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Short communication

Decrease in concentration of free catechins in tea over time determined by micellar electrokinetic chromatography

Dirk Stach, Oliver J. Schmitz*

Division of Molecular Toxicology, German Cancer Research Center, Heidelberg, Germany

Abstract

Cancer chemoprevention is a new and important medical science and much interest has been focused on catechins, not only for their antioxidant activity, but also because of their known antimutagenic and antitumorigenic properties. Green tea and black tea, which are among the most popular beverages consumed worldwide, contain many different catechins. Due to the instability of catechins in solutions with neutral or basic pH values the concentrations of catechins in tea decrease in time. In this presentation we used micellar electrokinetic chromatography to determine the real concentration of catechins between 0 and 60 min after the tea was brewed.

Keywords: Tea; Food analysis; Catechins; Phenolic compounds

1. Introduction

Recently several studies led to acceptance of green tea as an useful cancer preventive agent [1–3]. The main components of tea are polyphenolic compounds, commonly known as catechins, which represent a group of compounds belonging to the flavonoid family. Catechins are strong antioxidants, what might explain their protective effects. Other important features of epigallocatechin gallate (EGCG) and green tea as cancer preventive agents are their non-toxic nature for rodents and humans, their wide-range of target organs and their inhibitory effects on the growth of cancer cells and lung metastasis of B16 melanoma cells [4]. In addition, it was found that green tea consumption reduces the formation of polycyclic aromatic hydrocarbon-induced DNA adducts [5]. Moreover, the inhibition of human CYP1A gene expression by green tea components could be proved. CYP1A enzymes mediate the bioactivation of carcinogens [6].

Green tea and black tea are originally derived from the same plant, *Camellia sinensis*, and the six major tea catechins (Fig. 1) are (+)-catechin (C), (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-catechin gallate (CG), (−)-epigallocatechin gallate (EGCG) and (−)-epicatechin gallate (ECG). Tea is the most popular beverage consumed worldwide and contributed e.g. for 55% of the catechin intake in a representative study of the Dutch population [7]. Methods to determine catechins in tea have already been developed [8–10] such as HPLC [11–16], HPLC–MS [17] and capillary zone electrophoresis (CZE) [18,19] as well as micellar electrokinetic chromatography (MEKC) mainly under neutral conditions [20]. The resolution of the catechins was

*Corresponding author. Present address: Department of Analytical Chemistry, Bergische Universität-Gesamthochschule Wuppertal, Gauss-Strasse 20, 42119 Wuppertal, Germany. Tel.: +49-202-439-2492; fax: +49-202-439-3915.

E-mail address: olivers@uni-wuppertal.de (O.J. Schmitz).

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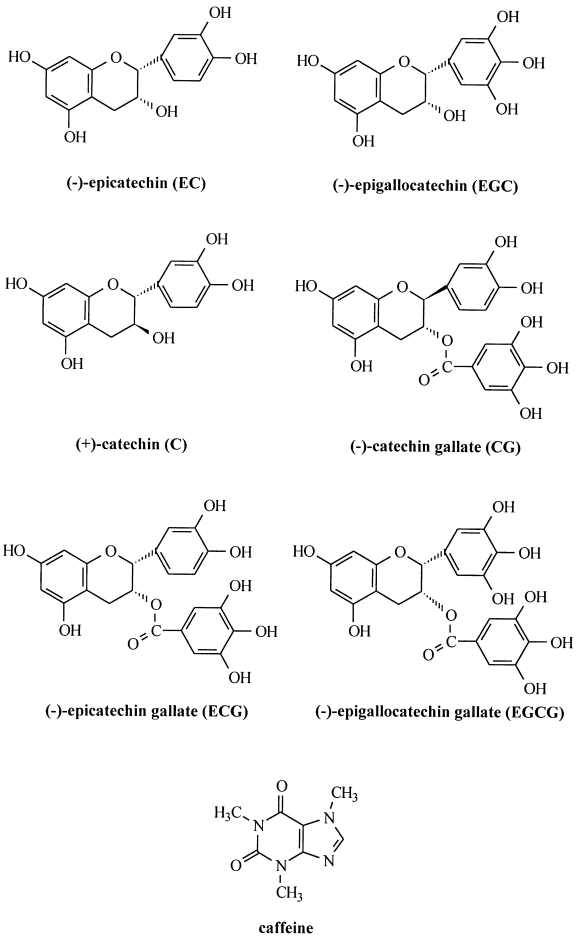

Fig. 1. Structures of the analyzed catechins and caffeine.

much better with MEKC than with CZE and, however, acidic conditions during the CE-analysis improve the stability of these compounds [21,22] and lead to higher efficiencies [23]. Here, a fast separation of six catechins and caffeine in a green Darjeeling tea by micellar electrokinetic chromatography under acidic conditions was used to determine the content of catechins and caffeine in dependency on time after brewing.

2. Experimental

2.1. Reagents

Six catechin standards (C, EC, EG, CG, EGCG and ECG) were purchased from Sigma (Deisenhofen, Germany). Caffeine and 4-amino-2-hydroxybenzoic acid (AHBA) were purchased from Aldrich (Steinheim, Germany). All other chemicals used were of highest purity. The analyzed green Darjeeling tea was purchased from a tea store in Heidelberg.

2.2. Instrumental

The instrument used for the analysis was a BioFocus 3000 TC with a fast-scanning UV detector from Bio-Rad (Munich, Germany). The separations were carried out at 25°C on an untreated fused-silica capillary [50 cm (effective length 45.4 cm)×50 μm I.D.] purchased from CS-Chromatography-Service (Langerwehe, Germany); the field strength was 400 V/cm. The capillary was conditioned by rinsing with 1 *M* NaOH (15 min), 1 *M* HCl (15 min) and electrolyte (15 min). Before each run the capillary was rinsed with 1 *M* HCl (1.5 min), bidistilled water (0.5 min) and finally for 2 min with electrolyte. The samples were injected hydrodynamically (10 p.s.i. s; 1 p.s.i.=6894.76 Pa) and the capillary outlet was the anode in all runs. The separations were achieved with an electrolyte consisting of 100 m*M* sodium dodecylsulfate (SDS) in a solution of 90% (v/v) sodium phosphate buffer (20 m*M*, pH 2.7) and 10% (v/v) methanol as organic modifier.

2.3. Sample preparation

The content of individual catechins and caffeine were analyzed in a green Darjeeling tea after different times. The tea was prepared by pouring 400 ml 90°C hot water over 1.4 g tea leaves and drawing for 3 min with stirring. After 3 min the tea water was 70°C warm. After 0, 15, 30, 45 and 60 min at this temperature 1 ml of the tea sample were filtered through 0.45 μm Millipore filters and 3×150 μl of this filtrate were diluted each with 50 μl 80 m*M* HCl and 20 μl AHBA (500 mg/l). A fronting of the caffeine peak was observed, which led to a lower reproduciblity of the peak area at high concentrations. Therefore, the determination of caffeine was achieved by dilution of the tea sample.

2.4. Construction of calibration curves for catechins and caffeine

The linear range for the six catechins and caffeine

was investigated by four or five calibration mixtures between 5 and 52 mg/l (C, EGC, caffeine) and 5 and 65 mg/l (CG, EGCG, ECG, EC). The concentration of the internal standard (AHBA) was 45.5 mg/l in all samples. These mixtures were analyzed by MEKC with UV detection at 200 nm ($n=5$). The resulting peaks are time and peak area corrected with the internal standard to reduce the problem of nonreproducible injection volumes and migration times. The data were then subjected to linear least-squares analysis. The correlation coefficient obtained was between 0.9996 and 0.9998. The detection limit of all analytes was between 2 and 3 mg/l, which is suitable for the direct analysis of the main polyphenols and caffeine in tea.

3. Results and discussion

Optimized rinsing steps between each run and the change of electrolytes after each run are helpful to improve the reproducibility of the analysis. The use of an internal standard is indispensible for a quantitative analysis and corrects the migration shift of the analytes during long-time measurements. In this work we analyzed a mixture of six catechins- and caffeine-standards as well as tea samples after different time of storage and used AHBA as an internal standard. Due to the high reproducibility of the migration times achieved by previous work [23], tea compounds can easily be identified without any spiking. The six catechins and caffeine were separated by MEKC under acidic conditions. The optimized electrolyte consisted of 90% (v/v) sodium phosphate (20 m*M*, pH 2.7), 10% (v/v) methanol and 100 m*M* SDS. Tea samples were diluted with 80 m*M* HCl and an acidic electrolyte was used due to the unstability of catechins above pH 7. For quantification of the analytes in tea samples the corrected peak areas were compared with the calibration curves yielded from four or five standard solutions with different concentrations of catechins and caffeine and always the same concentration of AHBA (45.5 mg/l). Fig. 2 shows the separation of a standard solution in which the concentration of each catechin and caffeine is 26.0 mg/l.

The use of an internal standard led to excellent reproduciblity; this method is thus suitable for determining small changes in the catechin content. In

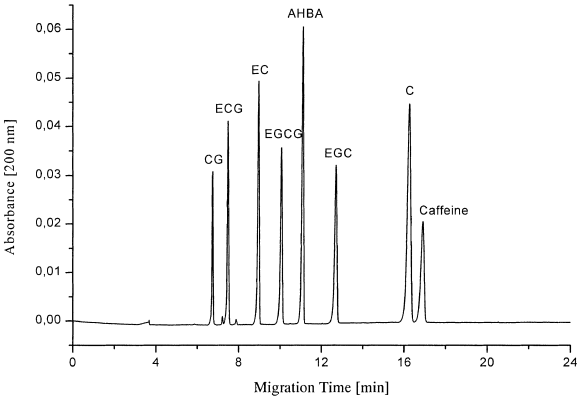

Fig. 2. Electropherogram showing the separation of a standard solution containing six catechins and caffeine (each 26.0 mg/l) and AHBA as an internal standard (45.5 mg/l). Experimental conditions: Buffer solution: 90% (v/v) 20 m*M* sodium phosphate, pH=2.7, 10% (v/v) methanol, 100 m*M* SDS; field strength: 400 V/cm, outlet/anode; fused-silica capillary: 50 cm (effective length 45.4 cm)×50 μm I.D.; wavelength: 200 nm.

epidemiological studies, for example, the intake of catechins has normally been determined by tea consumption, but not all cups of tea were drunk immediately. Because catechins are unstable in basic or neutral solutions, a decrease of the concentrations with time was expected. Fig. 3 shows the analysis of a tea sample 45 min after brewing. The determination of catechins and caffeine in green tea 0, 15, 30, 45 and 60 min after brewing showed a significant decrease in the concentration of EGCG and EGC (14 and 21%, respectively; Fig. 4). Thus, the storage of

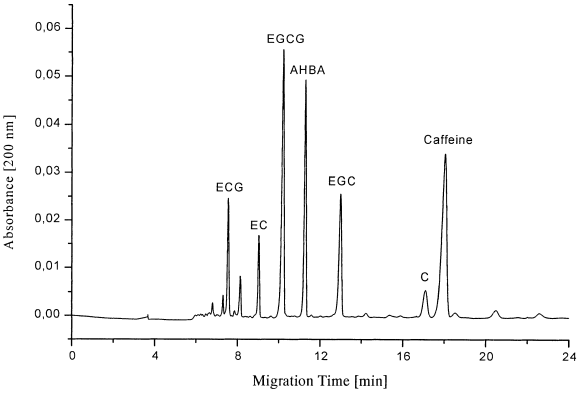

Fig. 3. Electropherogram showing the analysis of a tea sample 45 min after brewing. Separation conditions were as in Fig. 2.

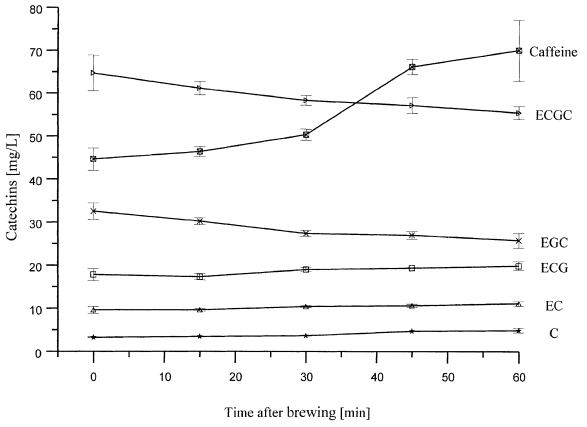

Fig. 4. Determination of the content of catechins and caffeine in dependency on time after brewing.

tea in a thermos bottle led to a reduction of the antioxidant- and cancer-preventing activities of tea. There is no significant change in the concentration of ECG and EC, but the amount of C and caffeine increased with time (53 and 48%, respectively, in 60 min). Because of the low concentration of C in the tea samples, this increase is not important. The reason for the increase of caffeine after 30 min may be the degradation of complexes of caffeine with polyphenols, which slowly releases caffeine. This could be the reason for the different effects of the same amount of caffeine in tea and in coffee. The caffeine in coffee is not complexed by polyphenols, and the stimulation occurs quickly after consumption. In tea the effect comes on more slowly, but lasts for a longer time. To verify this hypothesis, further experiments are necessary.

4. Conclusion

It was shown, that MEKC is a powerful method to determine the concentration of different catechins in tea extracts with high reproducibility. The instability of catechins under neutral and basic conditions allows a separation with high efficiencies of all catechins only with acidic buffer systems.

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