis of both tea leaves and tea infusions[6]. Since most important tea components can be extracted and analyzed simultaneously and the volume needed for the analysis is less than 50 μl, this CE method is also suitable for the analysis of samples of small amount. The examples to measure the components in the small volumes of tea infusions have already been shown in our report[14].

Four tea shoots of the same developmental stage from the same tree were collected and this CE method was applied to the analysis of single leaves of them. The weight of each part of shoot ranged from 2 mg to 40 mg.

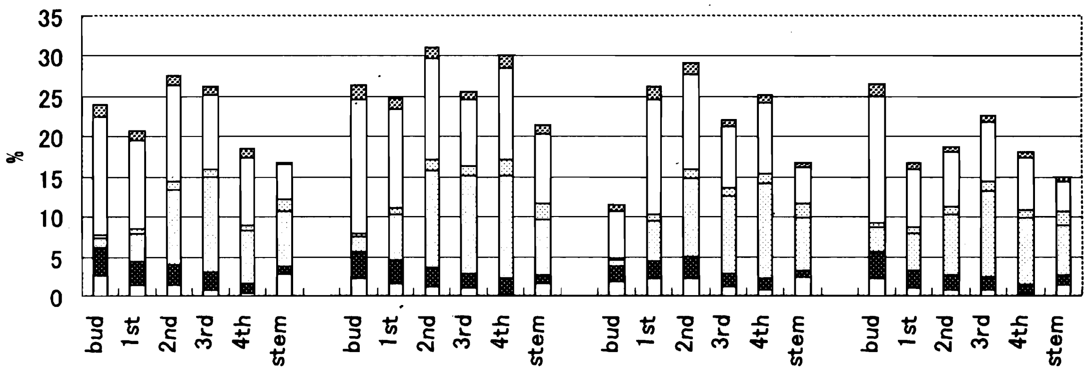

Fig. 2 Components in each part of four tea shoots
1st: first leaf, 2nd: second leaf, 3rd: third leaf, 4th: fourth leaf.

The pherograms of each part of one shoot were shown in Fig. 1. Although the weight of one bud was less than 10 mg, it was easy to analyze theanine, caffeine and four major catechins simultaneously using this CE method. It is noticeable that each peak area was significantly different among the parts of the shoot. In Fig. 2, the contents of each component of four shoots were compared. Even we selected the shoots of similar stages of development from the same tree, the contents of each component in each part were different among the shoots.

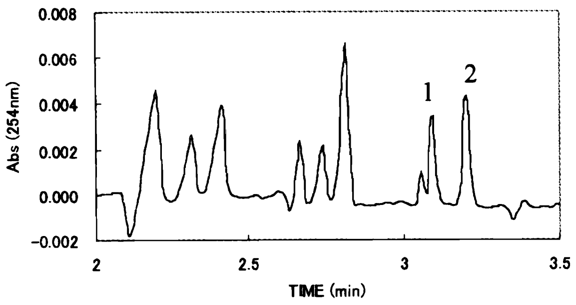

Fig. 3 Pherogram of tea extract
1: aspartic acid, 2: glutamic acid.

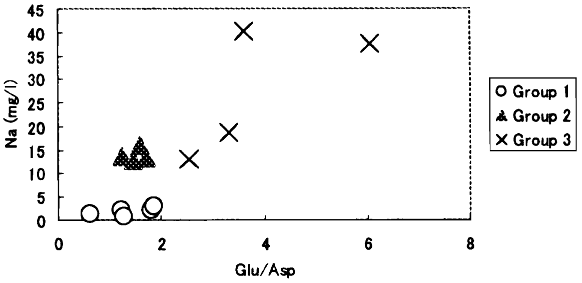

Fig. 4 Relationship of sodium ion concentration and the rate of Glu / Asp.
Group 1 and 2: unflavored tea, Group 3: flavored tea.

Discrimination of sodium glutamate added tea

For the additional method to evaluate the flavoring besides sodium ion analysis, analysis of glutamic acid and aspartic acid seemed to be useful. The pherogram of the analysis of these amino acids was shown in Fig. 3. It needs less than 10 min for the analysis of one sample.

Sodium ion was measured according to our CE method[12] and aspartate and glutamate were measured using the newly developed method. The sample solution prepared for the sodium analysis could be used for the analysis of these amino acids. The relationship between the concentrations of sodium ion and the rates of Glu / Asp (glutamic acid (mg/l)/ aspartic acid (mg/l)) were shown in Fig. 4. The sodium ion concentrations of teas belonging to group 2 (unflavored teas) exceed 10 mg/l and it is difficult to discriminate between the teas of group 2 and group 3 (flavored teas) from the data of sodium ion. The rates of glutamic acid / aspartic acid were less than two in the teas of group 1 and group 2 (unflavored teas), and they were lower than those of group 3. More precise discrimination of flavoring is possible if these two CE methods are used together.

We have also developed the CE methods to determine organic acids in tea infusions[11], and found that oxalic acid and citric acid are the important factors for the taste of green tea[15]. Our methods are more rapid and economical, and less laborious than the chromatographic methods currently in use. We hope these CE methods will be commonly used and contributed to the development of tea industries.

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