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Method for determining the content of catechins in tea infusions by high-performance liquid chromatography

W.E. Bronner*, G.R. Beecher

Food Composition Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service, US Department of Agriculture, Beltsville, MD 20705, USA

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Abstract

A high-performance liquid chromatography method employing diode array detection was developed to determine levels of the major catechins present in black, green, and Jasmine tea infusions. Reversed-phase separations were performed on a C_{18} column using three gradients: acetonitrile–acetate buffer, methanol–acetate buffer, and acetonitrile–acetate buffer with ascorbic acid. The identities of the tea catechins were established by comparing absorbance spectra and retention times to reference standards chromatographed under identical conditions. Epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate were found in all the tea infusions examined, ranging in concentration from 1–13 mg dl^{-1}. These levels indicate that even moderate tea consumption can contribute a substantial quantity of flavanols to the diet. Although some differences between the three brewed teas were evident, all were comparably good sources of these catechins. 1998 Published by Elsevier Science B.V.

Keywords: Tea; Food analysis; Catechins; Flavonoids; Polyphenols

1. Introduction

In addition to being the most widely consumed beverage worldwide, brewed tea is the only food product known to contain significant levels of the catechin flavonoids. The tea leaves used in the brewing process, themselves, have been found to contain large quantities of these polyphenols which may comprise as much as 30% of the dry mass of fresh or mildly processed commercial tea [1–4]. Comparable concentrations have not been reported in any other foodstuffs [3]. As a result, brewed tea is perhaps the only major dietary source for this potentially important group of compounds.

Recent findings indicate that flavonoids, in general, and catechins, in particular, possess rather potent antioxidant properties which may result in a number of beneficial health effects. (See, for example, [5–8] and Refs. therein.) Moreover, catechins may be one of the few groups of flavonoid compounds to also possess a significant degree of bioavailability. In contrast to quercetin, for example, which was not detected in plasma following a large oral dose [9], several researchers have reported small but measurable quantities in human plasma following ingestion [2,5,10,11].

The present paper reports an HPLC method designed to identify and quantitate low-molecular-mass catechins present in brewed teas. (For a general survey of chromatographic techniques applicable to

*Corresponding author.

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catechins and other tea constituents, interested readers are referred to the 1992 review article by Finger et al. [12].) However, unlike the more commonly used HPLC procedures which utilize low pH aqueous methanol or acetonitrile mobile phases to effect separation (dimethylformamide, propanone and tetrahydrofuran have also been used), the method described here using a weak acetate buffer in aqueous acetonitrile permits absorbance measurements at or below 210 nm. In addition, small quantities of ascorbic acid added to the mobile phase were found effective in improving peak shapes. Catechin levels measured using this new procedure are reported for three different tea infusions.

2. Experimental[1]

2.1. Materials and instrumentation

Lipton brand (Thomas J. Lipton, Englewood Cliffs, NJ, USA) black tea (mixture of orange Pekoe and Pekoe cut black tea) packaged in tea bags (2.2 grams of tea per bag) was purchased at a local grocery store. Jasmine tea produced in Fuanchachang Province, China, was obtained as loose boxed tea. Loose leaves of Dragon Well green tea were purchased in Taiwan.

Epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG) purchased from Indofine (Belle Meade, NJ, USA) were stored at −20°C. Epicatechin (EC) and naringenin (NGN) were purchased from Aldrich (Milwaukee, WI, USA), and Anotop 0.2 μm porosity syringe filters (25 mm diameter) from Alltech (Deerfield, IL, USA).

The HPLC system consisted of a Perkin-Elmer Series 4 LC pump (Norwalk, CT, USA), a 25 cm× 4.6 mm I.D. Alltima analytical column (Alltech) packed with 5 μm C_{18} modified silica, a 3 cm×4.6 mm I.D. Brownlee ODS-GU guard column (Rainin, Woburn, MA, USA) packed with 5 μm C_{18} modified silica, a 20 μl injection loop, and a Hewlett-Packard 1040M series II diode-array detector.

2.2. Chromatograhic conditions

All separations were conducted at ambient temperature by reversed-phase HPLC on a 25 cm C_{18} column using gradient elution. Quantitative levels of tea catechins were determined using an acetonitrile–aqueous acetate buffer (1.0 m*M* acetic acid, 1.0 m*M* sodium acetate in water, pH 4.5) mobile phase programmed linearly from 12–21% (0–18 min), then 21–65% (18–40 min) acetonitrile at a flow rate of 0.7 ml min^{-1}. For purposes of identification, qualitative data (spectra and retention times) were also collected using this same gradient. A second mobile phase of methanol–aqueous acetate buffer (1.0 m*M* acetic acid, 1.0 m*M* sodium acetate in water) programmed linearly from 30–50% methanol (0–40 min) at a flow rate of 0.5 ml min^{-1} was used to confirm the identity of the catechins found. In addition, an acetonitrile–aqueous ascorbate buffer solution (1.0 m*M* acetic acid, 1.0 m*M* sodium acetate, 0.10 m*M* ascorbic acid in water) programmed linearly from 15–19% (0–16 min), then 19–31% (16–40 min) acetonitrile at a flow rate of 0.7 ml min^{-1} was used as the mobile phase in determining analyte losses occurring during sample preparation.

UV–Vis absorbance spectra (λ=210–400 nm) were collected continuously during the course of each chromatogram. Peak areas were measured by integrating absorbance–time plots obtained at a wavelength of 210 nm.

2.3. Standard solutions

Stock solutions of epicatechin (0.98 mg ml^{-1}), epicatechin gallate (0.75 mg ml^{-1}), epigallocatechin (0.72 mg ml^{-1}), epigallocatechin gallate (0.76 mg ml^{-1}), and naringenin (1.4 mg ml^{-1}) were prepared by dissolving weighed quantities of commercial standards into methanol. Less concentrated solutions were prepared, as needed, by dilution with water. Methanolic solutions of the catechins were used within two days of preparation and stored at −20°C when not in use. (None of these solutions exhibited

[1] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the US Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

any measurable deterioration under these storage conditions.) A 0.7 mg ml^{-1} stock standard of caffeine in methanol was used to identify caffeine in the tea infusions.

Quantitative standards of EC, ECG, EGC, and EGCG were used to construct calibration plots for determining catechin levels in tea samples. Each catechin was prepared at four different concentrations (relative to naringenin) in aqueous methanol, and analyzed in duplicate. Levels were chosen to bracket those found in the tea infusions.

2.4. Tea preparation

A moderate to strong brew of black tea was prepared following the instructions provided on the package by pouring 237 ml (8 fluid oz.) boiling water into a glass flask and dipping a tea bag for 3 min.

Jasmine and green teas were prepared by pouring 80 ml boiling water over 0.25 g tea leaves (equivalent to 1/4 teaspoon Jasmine tea). At this point, a relatively mild brew was prepared by stirring for 3 min. A second, stronger brew was prepared by allowing the tea to steep for an additional 17 min without stirring.

After cooling, 100–200 μl of internal standard (naringenin in methanol) were added to 6.0 ml of black, green, or Jasmine tea infusion followed by mixing and filtration. (Note: Addition of internal standard can be omitted if fixed volumes of sample are injected.)

2.5. Measurement of analyte losses

Analyte losses (limited in this study to the amount of material lost by filtration) were determined by comparing catechin levels in twice-filtered tea infusions with those levels following a single filtration step. Experimentally, this involved analyzing two sets of filtrates: (1) brewed tea filtered once, and (2) combined equal portions of filtered and nonfiltered tea passed through a second filter. A total of eight HPLC runs (two batches each of once- and twice-filtered tea analyzed in duplicate) were collected for each of the three kinds of tea analyzed.

3. Results and discussion

3.1. Analyte losses

Analyte losses were calculated according to the procedure outlined in the Appendix. The experimental findings indicate losses of 4% or less occurred for epicatechin, epigallocatechin, epigallocatechin gallate and naringenin when filtering black, green, or Jasmine teas. Somewhat larger filtration losses (7%) were observed for epicatechin gallate in green and Jasmine teas. The overall percent losses measured were:

	EGC	EC	EGCG	ECG	NGN
Black tea	−3	0	3	4	−1
Green tea	−5	−1	0	7	4
Jasmine tea	−3	0	1	7	−5

Each of these values is based upon the average of eight data points, i.e. four ratios (see Section 2.5), with positive numbers reflecting loss of analyte and negative numbers an increase. (Reported increases are, obviously, experimental artifacts.) EGC losses were consistently negative in every case, but it is unclear whether this is of any particular significance.

3.2. Identification

Epigallocatechin, epicatechin, epigallocatechin gallate, and epicatechin gallate (Fig. 1) were identified as major components in black, green, and Jasmine teas based on comparisons of tea infusion chromatograms (including retention time data and UV–Vis absorbance spectra) with those of commercial catechin standards. Both acetonitrile–acetate buffer and methanol–acetate buffer mobile phases (Section 2.2) were used to confirm the presence of these components in the teas. No impurities were detected with the peak purity software used to analyze the diode-array data: signal ratios (relative absorbances) were constant across each peak profile for the entire wavelength range examined.

A representative chromatogram of the tea filtrates is shown in Fig. 2 and the corresponding retention times and absorbance data are reported in Table 1. Use of methanol in the mobile phase (not shown)

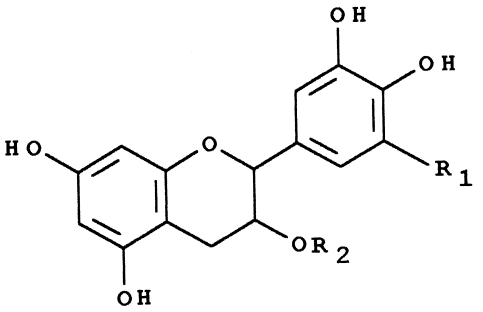

Fig. 1. Structures of four catechins in tea infusions. Epicatechin: R_1=H, R_2=H; epigallocatechin: R_1=OH, R_2=H; epicatechin gallate: R_1=H, R_2=3,4,5-trihydroxybenzoyl; epigallocatechin gallate: R_1=OH, R_2=3,4,5-trihydroxybenzoyl. Note: The absolute configuration of epicatechin is 2R,3R or 2S,3S; catechin is 2R,3S or 2S,3R [13–15].

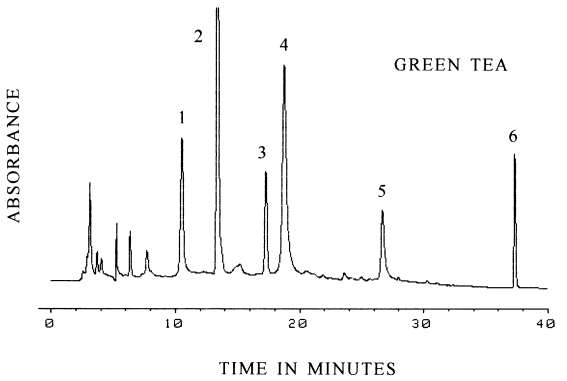

Fig. 2. Representative chromatogram of tea infusions. Conditions: C_{18} column; acetonitrile–aqueous acetate buffer gradient; absorbance at 210 nm. Peak identities: 1=EGC; 2=caffeine; 3=EC; 4=EGCG; 5=ECG; 6=internal standard (naringenin).

Table 1
Retention time and absorbance data

Mobile phase	Retention time (min)			
	EGC	EC	EGCG	ECG
Acetonitrile–acetate	10.4	17.0	18.4	26.3
Acetonitrile–ascorbate	8.7	15.3	17.4	28.4
Methanol–acetate	10.5	18.2	15.0	24.1
λ_{max}	271	279	275	279
$A_{210}/A_{\lambda max}$	32	14	8	6

Retention times are average of three runs.
λ_{max}=local absorbance maximum (in nm) in acetonitrile–acetate buffer and methanol–acetate buffer.
$A_{210}/A_{\lambda max}$=ratio of absorbances at 210 nm and λ_{max} in acetonitrile–acetate buffer (average of three runs).

resulted in a somewhat different elution order (EGC, EGCG, EC, ECG) than with acetonitrile (EGC, EC, EGCG, ECG). Separations performed with ascorbic acid added to the mobile phase gave sharper peak shapes (EGCG exhibited the most dramatic change) than those with ascorbic acid absent. This improvement, however, was partially offset by the somewhat higher background absorbance of the mobile phase at shorter wavelengths.

Spectra of EGC, EC, EGCG, and ECG all exhibited maximum absorbance at the shortest wavelength examined (210 nm). However, local absorbance maxima were also observed for each catechin at slightly longer wavelengths, between 270 and 280 nm. (Interestingly, these local maxima occurred at identical wavelengths in both acetonitrile and methanol mobile phases.) As the absorbance ratios in Table 1 indicate, signal intensities at 210 nm in the acetonitrile–acetate buffer were always several times larger than at λ_{max}. Thus, chromatograms collected at this wavelength showed considerable improvement in signal-to-noise, even with ascorbic acid present in the mobile phase.

3.3. Quantitative results

Calibration plots of peak-area ratios (analyte/internal standard) vs. mass ratios (analyte/internal standard) were obtained for solutions of the pure compounds. Each set of calibration standards was fitted to a least squares linear plot, the results of which are presented in Table 2. All plots were found to be linear across the ranges studied.

Quantitative values for catechin levels in the tea infusions are listed in Table 3. (Literature values from earlier studies are also included for comparison purposes.) The results obtained indicate that epigallocatechin gallate was in highest concentration in all the tea infusions, while epicatechin was the lowest, although minor differences are also apparent: in black tea the order of concentrations was EGCG>ECG>EGC>EC, in green tea EGCG>ECG, EGC>EC, and in Jasmine tea EGCG>EGC>ECG>EC.

Infusions prepared from black tea reached higher catechin concentrations in 3 min than either the green or Jasmine teas brewed for the same length of time. This finding is somewhat unexpected, as the processes used in the manufacture of black tea are

Table 2
Statistical data for calibration plots

	EGC	EC	EGCG	ECG
Number of data points	8	8	8	8
Range[a]	0.52–4.2	0.14–1.8	1.1–8.8	0.54–4.3
Slope	1.2	1.7	1.2	0.71
Intercept	−0.18	−0.086	−0.14	−0.045
Standard error of estimate	0.22	0.043	0.34	0.32
Correlation coefficient	0.993	0.999	0.997	0.966

Results derived from linear regression analysis of analyte/internal standard chromatogram peak-area ratios (ordinates) plotted vs. analyte/internal standard mass ratios (abscissae). Absorbances measured at 210 nm.
[a] Ranges given are analyte/internal standard mass ratios.

known to decrease levels of the monomeric catechins to a much greater extent than the less severe conditions applied to other teas. Results from other research [22] suggest that this disparity is due simply to the finer size of the black tea leaves used in the brewing process. Not surprisingly, the data also showed that longer brewing times result in more concentrated infusions. This effect is consistent with, but more pronounced than that observed in another investigation involving green tea infusions [20].

4. Conclusions

In the present study, catechin levels in tea infusions were determined by a simple analytical procedure requiring minimal sample preparation. Separations using a weak acetate buffer permitted detection at shorter wavelengths, resulting in an increase in sensitivity. Improved peak shapes were obtained by addition of ascorbic acid to the mobile phase, an effect similar to that seen with citric acid in at least one other study [23]. (See also Refs. [24,25].)

The quantitative data reported in this study show that consumption of just 200 ml of brewed tea contributes approximately 20–70 mg of total catechins to the diet. Based on these results, even moderate tea consumption (2–3 cups per day) may provide significant amounts of these potentially beneficial compounds.

Table 3
Catechin levels in tea infusions, mg dl^{-1}

		EGC	EC	EGCG	ECG
Black tea	3 min	6	4	12	11
	Literature[a]	1–39	1–44	3–48	4–218
Green tea	3 min	2	1	6	3
	20 min	4	2	9	5
	Literature[b]	5–87	2–65	5–190	4–377
Jasmine tea	3 min	5	2	5	3
	20 min	7	3	8	5
	Literature[c]	1–27	1–12	2–47	2–34
	R.S.D. (%)[d]	5	4	12	7

Reported concentrations are averages of six measurements (two batches of tea, each analyzed in triplicate).
[a] Reflects range of data compiled from Refs. [16–18].
[b] Reflects range of data compiled from Refs. [16–21].
[c] Reflects range of data for oolong tea compiled from Refs. [16,18].
[d] R.S.D.=within-run (separate injections, same batch) relative standard deviations averaged for all three teas.

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Appendix

Analyte losses were calculated as follows: From the formulae for the concentration of analyte present following a single filtration, C_1=(initial concentration)(1−fraction lost per filtration), and after a second filtration, C_2=(initial concentration)[0.5(2−fraction lost per filtration)](1−fraction lost per filtration), it follows that the ratio of concentrations C_2/C_1 equals 0.5(2−fraction lost per filtration). Consequently, the fraction of analyte lost per filtration step is $2(1-A_2/A_1)$, where A_2 and A_1 are the experimentally determined chromatogram peak areas corresponding to the second and first filtration steps, respectively.

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