Analysis of organic anions in tea infusions using capillary electrophoresis

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Abstract

Major organic acids and acidic amino acids in tea infusions were analyzed simultaneously using capillary zone electrophoresis with indirect detection at 254 nm. Electropherograms were obtained with chromate buffer (10 mM) containing tetradecyltrimethylammonium bromide (0.5 mM) and ethylenediaminetetraacetic acid (disodium salt, Na$_2$EDTA, 0.1 mM), which was added to reduce the influence of metal cations. For the sample preparation, tea infusions, with Na$_2$EDTA (0.25 mM) were diluted five times. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Organic anions in tea are considered to be important from the viewpoint of taste and quality. However, analytical data on these components are more scarce than those of catechins and other polyphenols.

Tea plant (*Camellia sinensis*) contains relatively high amounts of oxalic acid [1]. Oxalic acid is known to be an undesirable compound and the excess intake of it increases the risk of renal calculus formation [2]. Recently we found that the white precipitation produced when green tea was infused with hard water is calcium oxalate [3]. As such tea infusion is less harsh than that prepared with distilled water, we are now investigating the relationship between the taste and oxalate contents in tea infusions. Monosodium glutamate has a strong umami or brothy taste. Green tea with a strong umami taste is favored in Japan, and high-grade green teas contain a large amount of glutamate [4]. Thus, glutamic acid is

one of the key components in the quality of green tea. Sakata et al. [5] found that quinic acid is one of the major organic acids in tea leaves, but no further study on this compound has been carried out.

Ding et al. [6] have developed a method to determine organic and inorganic anions in tea, using ion chromatography. However, it could not separate the anions of our interest, and the amounts of some anions in their report were much higher than those of published data [4,7], making it necessary to develop a better method.

We tried to develop a method to determine oxalic acid, glutamic acid and quinic acid together with other major anions in tea. Several analytical methods are known to analyze organic anions. Tedious preparation and derivatization is needed to analyze them using gas chromatography [8], and very expensive columns must be used to measure them using high-performance liquid chromatography [9] or ion chromatography [6,10]. In contrast, analysis using capillary electrophoresis (CE) is generally quite simple and economical. Therefore, we tried to develop a method to measure the anions of tea infusions using

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CE. The organic anions selected for analysis, oxalic acid, malic acid, citric acid, quinic acid, aspartic acid and glutamic acid, are the major ones in tea according to the literature [4,5,7]. The inorganic anion, fluoride, is also of interest for analysis, because it is supposed to prevent teeth from decaying.

2. Experimental

2.1. Chemicals and tea samples

Sodium chromate, tetradecyltrimethylammonium bromide (TTAB) and ethylenediaminetetraacetic acid (disodium salt, Na_2EDTA) were purchased from Kanto (Tokyo, Japan), Wako (Osaka, Japan) and Dojin Labs. (Tokyo, Japan), respectively. Other chemicals used were of analytical grade. A fused-silica capillary tube was purchased from J&W Scientific (Folsom, CA, USA).

Green tea (Sen-cha) was produced at our institute. High-grade green tea (Gyokuro) and post-fermented teas (Goishi-cha and Pu-erh) were purchased from a market. Black teas (Darjeeling and Uva) were a kind gift from Lipton Japan.

Three grams of each tea were infused with 180 ml of boiling water for 3 min, and the infusion was filtered. Na_2EDTA (0.25 mM, final concentration) was added to the filtrate and diluted five times. The CE samples were prepared by microfiltration (0.45 μm, Advantec, Tokyo, Japan) of these diluted tea infusions.

2.2. Instrument and analytical conditions

A Beckman (Fullerton, CA, USA) P/ACE 5000 CE instrument and an IBM personal computer with System Gold software was used. UV absorption was detected indirectly at 254 nm and capillary temperature was set at 20°C. Samples were introduced into the capillary by pressure injection for 5 s. An open tubular fused-silica capillary (57 cm×360 μm O.D.×75 μm I.D.) was used throughout the experiments. The analysis was performed with an applied voltage of −20 kV. Between each run the capillary was rinsed with 0.1 M hydrochloric acid, 0.1 M sodium hydroxide and then the electrolyte.

The electrolyte solution contained 10 mM sodium chromate, 0.5 mM TTAB and 0.1 mM Na_2EDTA. The electrolyte was prepared using Milli-Q water (Millipore) and the pH of the electrolyte was not adjusted.

3. Results and discussion

3.1. Effect of EDTA

It is known that metal cations influence the migration times and the peak areas of anions in CE analysis [11,12]. There is a possibility that the metals in tea infusion would influence the performance of the CE analysis. To reduce the effect of metals, we tried to add Na_2EDTA (0.1 mM) to the electrolyte according to Nelson et al. [12]. Even with Na_2EDTA in the electrolyte, the peak area of citric acid was still significantly influenced by metals. The major divalent or trivalent cations in tea are calcium, magnesium, aluminum and manganese, so the peak areas of citric acid with and without these metals were compared. In the presence of 1 mg/l of each of these metal cations, the peak area of citric acid was smaller than that without metal ions (Fig. 1,
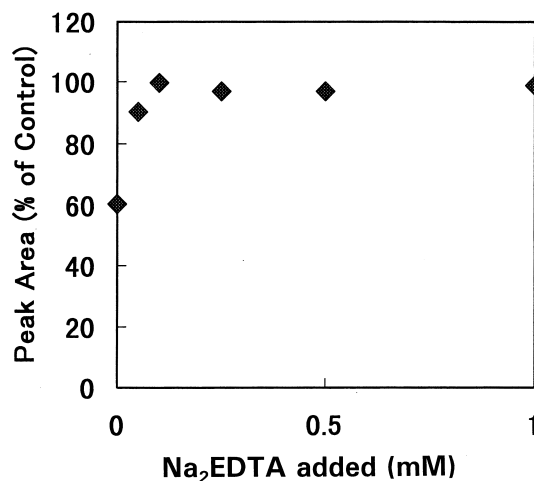

Fig. 1. Effect of EDTA on the peak area of citric acid in the presence of metal ions. The electrolyte was 10 mM chromate, 0.5 mM TTAB and 0.1 mM EDTA. The concentration of citric acid was 25 mg/l. Calcium, magnesium, aluminum and manganese ions were added to citrate solution at the concentration of 1 mg/l each. Control was the peak area of citric acid without metals.

Na$_2$EDTA; 0 mM). To eliminate the effect of metals, Na$_2$EDTA was also added to the sample solutions. By the addition of more than 0.1 mM of Na$_2$EDTA into the mixtures of citrate and metals, the peak area of citric acid was recovered perfectly (Fig. 1). In cases of higher Na$_2$EDTA concentration, a large EDTA peak would interfere with other peaks, so we chose 0.25 mM as EDTA concentration to add to sample solutions. In our study, 0.25 mM of Na$_2$EDTA was added to both standard and sample solutions and 0.1 mM of Na$_2$EDTA was added to the electrolyte.

3.2. Analytical conditions

During the analysis of anions using CE, chromate and phthalate buffers are the most frequently used as electrolytes. When phthalate buffer was mixed with tea infusions, precipitation occurred. Because the precipitation would occur in the capillary during the analysis and reduce the analytical performance, chromate buffer was selected as the electrolyte instead.

It was not easy to adjust the pH of the chromate buffer correctly, because of its weak buffer capacity, so the pH was not adjusted. In this condition the performance was satisfactory for our purpose (Tables 1 and 2). The anions of interest could be clearly separated by this method (Fig. 2).

3.3. Analysis of anions in tea infusions

This method was applied to the analysis of actual tea infusions. The electropherogram of green tea (Sen-cha) is shown in Fig. 3. Oxalic acid, citric acid, malic acid, aspartic acid, glutamic acid and quinic acid were clearly separated. In this method the peak of fluoride can be separated from other anions, however the concentrations of it in tea infusions were too low to analyze precisely. The large peaks at the migration time around 4.5 min were estimated to be EDTA–metal complexes.

The results of several kinds of teas are shown in Table 3. The peak areas of fluoride were too small to determine precisely in all the infusions tested. In the infusions of green teas (Sen-cha and Gyokuro), the concentration of glutamic acid was higher than that in the other tea infusions. Between green tea infusions, the concentrations of oxalic acid and glutamic acid of Gyokuro was higher than those of Sen-cha. These results are consistent with published data [4,13]. The concentration of glutamic acid measured

Table 2
Recovery tests of organic anions

	Mean recovery (%) ($n=3$)	
	Green tea	Black tea
Oxalic acid	99	97
Citric acid	105	100
Fluoride	105	102
Malic acid	97	97
Aspartic acid	97	98
Glutamic acid	97	102
Quinic acid	104	99

The concentration of each anion spiked to the infusion of green or black tea was 20 mg/l.

Table 1
Performance of the analysis

Analyte	Migration time R.S.D. (%) ($n=4$)	Peak area		
		R.S.D. (%)	Linearity	r^2
Oxalic acid	0.40	0.93	0.2–1000	0.999
Citric acid	0.43	1.38	0.5–1000	0.998
Fluoride	0.44	1.00	0.5–250	0.998
Malic acid	0.40	1.04	0.5–250	0.997
Aspartic acid	0.75	2.20	0.5–500	0.999
Glutamic acid	0.83	3.53	1.0–500	0.999
Quinic acid	0.65	3.11	1.0–1000	0.999

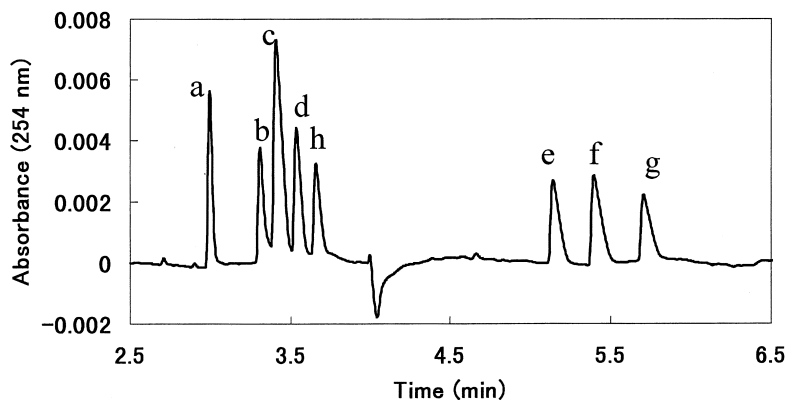The concentration of each analyte was 20 mg/l.

Fig. 2. Electropherogram of standard anions. (a) Oxalic acid, (b) citric acid, (c) fluoride (d) malic acid (e) aspartic acid, (f) glutamic acid, (g) quinic acid, (h) EDTA. The concentration of each anion was 20 mg/l.

by this method could be a good indicator of the quality of green tea.

There are fermentation steps by microorganisms in the manufacturing process of post-fermented teas (Pu-erh and Goishi-cha). We had expected that these tea infusions would contain high concentrations of organic acids caused by microbiological fermentation. Several unidentified peaks were observed in the infusion of Goishi-cha (Fig. 4), and the largest one was identified as lactic acid (320 mg/l). A high content of lactic acid in Goishi-cha has already been observed by Kato et al. [14]. While the concentrations of organic anions were quite low in the infusion of Pu-erh, which may reflect the difference of the fermentation processes between these two post-fermented teas.

There are few reports on the organic acids in tea, however the concentrations of them in tea infusions can not be ignored. More than 100 mg/l of quinic acid was found in the infusions of green and black teas, while its relationship to the quality or taste of teas is still unknown. Oxalic acid shows harsh taste at the level of tea infusions, and oxalic acid and other organic acids influence the bioavailability of aluminum, calcium and other metals [13,15,16]. It is expected that our method will proceed the research works on these fields.
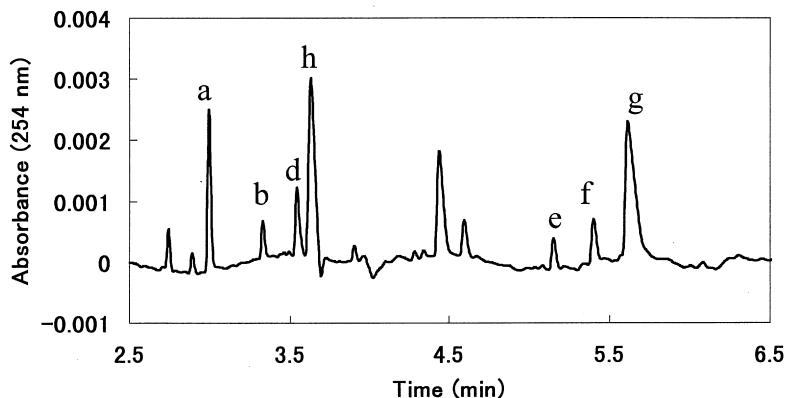
Acknowledgements

Fig. 3. Electropherogram of green tea (Sen-cha) infusion. The infusion was diluted five times. For peak identification, see Fig. 2.

Table 3
Analytical results of anions in tea infusions

Compound	Concentration (mg/l)					
	Sen-cha	Gyokuro	Darjeeling	Uva	Pu-erh	Goishi-cha
Oxalic acid	76.0 (1.9)[a]	166.3 (3.2)	81.9 (3.3)	76.0 (3.2)	45.2 (1.5)	12.1 (0.9)
Citric acid	19.8 (0.5)	31.7 (0.6)	20.3 (0.7)	27.1 (1.3)	3.7 (0.2)	trace
Malic acid	40.9 (1.7)	32.3 (1.6)	23.1 (1.1)	18.3 (0.9)	trace	15.8 (1.9)
Quinic acid	275.2 (8.9)	132.4 (7.1)	272.1 (4.3)	316.4 (19.0)	13.5 (1.4)	14.3 (1.3)
Aspartic acid	12.7 (0.6)	64.5 (1.8)	5.5 (0.2)	6.7 (0.3)	trace	3.1 (0.2)
Glutamic acid	33.8 (1.2)	64.5 (1.3)	7.6 (0.3)	10.4 (2.8)	trace	2.6 (0.3)

[a] Standard deviation of the three differently prepared infusions. Three grams of tea was infused with 180 ml of boiling distilled water for 3 min.
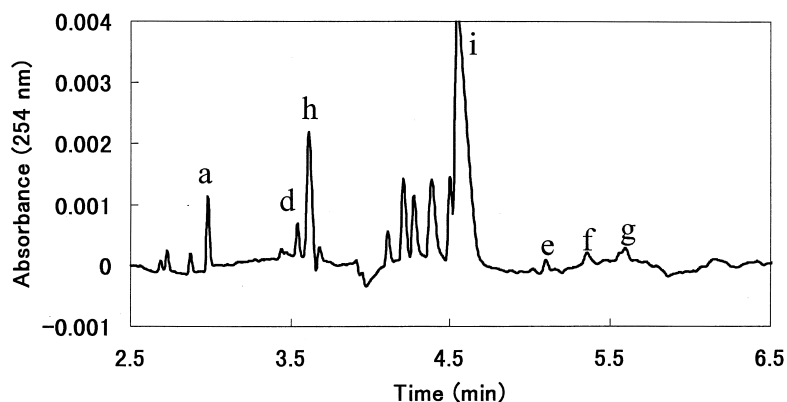

Fig. 4. Electropherogram of Goishi-cha infusion. (i) Lactic acid. For the identification of other peaks, see Fig. 2.

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