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Determination of Tea Components by Enzymatic Flow Injection Analysis

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Abstract
Rapid and easy analysis of qualitatively important components is required for the fair-trading of green teas. Flow injection analysis combined with immobilized enzymes was applied for that purpose, and the target compounds were amino acids (total amino acids, L-glutamic acid and γ-amino butyric acid) and ester-type catechins.

Discipline: Food / Tea industry
Additional key words: immobilized enzyme, biosensor, theanine, catechins, GABA

Introduction

Studies on green tea have revealed the relationship between the quality or taste and its chemical constituents[3]. Amino acids (such as theanine and glutamic acid) give the UMAMI taste and high-grade teas contain a large amount of them. Catechins are related to the astringency and bitterness. The balance between amino acids and catechins is important for the optimum taste of green tea. Although high-performance liquid chromatography (HPLC) or colorimetric methods can be applied to the determination of the concentrations of amino acids and catechins, these methods are time-consuming and laborious. Recently, near infrared spectroscopy has been applied for the rapid analysis of tea components[1]. The spectrometer itself can be used even by untrained persons, while the determination of calibration curve parameters for each tea component is very laborious and the parameters determined can only be applied to the samples which show similar characteristics as those used for calibration. Moreover the instrument itself is expensive. Thus other methods for rapid measurement of the contents of chemical components of tea are required.

Flow injection analysis (FIA) may be an effective method for that purpose. The selectivity of the analysis can be provided using the enzyme reactions. This paper describes the simultaneous flow injection determination of the concentrations of total amino acids and glutamic acid by FIA using biosensors, combined with enzyme-immobilized membranes and oxygen electrodes. The analysis of ester-type catechins ((-)-epigallocatechin gallate and (-)-epicatechin gallate) using enzyme reactors and pH-sensitive transistors is outlined. Finally, γ-aminobutyric acid (GABA) determination using an enzyme reactor and a fluorometric detector is described.

Simultaneous determination of total amino acids and glutamic acid[4]

High-grade teas are rich in amino acids, and theanine is the dominant amino acid in tea leaves. We observed that the biosensor for amino acids (amino acid sensor), when combined with L-amino acid oxidase-immobilized membrane and an oxygen electrode, responded linearly to the concentration of theanine[5]. The reaction is as follows.

$$\text{L-amino acid} + H_2O + O_2 \longrightarrow \text{keto-acid} + NH_3 + H_2O_2$$

The oxygen consumed by this enzyme reaction was monitored by the oxygen electrode. The sensor responded to theanine, asparagine, arginine, glutamine and serine but did not respond to glutamic acid or aspartic acid among the major amino acids found in tea leaves. The amino acid sensor was then calibrated with theanine, which gave the highest response among the major amino acids in tea leaves. The contents obtained by this method are referred to as "total amino acids". As the amino acids to

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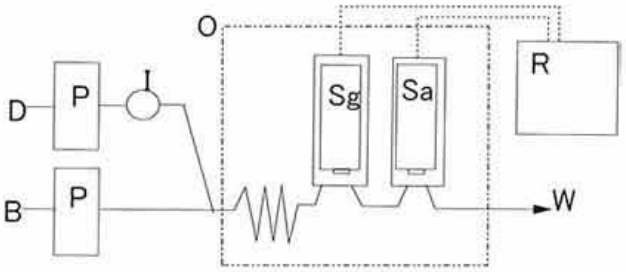

Fig. 1. Flow injection system for the simultaneous determination of the concentrations of glutamic acid and total amino acids

D: distilled water (flow rate 0.7 mL/min), B: phosphate buffer (1/15 M, pH 6.4, flow rate 2.7 mL/min), P: peristaltic pump, I: injection valve (loop 100 μL), O: column oven for HPLC (30°C), Sg: glutamate sensor, Sa: amino acid sensor, R: recorder, W: waste.

which the sensor responded are abundant in high-grade green teas, the sensor is expected to show a higher response to the infusions of high-grade teas. The use of this amino acid sensor was a convenient method to analyze the concentrations of total amino acids in tea infusions and estimate the quality of teas, but still it took about 10 min for the analysis of one sample[5].

The amino acid sensor did not respond to glutamic acid, while glutamic acid gave the strongest UMAMI and is one of the major components for tea quality. Sometimes sham teas are produced by the addition of sodium glutamate to enhance the taste of tea in the manufacturing process. Determination of the content of glutamic acid in tea is also important, and we found that the glutamate sensor, which was composed of a glutamate oxidase-immobilized membrane and an oxygen electrode, was effective for the analysis of the concentration of glutamic acid in tea infusions[6].

For more rapid and simultaneous analysis of the concentrations of total amino acids and glutamic acid, a flow injection system was developed by setting the glutamate sensor upstream of the amino acid sensor (Fig. 1)[4]. The filtered tea infusions could be directly injected into the flow system. Glutamic acid and total amino acids in the infusions were then analyzed using the glutamate sensor and the amino acid sensor, respectively.

The results of determinations in tea samples by this method were compared with those obtained using the HPLC method (Fig. 2). Correlation coefficients were significant for the practical use of both sensors. Using this flow injection system, 30 tea infusions could be analyzed in one hour for both the concentrations of glutamic acid and total amino acids. High-grade green tea showed a higher response to the amino acid sensor, as expected,

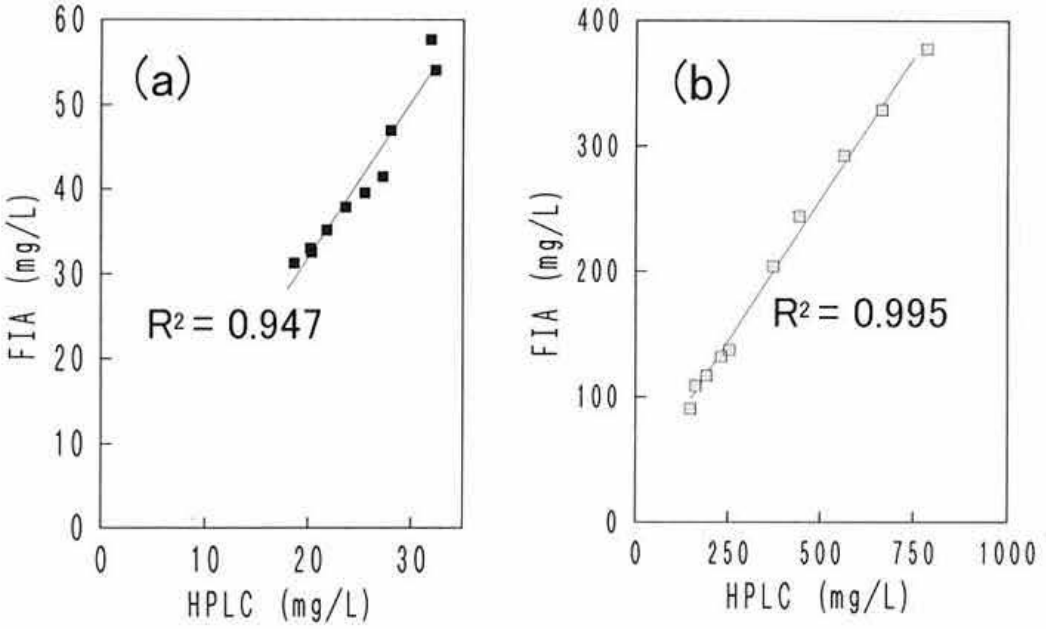

Fig. 2. Comparison of the composition of tea infusions determined by FIA and HPLC methods

(a): glutamic acid, (b): total amino acids.
To 3 g of tea, 180 mL of boiling water was added. The infusion was used as the sample for FIA analysis, after filtration with No.2 filter.

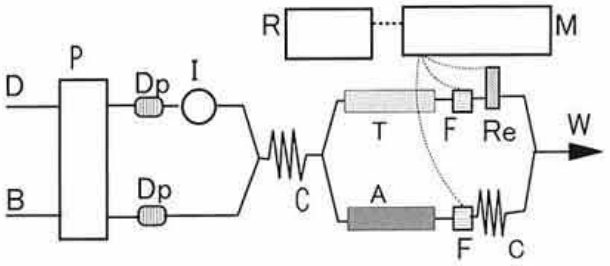

Fig. 3. Flow injection analysis system for ester-type catechins

D: distilled water, B: buffer (10 mM phtalate buffer, pH 5.5), P: peristaltic pump (total flow rate 0.7 mL/min), Dp: pulse-damper, I: injection valve (sampling loop 100 μL), C: coil, T: tannase-immobilized reactor (1 mm i.d. × 20 mm), A: bovine serum albumin-immobilized reactor, F: pH-ISFET electrode, Re: reference electrode, W: waste, M: ISFET pH/mV meter, R: recorder.

while sham teas to which glutamate had been added showed a high response to the glutamate sensor. This system was therefore effective for the rapid and objective estimation of tea quality and could be used to detect glutamate addition to green treas.

FIA determination of ester-type catechins[7]

Catechins are associated with the astringent and bitter taste of tea. Tea catechins that have a gallate moiety through ester linkage show a stronger astringency[3] and such catechins are called ester-type catechins. Major ester-type catechins in tea are (-)-epigallocatechin gallate (EGCg) and (-)-epicatechin gallate (ECg), and usually the content of the former is several times higher than that of the latter.

The analysis of ester-type catechins is therefore important for the evaluation of the taste and quality of green teas. A rapid analytical system was developed for ester-type catechins (Fig. 3)[7]. In this system the enzyme tannase was used to catalyze the following reaction.

(-)-epigallocatechin gallate + H_2O ⟶ (-)-epigallocatechin + gallic acid

The gallic acid formed can be detected by the pH shift using a pH electrode. The transistor electrode (pH-ISFET) was used here as a pH electrode to reduce the size of the system, by setting it in the flow cell. The enzyme reactor, which was filled with tannase-immobilized glass beads, was connected upstream of the electrode. After a standard EGCg solution was injected, the gallic acid formed in the reactor was detected by the change of the

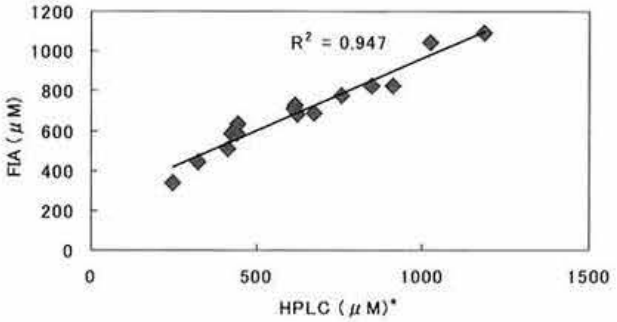

Fig. 4. Comparison of the concentrations of ester-type catechins in tea infusions determined by HPLC and FIA methods

*[EGCg] + [ECg]

electric potential of the pH-ISFET. However when the tea infusion was introduced, the pH shift due to the tea interfered with the analysis. A dummy reactor, which immobilized albumine instead of the enzyme, and a pH-ISFET electrode were prepared as control.

In this system, a linear response to EGCg was observed with selected buffers. The gallate-releasing activity of the immobilized tannase from ECg was the same as that for EGCg. Filtered tea infusions could be introduced every 3 min, and the results were compared with the HPLC method (Fig. 4). The correlation was significant for practical and rapid evaluation of the astringency of the green tea infusions.

Determination of γ-aminobutyric acid in GABA-enriched tea[8]

In the normal production of green tea, the content of γ-aminobutyric acid (GABA) is less than 0.1%, while anaerobic treatment of fresh leaves leads to a considerable increase of the content of GABA[2]. Since the consumption of GABA-enriched tea decreases the blood pressure in humans with hypertension, such teas are sold as "GABARON CHA" in Japan. As the effective component is GABA, it is necessary to analyze GABA easily and rapidly at the site of tea manufacturing and trading.

The enzyme complex called GABAse has been identified and it catalyzes the following reaction.

GABA + 2-ketoglutaric acid + H_2O + $NADP^+$ ⟶
succinic acid + glutamic acid + NADPH + H^+

NADPH is a fluorescent compound, and the fluorescence due to the enzyme-catalyzed reaction can be measured with a fluorescence detector. In the FIA (Fig. 5), GABAse was immobilized on glass beads and the beads were set in a silicone tube, which was used as an enzyme

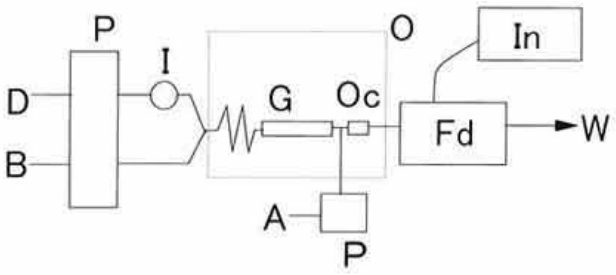

Fig. 5. Flow injection system for GABA analysis
D: distilled water, B: buffer (100 mM tris-HCl pH 8.0, 400 mM sodium sulfate, 1 mM $NADP^+$, 5 mM 2-ketoglutaric acid, 2 mM mercaptethanol), P: pump (flow rate 0.2 mL/min), I: injection valve (sampling loop 20 μL), G: GABAse-immobilized reactor (1 mm i.d. × 100 mm), Oc: C_{18} column (4.6 mm i.d. × 10 mm), A: acetic acid (1%), Fd: fluorescent detector (EX. 360 nm, Em. 460 nm), In: integrator, O: column oven (30°C), W: waste.

reactor. The injection valve was connected upstream and the detector was located downstream of the reactor, respectively[8].

For tea samples, the fluorescence from tea components interfered with the precise analysis of the sample. A guard column for HPLC packed with C_{18}-silica was connected upstream of the detector to prevent the interference. The flow from the reactor was acidified by acetic acid before entering the C_{18} column to improve the efficiency of the removal of the interfering substances and prevent the packed material in the column from deteriorating.

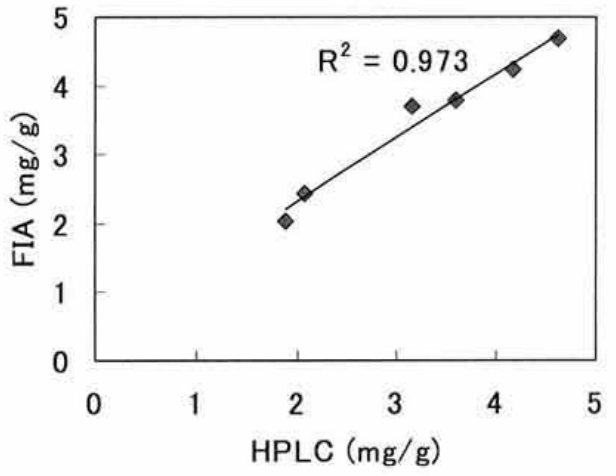

Fig. 6. Relationship of the GABA content in GABA-enriched teas between FIA and HPLC methods
GABA-enriched teas (1.0 g) were extracted with 100 mL of distilled water for 40 min. The concentrations of GABA in the filtrates were determined by FIA and HPLC methods.

Using this system, the sample could be injected every 4 min. The results of determinations by this method were compared with those by the authentic HPLC method (Fig. 6). The correlation was significant for the rapid determination of the GABA content of GABA-enriched teas at the tea manufacturing factories.

Conclusion

We developed flow injection determination systems for the major components of green tea. These methods significantly reduced the time required for the analysis compared with the authentic methods. Moreover, since the buffer solutions and the wastes are harmless, they can be safely used by untrained workers at the tea factories. These newly developed methods are suitable for the estimation of the quality of teas by tea manufacturers and traders.

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