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Review

Analysis of tea components by high-performance liquid chromatography and high-performance capillary electrophoresis

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Abstract

Tea is one of the most popular beverages in the world. The number of reports on the analysis of tea components, especially for catechins, has recently been increasing. We review the recent reports on the analysis of tea components using the analytical methods of high-performance liquid chromatography and high-performance capillary electrophoresis.

Keywords: Reviews; Tea; Food analysis; Polyphenols; Vitamins; Amino acids; Catechins; Caffeine; Alkaloids; Inorganic cations; Organic acids

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1. Introduction

Tea is a very popular beverage and about 2.5 million tons of tea are produced in the world every year. The most popular type of tea in the world is black tea, which is produced in India, Sri Lanka, Kenya and many other countries. Most of the tea produced in Japan is green tea, while China produces both green and black teas and several other types of teas, such as Oolong tea and Pu-er tea.

The various methods for the analysis of tea

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Table 1
HPLC analysis of tea components

Analyte	Column, length×I.D. (mm)	Temperature (°C)	Mobile phase 1	Mobile phase 2	Detection	Internal standard	Ref.
Amino acids, s-methylmethionine	MCI gel CK-10S 100×4.6	40	Lithium citrate step-wise		Post-column 394 nm		[71]
Amino acids (OPA)	TSK gel-120A ODS-SIL 250×4.6	40	3.5 m*M* Citrate buffer (pH 6.0), 12% ethanol	3.5 m*M* Citrate buffer, 50% ethanol	FL Ex 340, Em 455 nm	Norvaline	[18]
Amino acids (OPA)	Develosil ODS-HG-5 150×4.6		95% Citrate buffer (5 m*M*, pH 6.0), 5% acetonitrile	30% Citrate buffer (5 m*M*, pH 6.0) 70% acetonitrile	FL Ex 340, Em 450 nm	Fly-Gly	[19]
Amino acids (PTC), caffeine	Wakopack WS-PTC 200×4.0	40	6% Acetonitrile 94% acetate buffer (pH 6.0)	60% Acetonitrile, 40% acetate buffer (60 m*M*)	254 nm	Norvaline	[20]
D-Theanine, L-theanine (FMOC-Gly)	Cyclobond II-200 γ-CD 250×4.6		Acetonitrile, methanol, triethylamine, acetic acid		FL Ex 266, Em315 nm		[21]
EGCg, ECg, EGC, EC, C	Ultron N-C_{18} 150×4.6	43	0.1% acetonitrile 5% *N,N*-Dimethylformamide 0.1% phosphoric acid waste	Acetonitrile	280 nm		[72]
Catechins	Hypersil ODS 3 μm 250×4.0		5% Acetic acid	Methanol	Post-column derivatization		[30]
EGCg, ECg, EGC, EC, GA	TSKgel-120A ODS-SIL 250×4.6	40	11.3% Acetonitrile, 0.5% acetic acid, 20% methanol, 86.2% water		280 nm	Homocatechol	[18]
EGCg, ECg, EGC, EC, caffeine	Wakosil 5C_{18} 250×4.0		20% Methanol, 75% water, 5% acetic acid		280 nm	Tryptophan	[73]
EGCg, ECg, EGC, EC, C, TB, caffeine	Tosoh ODS-80Ts 250×4.6	45	20 m*M* Phosphate buffer (pH 3.0)	Acetonitrile	207 nm		[74]
EGCg, ECg, EGC, EC, C, TF	Hypersil 3 μm ODS 100×4.6		0.5% Acetic acid	30% Acetonitrile, 0.5% acetic acid, 69.5% water	280 nm (450 nm TF)		[75]
EGCg, ECg, EGC, EC, C, GC, GCg, G	Capcell pak C_{18} 100×4.6		0.1 m*M* EDTA, 100 m*M* phosphate, 10% Acetonitrile (pH 2.5)		550 mV ED		[26]
EGCg, ECg, EGC, EC, GC, C, GCg, Cg, caffeine	Develosil ODS-HG 150×4.6	40	94.5% Water, 4.5% acetonitrile, 0.05% phosphoric acid	49.95% Water, 50.00% acetonitrile, 0.05% phosphoric acid	231 nm		[25]
EGCg, ECg, EGC, EC, caffeine	LiChrosorb RP-18 250×4.0		20 m*M* Phosphate buffer (pH 3.0)	Acetonitrile	280 nm		[76]
EGCg, ECg, EGC, EC, caffeine	TSKgel ODS-80Ts 250×4.6	40	0.1% Phosphoric acid	40% Acetonitrile 0.1% phosphoric acid 59.5 water	230 nm	Catechol	[77]

EGCg, ECg, EGC, EC, caffeine	Capcell pak C_{18} UG120 250×4.6	40	6% Acetonitrile, 11% Methanol, 0.5% phosphoric acid, 82.5% water		270 nm		[78]
EGCg, ECg, EGC, EC, caffeine	Hypersil ODS 5 μm 250×4.6		12% Acetonitrile, 2% ethyl acetate, 0.04% sulfuric acid		280 nm	(+)-Catechin	[23]
EGCg, ECg, EGC, EC, C, caffeine	Wako Pack HG C_{18} 250×4.6		22% Methanol, 78% water, 0.1% phosphoric acid		280 nm		[79]
EGCg, ECg, EGC, EC, caffeine	Altima C_{18} 250×4.6	Ambient	Acetate buffer (1 m*M*, pH 4.5)	Acetonitrile	210 nm	Naringenine	[32]
EGCg, ECg, EGC, EC, GCg, C	Zorbax Eclipse XDB-C_{18} 250×4.6		0.05% TFA	60% Methanol, 40% acetonitrile 0.05% TFA	210 nm	Naringenine	[27]
EGCg, ECg, EGC, EC, caffeine, TP, TB, GCg, Cg	Cosmosil C_{18}-MS 250×4.6		20% Methanol, 0.3% formic acid, 79.7% water	99.7% Methanol, 0.3% water	280 nm		[28]
EGCg, ECg, EGC, EC, minor catechins	Nucleosil 100-5 C_{18} 250×4.6		0.05 *M* Phosphoric acid	60% Water, 40% acetonitrile, 0.05 *M* phosphoric acid	280 nm		[29]
EGCg, ECg, EGC, EC	Waters C_{18} 250×2.0		30% Methanol, 0.05% TFA		$[M+H]^+$ ions MS–MS, CID		[33]
EGCg, ECg, EGC, EC, C, caffeine, TB, theanine			0.1 *M* Ammonium acetate, 10% methanol		$[M+H]^+$ ions thermospray		[34]
EGCg, ECg, EGC, EC	Zolbax Eclipse C_{18} 300×0.256		5 m*M* Ammonium acetate 0.05% TFA	5 m*M* Ammonium acetate, 0.05% TFA 40% acetonitrile, 60% methanol	$[M+H]^+$ ions electrospray		[35]
TF	TSKgel-120A ODS-SIL 250×4.6		77.7% Water, 22% acetone 0.3% phosphoric acid		375 nm	Purpurogalline	[18]
TF, flavonol glycoside	Hypersil 3 μm ODS 100×4.6		0.5% Acetic acid	0.5% Acetic acid, 30% Acetonitrile	370–550 nm		[44]
TF	Nucleosil C_{18} 250×4.6		74% Acetic acid (5%) 26% Acetonitrile		278 nm		[80]
TF, flavonol glycoside	Hypersil 5 μm ODS 250×4.9		1% Citric acid (pH 2.8)	Acetonitrile	280–460 nm		[81]
TF	Hypersil 5 μm ODS 250×4.6		1% Acetic acid	Acetonitrile	375 nm		[82]
TF	Spheriosorb ODS2 250×4.6		25% Acetonitrile, 1% acetic acid, 74% water		375 nm		[83]
Caffeine, TB, ECg, EGCg, chrologenic acid	Hypersil 5 μm ODS 250×4.6		2% Acetic acid	Acetonitrile	280, 380, 460, 510 nm		[43]

Table 1. Continued

Analyte	Column, length×I.D. (mm)	Temperature (°C)	Mobile phase 1	Mobile phase 2	Detection	Internal standard	Ref.
TF, TR	Hypersil 3 μm ODS 100×4.6		0.5% Acetic acid	0.5% Acetic acid 30% Acetonitrile	450 nm		[84]
Flavonol glycoside	Prodigy 5 μm ODS3 250×4.6		98% Water, 2% THF 0.1% TFA	acetonitrile	200–450 nm		[85]
Caffeine	TSKgel–120A 250×4.6		74% Methanol, 1% acetic acid		272 nm		[49]
Caffeine	Nova-Pak C_{18} 100×5		15% Acetonitrile, 85% water		254 nm		[50]
Caffeine, TB, TP	FLC-ODS 50×4.6		7% Acetonitrile, 93% water		280 nm		[86]
Caffeine, TB, TP	LiChrosorb RP-8 150×4.0	45	9% Methanol, 36% water, 5% phosphate buffer (pH 5.0, 0.2 *M*)		275 nm		[46]
Caffeine, TB	LiChrospher 100 RP-18 250×4.6		5 m*M* Octylamine (pH 6.4)		274 nm		[52]
Caffeine	μm Bondapak C_{18} 150×3.9		20% Acetonitrile, 80% water		276 nm		[50]
Caffeine, TB, TP	Two HPIC-CS3 Ion Chromatography		100 m*M* HCl		274 nm		[53]
Caffeine, TB	Capcell Pak C_{18} UG120A 250×4.6		82.5% Water, 11% acetonitrile, 6% methanol, 0.5% phosphoric acid		272 nm		[51]
Tocopherols	Finpack-SIL 250×4.6		99.5% Hexane, 0.5% isopropanol		280 nm	6-Hydroxy-2,2,7,8-pentaethyl chroman	[55]
Tocopherols	LiChrosorb NH_2 250×4.0	20	97.5% Hexane, 2% dioxane, 0.5% ethanol		FL Ex 296, Em 323 nm		[56]
Ascorbic acid	Intersil ODS-2 150×4.6	20	5 m*M* Tetra-*n*-amylammonium bromide, 0.03 *M* Na_2EDTA, 5% methanol, 95% KH_2PO_4 (0.01 *M*)		EC 500 mV		[56]
Ascorbic acid	TSKgel-120A ODS-SIL 250×4.6		1% Metaphosphoric acid		242 nm		[18]
Chrolophyl, carotenes	Zorbax ODS 150×4.6	40	80% Acetonitrile, 10 methanol, 10% dichloromethane		450, 440 nm		[56]
Chlorophylls, carotenes, phaeophorbides	Hypersil μm ODS 250×4.6		90% Acetonitrile, 10% water	Ethylacetate	450 nm		[57]

Chlorophylls, pheophorbides	TSKgel ODS-80 150×4.6		80% Ethanol containing 5 m*M* sodium chloride	95% Ethanol containing 5 m*M* sodium chloride	405, 425 nm		[58]
Free sugars	Shodex Ionpak S-801 500×8	60	Water		RI	Xylitol	[18]
Free sugars	YMC-Pack-PA3 250×4.6	35	75% Acetonitrile		RI		[87]
Free sugars	Polyspher CH PB 300×7.8	80	Water		RI		[76]
Riboflavin	ODS 120A 250×4.6	40	88.5% Water, 11% acetonitrile, 0.5% acetic acid		FL Ex 360, Em 500 nm		[60]
Oxalic acid	C_{18} 150×4.6		0.02 *M* Ammonium acetate		Postcolumn chemiluminescence		[67]

Table 2
HPCE analysis of tea components

Analyte	Method	Capillary, length (cm) ×I.D. (μm)	Voltage (kV)	Temperature (°C)	Buffer	Detection (nm)	Internal standard	Ref.
EGCg, ECg, EGC, EC, C theanine, caffeine, ascorbic acid	CZE	77×50	30	23	20 m*M* Borax (pH 8.0)	200		[36]
EGCg, ECg, EGC, EC, C gallic acid, adenine	CZE	75×57	20	20	150 m*M* Borate (pH 8.5)	210		[39]
EGCg, ECg, EGC, EC, theanine, caffeine, ascorbic acid	MEKC	75×77	25	30	80 m*M* Boric acid, 50 m*M* SDS (pH 8.4)	194 270	*p*-Hydroxybenzoic acid	[37]
EGCg, ECg, EGC, EC, theanine, caffeine, ascorbic acid	MEKC	75×57	25	20	50 m*M* Borate, 10 m*M* phosphate, 50 m*M* SDS, 10% methanol (pH 8.2)	200 270		[38]
EGCg, ECg, EGC, EC, C, Cg, GCg caffeine, ascorbic acid	MECC	50×36	20	20	25 m*M* SDS, 25 m*M* phosphate, 50 m*M* Borate buffer (pH 7.0)	280		[40]
EGCg, ECg, EGC, EC, C, GCg caffeine	MEKC	50×85	30	21	20 m*M* Borate-phosphate, 25 m*M* SDS (pH 7.0)	200	*N*,*N*-Dimethylformamide	[88]
EGCg, ECg, EGC, EC, C, GCg	MEKC	50×67	20	20	20 m*M* Borax, 110 m*M* SDS, 14% methanol 1.5% urea, 1 m*M* β-CD (pH 8.0)	280	L-Tryptophan	[41]
EGCg, ECg, EGC, EC, caffeine, TB, chlorogenic acid	MEKC	50×64.5		25	20 m*M* SDS, 50 m*M* phosphate, 50 m*M* sodium tetraborate, 10% acetonitrile	278		[45]
TB, caffeine	MEKC	50×57	17		20 m*M* Borate, 45 m*M* SDS, 10% methanol (pH 8.25)	214		[54]
Sodium, calcium, magnesium, manganese	Indirect CZE	75×60	20	23	5 m*M* Imidazole, 6.5 m*M* HIBA, 20% methanol 0.53 m*M* 18-crown-6 (pH 4.5)	214		[61]
Ammonium, sodium, ethylamine	Indirect CZE	75×57	25	20	10 m*M* Imidasole 20 m*M* 18-crown 6-ether (pH 4.3)	214		[64]
Organic acids, glutamate	Indirect CZE	75×57	−20	20	10 m*M* Sodium chromate, 0.5 m*M* TTAB, 0.1 m*M* Na_2EDTA	254		[68]

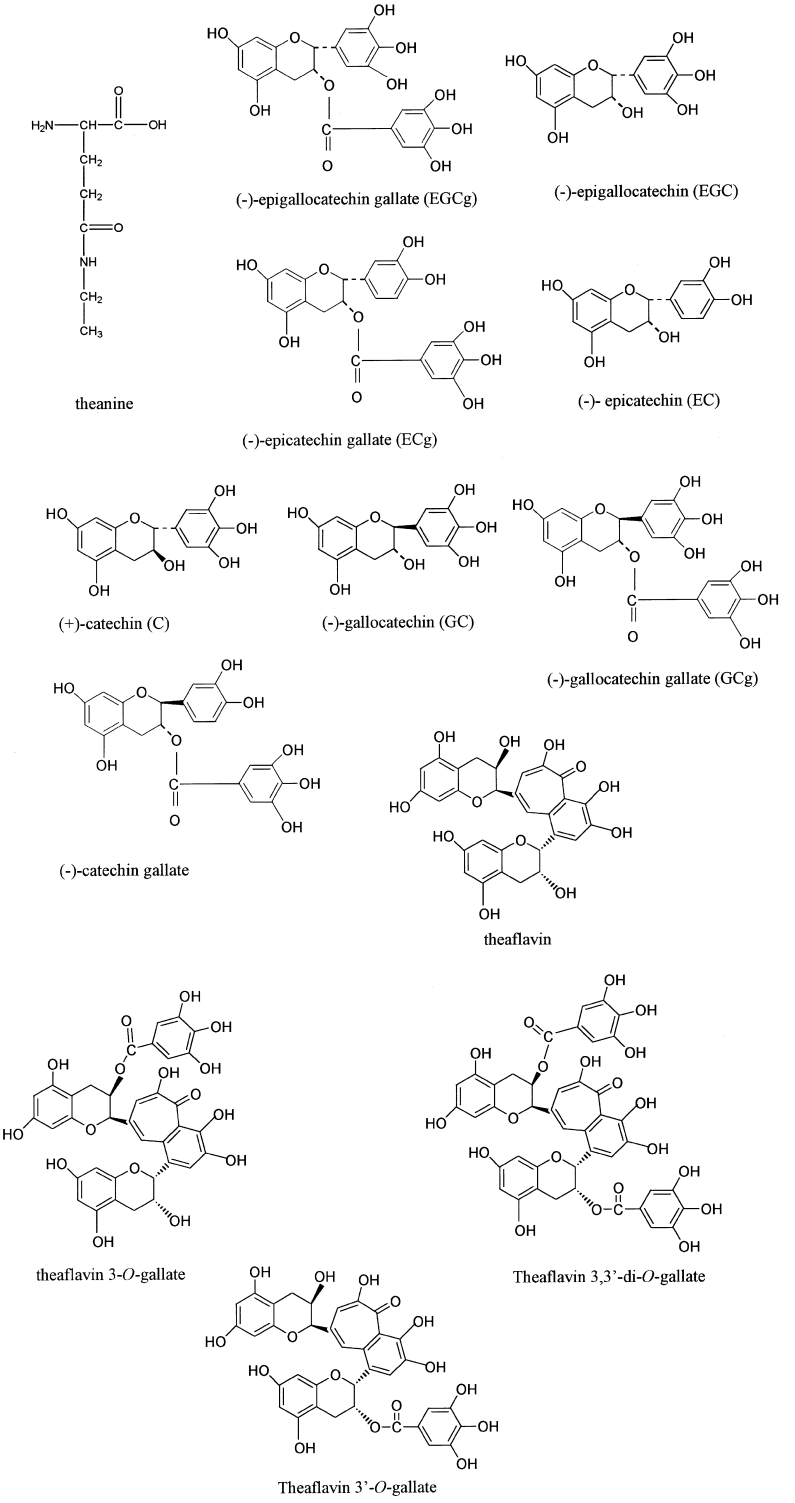

Fig. 1. Structures of tea components.

components were reviewed in this journal in 1992 [1]. One of the analytical methods that was not reviewed, but which is popular now, is near infrared spectroscopic (NIR) analysis [2–7]. The 'tea components analyzer' based on NIR has already sold more than 100 units in Japan. Since the review was published, studies on the positive effects of tea catechins on human health have become popular and now it is possible to get major tea polyphenols, (−)-epigallocatechin gallate (EGCg), (−)-epicatechin gallate (ECg) and (−)-epigallocatechins (EGC) etc., from commercial sources. The experiments using tea catechins have become easier for scientists. Also, new techniques like liquid chromatography LC–MS or high-performance capillary electrophoresis (HPCE) have been introduced into this field. In this review newly developed methods in high-performance liquid chromatography (HPLC) and HPCE for the analysis of tea components will be discussed, with emphasis on the important qualitative methods (Tables 1 and 2).

2. Amino acids

Except for aroma formation, amino acids are not very important in the quality of black tea [8]. Amino acids are the most important components in Japanese green tea and many studies have demonstrated the relationship between the quality and the content of amino acids in green tea [9–13]. Tea leaves contain theanine (γ-glutamylethylamide, Fig. 1), and higher grade Japanese green teas contain higher amounts of theanine and arginine.

Theanine has a delicate taste, similar to that of sodium glutamate, and many biological uses of this compound have been claimed. For instance, it has been reported that theanine decreases the level of norepinephrine and serotonin in the brain [14], and in naturally hypertensive rats, its administration results in decreased blood pressure [15]. Recently, cooperative effects of anti-tumor agents and theanine to cancer have been reported [16].

Another amino acid of interest contained in tea is γ-aminobutyric acid (GABA). Normal green tea contains less than 0.1% of this amino acid, while the anaerobic treatment of fresh leaves before the normal manufacturing process increases its content enormously. The continuous drinking of anaerobically treated tea has been shown to produce a decline in blood pressure [17], and such specially processed teas are sold in Japan to people suffering from hypertension.

In the review on the analytical methods of green tea by Ikegaya et al. [18], *o*-phthalaldehyde derivatives of amino acids were separated by reversed-phase HPLC (RP-HPLC). Goto et al. [19] improved the method for the rapid analysis of some major amino acids. They separated eight major amino acids, including theanine and GABA, within 25 min using a gradient of acetonitrile.

Ohta et al. [20] developed a HPLC method for a more rapid analysis of amino acids. They separated phenylthiocarbamoyl (PTC)-derivatized amino acids with a specially prepared column, which were then detected by a UV detector. They could analyze 14 amino acids and caffeine simultaneously within 16 min; however, in their method the sample preparation and derivatization were not simple.

Ekborg-Ott et al. [21] measured the enantiomeric composition of theanine in tea using HPLC. Derivatized theanine, using 9-fluoromethoxycarbonylglycine chloride, was separated from other amino acids using a C_{18} column and was introduced to a γ-cyclodextrin (CD) column for enantiomeric separation using the column-switching technique. They found that the average relative level of D-theanine in various teas was 1.85% and that theanine racemizes slowly in aqueous solution.

3. Polyphenols

3.1. Catechins

In fresh tea leaves and green teas, catechins are the major polyphenols and are mainly composed of (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECg) and (−)-epigallocatechin gallate (EGCg). Recently, these compounds have attracted the attention of the general public due to their physiological, antioxidative and antihypertensive properties, together with their ability to slow down tooth decay [22]. Tea catechins are becoming popular as additives to food.

Catechins are not stable in alkaline conditions and

heating increases their degradation [23]. They cannot be extracted completely by infusion with hot water, hence they are commonly extracted from powdered tea leaves using aqueous organic solvents [24,25]. They are easily absorbed on membrane filters [25], hence care is needed during sample preparation.

In the analysis of these catechins using HPLC, caffeine is simultaneously determined in most cases, and minor catechins, like catechin gallate and gallocatechin gallate, were simultaneously determined in some cases [25–28]. Saijo and Takeda [29] also analyzed major catechins, catechin gallate, gallocatechin gallate, gallocatechin, epigallocatechin-3-(3″-*O*-methyl) gallate and other catechins in an acetone extract from tea leaves.

In the HPLC study of catechins, many detection methods have been tried. Treutter [30] used a post-column reaction with 4-methylaminocinnamaldehyde and detection at an absorbance of 640 nm. Umegaki et al. [26] detected catechins using an electrochemical detector with an applied potential of +550 mV. The sensitivity of this method was 1000 times superior to the UV detection at 280 nm. Nakagawa and Miyazawa [31] detected picomole levels of EGCg in human plasma using a chemiluminescent reaction. They measured the chemiluminescence intensity of EGCg in the presence of acetaldehyde and hydrogen peroxide without interference in biological fluid. Other studies mostly used a conventional UV detector of varying wavelengths. Bronner and Beecher [32] mentioned the use of 210 nm to improve the signal-to-noise ratio, while Goto et al. used 231 nm to reduce the interference from other compounds [25].

In most cases, separation was made using a C_{18} column, and in case of gradient separation, the concentration of acetonitrile or methanol was gradually increased. Dalluge et al. [27] compared a variety of stationary phases and elution conditions, and observed that the stationary phases which utilized ultrapure silica and maximized coverage of the silica support improved the separation and that the presence of acid in the mobile phase was essential. According to their results, the separation using a methanol-based mobile phase was poor.

LC–MS has been applied to the analysis of catechins and other polyphenols. Lin et al. [33] applied LC–MS–MS for the characterization of the structures of catechins. They prepared catechin mixtures from a tea extract by precipitation with $CaCO_3$. The $[M+H]^+$ ions of the major catechins could be obtained by thermospray LC–MS, and the fragment patterns of them were observed by collision-induced dissociation (CID) spectra. Kiehne and Engelhardt [34] used thermospray HPLC–MS for the analysis of catechins, flavonol *O*-glycosides and flavone *C*-glycosides. They observed the $[M+H]^+$ ions of catechins from green tea brew without further treatment. From flavonol *O*-glycoside, the molecular ion of the aglycone $[A+H]^+$ was observed, because the glycoside bond is labile. Dalluge et al. [35] developed a method to determine six catechins using capillary liquid chromatography–electrospray ionization mass spectrometry (cLC–ESI–MS). In this microscale technique, 20 ng of catechins could be detected as pseudo-molecular ions.

Recently HPCE has been applied to the analysis of tea catechins. Horie et al. [36] separated major catechins simultaneously with caffeine, theanine (as the major amino acids are an indicator of green tea quality) and ascorbic acid (indicator for the freshness of green tea), using capillary zone electrophoresis (CZE) or micellar electrokinetic chromatography (MEKC) [37,38]. The separation of catechins is dependent on their complex formation with the borate which is a component of the electrolyte. Arce et al. [39] have developed a system that combined the flow injection (FI) system and capillary electrophoresis. The FI system was used for the extraction and the pretreatment of the teas, and the samples that were prepared automatically were analyzed by CZE. For a better separation between caffeine and EOF, MEKC was more effective. Watanabe et al. [40] separated catechins, caffeine and ascorbic acid in less than 10 min using MEKC. Nelson et al. [41] used β-cyclodextrin as a modifier to separate EGCg and its isomer GCg. The use of HPCE significantly reduced the time for the analysis of catechins compared with HPLC, while some minor catechins and theaflavins have not been separated by HPCE.

3.2. Other polyphenols

Theaflavins are reddish-colored pigments formed from the oxidative condensation of catechins. These compounds are found in black teas but not in green

teas. The stability of theaflavins was a problem when measuring them overnight using an autosampler, as more than 1 h was needed for the analysis. Temple and Clifford [42] declared that theaflavins are stable enough for overnight analysis, even for the decaffeinated tea extracts.

Thearubigins are red–brown colored components, and their structures have not been fully declared. Thearubigins, theaflavins and flavonol glycosides can simultaneously be measured using HPLC with a linear gradient of acetonitrile [43]. When strongly infused tea is cooled, an orange– brown precipitation appears and this precipitation is called 'tea cream'. Using HPLC, Powell et al. [44] analyzed the tea solution after the insoluble complexes were formed and they concluded that thearubigins are the principal components of tea cream.

Lager et al. [45] applied HPCE to the analysis of tea polyphenols, using MEKC with diode array detection. They separated some organic acids and flavonol glycosides together with catechins, caffeine and theobromine; however, they could not observe peaks of the theaflavins or thearubigins. MEKC is complementary to RP-HPLC for the separation of polyphenols.

4. Purine alkaloids

The stimulating effect of tea is due to the presence of purine alkaloids. Tea leaves contain caffeine and lesser amounts of theobromine. Theophyllin contents are negligible in tea. These alkaloids have been analyzed using RP-HPLC.

These alkaloids can be analyzed simultaneously with catechins or other polyphenols, but these methods are time consuming. For rapid analysis, samples must be pretreated to reduce the interference from other compounds. Terada and Sakabe [46] and Naik and Nagalakshmi [47] pretreated tea samples with Sep-Pak C_{18} to eliminate interference.

Batch treatment with polyvinylpolypyrrolidone (PVPP), which selectively absorbs polyphenols [48], was used by Ikegaya et al. [49] to eliminate tea polyphenols. Kuwano and Mitamura [50] combined Sep-Pak C_{18} and a PVPP-column to shorten the analysis time to within 2 min.

Nakakuki et al. [51] used a pre-column packed with PVPP for the on-line reduction of interference from polyphenols. This column was connected upstream to a conventional RP-HPLC column. The PVPP pre-column removed catechins and theaflavins perfectly, and it was possible to analyze the amount of caffeine and theobromine in the tea brew without pretreatment.

Gennaro and Abrigo [52] developed a reversed-phase ion-interaction HPLC method. Octylamine was added to the mobile phase to interact with caffeine and theobromine. Although it took more than 1 h for the analysis, there was no need for any pretreatment.

Chen et al. [53] developed a method to separate caffeine, theobromine and theophylline using ion chromatography. They revealed that these alkaloids in tea, coffee or cocoa could be analyzed using either a cation- or anion-exchange method without any pretreatment.

Vogt and Conradi [54] developed a HPCE method to determine purine alkaloids using MEKC and measured them in tea and cocoa. The preparation of tea samples was simple, while proteins had to be removed from the cocoa samples before the analysis.

5. Vitamins and photo-pigments

Green tea contains ascorbic acid and tocopherols but black tea does not because of differences in the manufacturing process. Tocopherols do not extract into tea brew. These days, eating green tea leaves is becoming popular due to the perceived health-benefits and as such the contents of tocopherols have become nutritionally meaningful. Ikegaya et al. [55] measured α-, β-, γ-, δ-tocopherols in green tea using HPLC. In their report, tea samples were pretreated with saponification. The samples dissolved in hexane were introduced to HPLC with a silica gel column. Kitada et al. [56] also measured tocopherols in green tea by HPLC using a NH_2 column. They did not saponify, but samples were treated with Sep-Pak Florisil to clean up.

Kitada et al. [56] also measured ascorbic acid by HPLC using a reversed-phase column and an electrochemical detector. Ikegaya et al. [18] used the RP-HPLC method to determine ascorbic acid by using metaphosphoric acid as the mobile phase.

α-, β-Carotenes and chlorophylls in green teas

were also determined by Kitada et al. [56] using RP-HPLC. Taylor and McDowell [57] analyzed chlorophylls and chlorophyllides, carotenes and other pigments of fresh tea leaves simultaneously using gradient RP-HPLC. The peaks were classified by the absorption spectra obtained by diode-array detector. Pheophorbide a is formed after the degradation of chlorophyll and excessive intake of it could cause dermatitis to human skin. Kohata et al. [58] developed a RP-HPLC method to determine chlorophylls and related compounds (including pheophorbide a) in teas, and they showed that the levels of pheophobide a were sufficiently low in green teas. Since the green color is very important for Japanese green teas, they also applied this method to evaluate the quality of the teas [59].

The riboflavin content of green tea was determined by Anan et al. [60] using RP-HPLC with fluorescent detection. Pretreatment using taka-diastase was necessary for complete extraction from tea samples.

6. Cations and anions

Yang et al. [61] developed a method to determine ammonium, alkali and alkaline earth ions by capillary electrophoresis. They used the electrolyte system composed of imidasole, 2-hydroxyisobutylic acid (HIBA), 18-crown-6 and methanol and detected indirectly at 214 nm. 18-Crown-6 and HIBA were added to moderate the mobility of the K^+ and alkaline earth metal cations, respectively. They applied this method to tea infusions, but the ammonium ions could not be separated well due to the interference from the high concentration of potassium ions in the infusion.

Some green teas are treated with ammonium hydrogencarbonate to enhance the green color [62] or have monosodium glutamate added to improve the taste [63]. Analyzing ammonium and sodium ions is effective in the detection of such sham teas. A HPCE method was developed by Horie et al. [64] in which the ammonium and sodium ions in tea infusions could be separated even in the presence of high concentrations of potassium ions by increasing the concentration of 18-crown-6.

Tea plants accumulate aluminum, which could possibly show toxicity to the human body if it is absorbed across the gastrointestinal tract. As the bioavailability of elements depends on their chemical species, the speciation of aluminum in tea infusions is important. Flaten and Lund [65] developed a post-column reaction HPLC method for that purpose. In this system Al–organic species were separated by size-exclusion chromatography and detected by the reaction with pyrocatechol violet. The results showed that the sizes of the Al–organic species were below 6500. The same group also tried to determine the speciation of various metals in tea infusion using inductively coupled plasma-MS combined with HPLC [66].

Too much intake of oxalate could cause kidney stones. Wu et al. [67] developed a HPLC method to detect oxalate with a chemiluminescent reaction. Oxalic acid in tea extracts was separated by a C_{18} column, reacted with $Ru(phen)_3^{2+}$ and then monitored by chemiluminescence. A white precipitate was observed when the green tea was infused with hard (calcium rich) water. Horie et al. [68] developed a HPCE method to analyze oxalic acid in tea infusion and found that the precipitation is calcium oxalate. Simultaneous measurement of oxalic, malic, citric, quinic, aspartic and glutamic acids using HPCE with indirect detection was carried out [69]. EDTA was added to the electrolyte to reduce the effect of divalent or trivalent cations in tea.

Ding et al. [70] used anion-exchange chromatography and measured acetic acid, ascorbic acid, succinic acid, malic acid, citric acid, tartalic acid, phosphoric acid, chloride ions and sulfuric ions simultaneously in tea without special pretreatment. The eluent used was the mixture of potassium hydrogenphtalate and phthalic acid, which showed higher sensitivity and resolution than phthalic acid alone, which is most widely used.

7. Conclusion

Progress in HPLC and HPCE methods has made it easier to analyze low-molecular-weight compounds in tea. It is possible to determine the variations of the chemical components at various points of the teas, place of production, taste, quality, character of the cultivars, manufacturing styles and cultivation methods.

It is still not possible to characterize tea components of high-molecular-weights, such as thearubigins, pectins and proteins. Since these compounds possibly relate to taste, color and the bioavailability of metals, the development of analytical methods to determine the compounds still remains a challenge.

In the review of Finger et al. [1], the need for interdisplinary research on tea polyphenols for human health was described. To promote this research, analytical methods for such a purpose, which show higher sensitivity and selectivity, have to be developed. Methods of detecting polyphenol metabolites from a small amount of serum or tissues are required in order to investigate the bioavailability study of tea polyphenols.

We did not mention tea aroma here. Aroma compounds are usually analyzed by gas chromatography or gas chromatography–MS, while HPLC or HPCE analysis of aroma precursors and their metabolites could be another interesting study.

8. Nomenclature

C	catechin
CD	cyclodextrin
Cg	catechin gallate
CZE	capillary zone electrophoresis
EC	(−)-epicatechin
ED	electrochemical detection
ECg	(−)-epicatechin gallate
EDTA	ethylenediaminetetraacetic acid
EGC	(−)-epigallocatechin
EGCg	(−)-epigallocatechin gallate
Em	emission
EOF	electro-osmotic flow
Ex	excitation
FL	fluorescence
FMOC-Gly	9-fluorenylmethoxycarbonylglycine
G	gallic acid
GC	gallocatechin
GCg	gallocatechin gallate
Gly–Gly	glycylglycine
HIBA	2-hydroxyisobutyric acid
HPCE	high-performance capillary electrophoresis
HPLC	high-performance liquid chromatography
LC–MS	liquid chromatography - mass spectroscopy
MEKC	micellar electrokinetic chromatography
Na_2EDTA	ethylenediaminetetraacetic acid, disodium salt
NIR	near infrared (spectroscopy)
OPA	*o*-phthalaldehyde
Phen	Phenanthroline
PTC	phenylthiocarbamoyl
PVPP	polyvinylpolypyrrolidone
RI	refractive index
RP-HPLC	reversed-phase HPLC
SDS	sodium dodecyl sulfate
TB	theobromine
TF	theaflavins
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TP	theophylline
TR	thearubigins
TTAB	tetradecyltrimethylammonium bromide
UV	ultraviolet

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