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Analytical Methods

Estimation of tea catechin levels using micellar electrokinetic chromatography: A quantitative approach

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ABSTRACT

A simple, inexpensive micellar electrokinetic chromatography (MEKC) method with UV detection was used to determine seven catechins and one xanthine (caffeine) in tea. All the compounds were successfully separated (15 kV) within a 15-min migration period with a high number of theoretical plates ($>8.0 \times 10^4$) in a running buffer (pH 7) containing 10 mmol l^{-1} sodium tetraborate, 4 mmol l^{-1} sodium phosphate, and 25 mmol l^{-1} SDS. The regression lines of all standard catechins were linear within the range of 0.03–4 μg ml^{-1}. Green tea infused at 95 °C for 10 min showed higher levels of catechins (especially epigallocatechin galate, epicatechin gallate, and epicatechin) than tea infused at 80 °C. In addition, major differences were observed in the levels of catechins in the first and second infusions (both brewed at 95 °C for 10 min). Finally, green tea leaves were infused separately with tap water, deionised water, spring water, reverse osmosis water, and distilled water at 95 °C, and the catechin content of the infusions was investigated by the proposed method. In the infusion brewed with tap water, catechins appeared to be epimerisation from the epistructure to the nonepistructure. This epimerisation may take place more readily in tap water than in distilled water owing to the complexity of the ions present in tap water.

1. Introduction

Tea is consumed throughout the world and it is one of the most popular non-alcoholic beverages. Green tea consumption may contribute to a reduction in cardiovascular disease and some forms of cancer (Connors, Chornokur, & Kumar, 2012; Di Domenico, Foppoli, Coccia, & Perluigi, 2012; Hessien, El-Gendy, Donia, & Abou Sikkena, 2012). The health benefits of green tea have been attributed to phenolic compounds, and it has been shown that phenolic compounds demonstrate strong antioxidant potential and may also possess anticarcinogenic and antiviral properties (Calland et al., 2012; Daglia, 2012; Di Domenico et al., 2012; Manikandan et al., 2012; Roh, 2012). The major constituents of tea polyphenols are catechins with a flavan-3-ol structure, and their polymerised products. The principal naturally-occurring catechins in tea leaves are epigallocatechin galate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), epicatechin (EC), gallocatechin (GC) and catechin (C). During the green tea manufacturing processes, some of the catechins undergo isomerisation at the C-2 position of flavan-3-ol, and EGCG, EGC, ECG and EC are turned to gallocatechin gallate (GCG), GC, catechin gallate (CG) and C, respectively. In black tea, most of the catehcins are oxidised and polymerised by enzymes derived from tea leaves during the fermentation processes. Thus, green tea contains a higher total catechin contents than black tea.

Reported methods for the analysis of tea infusions are based on reversed phase high performance liquid chromatography (HPLC) with UV and mass spectrometric detection (MS) following an extraction procedure and filtration (Dou, Lee, Tzen, & Lee, 2008; Dou, Lee, Tzen, & Lee, 2007; Lee, Lee, Tzen, & Lee, 2010; Lee et al., 2008; Li et al., 2012; Scoparo et al., 2012; Wang et al., 2012). More recently, capillary electrophoresis (CE) has been used (Arribas, Martinez-Fernandez, & Chicharro, 2012; Rabanes, Guidote, & Quirino, 2012). It has the advantages of speed, simple sample preparation, efficiency of separation, low column costs, and reduced sample/solvent consumption. Stable neutral and ionic groups in catechins make it possible for these molecules to be separated by CE (Ballus, Meinhart, de Oliveira, & Godoy, 2012; Xiao & Kai, 2012). Several methods have been described for catechin separation and quantification using micellar electrokinetic chromatography (MEKC) (Aucamp, Hara, & Apostolides, 2000; Bonoli, Colabufalo, Pelillo, Toschi, & Lercker, 2003; Hsiao, Chen, & Cheng, 2010; Huang, Huang, Liang, & Lee, 2007; Huang & Lien, 2005; Lopez, Vilarino, Rodriguez, & Losada, 2011; Peres, Tonin, Tavares, & Rodriguez-Amaya, 2011; Pomponio, Gotti, Luppi, & Cavrini, 2003a; Pomponio, Gotti, Santagati, & Cavrini, 2003b; Stach &

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Schmitz, 2001; Weiss, Austria, Anderton, Hompesch, & Jander, 2006). For example, in one report using MEKC to quantify seven tea catechins, all the compounds were separated within 8 min using a running buffer (pH 7) containing 10 mmol l^{-1} phosphate, 4 mmol l^{-1} sodium tetraborate, 45 mmol l^{-1} sodium dodecyl sulfate (SDS), and 0.5% ethanol (Hsiao et al., 2010). However, high concentration of SDS may lead to joule heating problems. Recently, a sulfated-β-cyclodextrin-modified reduced flow MEKC method was developed and validated for the analysis of catechins in green tea. The optimal electrolyte consisted of 0.2% triethylamine, 50 mmol l^{-1} SDS, and 0.8% sulfated-β-cyclodextrin (pH 2.9) and offered baseline separation of five catechins in 4 min (Peres et al., 2011). Although the separation performance was improved by adding sulfated-β-cyclodextrin, the amount of sulfated-β-cyclodextrin and SDS needed to be optimised because of complicated interactions between the catechins and the enhanced electrolyte.

Herein, we present a simple, inexpensive MEKC method with UV detection offering improved separation and quantification, and enabling detailed chemical characterisation of tea infusions. The effects of brewing temperature and duration, and the number of repeated brewing cycles on green tea catechin levels, were investigated using the proposed method. In addition, because a cup of tea is generally brewed with tap water, it was important to understand the fate of catechins in a tap water base. However, all the results from the analysis of tea catechins reported in the literature were obtained using purified water or a buffer solution based on purified water. As a result, little attention has been given to tracing the changes that occur when brewing tea with tap water. Therefore, we also report the characterisation of catechins in green tea infusions made with tap water, deionised water (D.I. water), sprint water, reverse osmosis water (R.O. water), and distilled water at 95 °C over a 10-min period.

2. Chemicals and methods

2.1. Chemicals

Several commercial tealeaf brands from different cities in Taiwan were analysed including Jin-Xuan tea (Taiwanese No. 12 at Mountain Ali of Chia-Yi County), green tea (Pi-Lo-Chun at Sanxia District of New Taipei City), black tea (Taiwanese No. 8 at Yuchih Township of Nantou County), Tie Kuan Yin (Shimen District of New Taipei City), and Oolong tea (Lugu Township of Nantou County). Caffeine, (+)-catechin (C), (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), (−)-epigallocatechin gallate (EGCG), (−)-gallocatechin (GC), (−)-gallocatechin gallate (GCG), dimethyl sulfoxide (DMSO), SDS, N,N-dimethylformanide (DMF), sodium tetraborate, sodium phosphate, and sodium hydroxide were purchased from Sigma–Aldrich (Milwaukee, WI, USA). All chemicals were of analytical grade. Stock solutions of the different phytochemicals (1000 μg ml^{-1}) were prepared in DMSO and stored carefully under refrigeration to avoid decomposition. The required dilutions to form the desired final concentrations were made using ultrapure water (Millipore, MA, USA). All buffer solutions were prepared in ultrapure water and then passed through a 0.45-μm filter.

2.2. Apparatus

A high-voltage power supply (Gamma High Voltage Research, Ormond Beach, FL, USA) was used to drive electrophoresis. For safety reasons, the high voltage end of the separation system was housed in a plexiglass box. The CE system was coupled to a variable wavelength UV detector (SAPPHIRE 600, ECOM, Praha, Czech Republic). Data acquisition and processing were accomplished using a PC equipped with a Peak-ABC Chromatography Data Handling System (Shanghai Qianpu Software Company Ltd., Shanghai, China).

2.3. Capillary conditioning

Bare fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with a 75-μm I.D. and 365-μm O.D. were used for the phytochemical separations. Before use, new capillaries were flushed with 0.5 mol l^{-1} NaOH for 3 h, rinsed extensively with water, and finally conditioned with the separation buffer solution for 30 min. The capillary length was 45 cm, and the detection window was located 10 cm from the outlet side. When not in use, the capillaries were stored in water to prevent buffer crystallisation.

2.4. Tea sample extraction

The dried tea-leaves (1 g) were extracted in different brewing cycles (first, second, and third extractions) with 50 mL of solvent (tap water, D.I. water, spring water, R.O. water and distilled water) at different temperatures (80, 85, 90, and 95 °C) for different periods of time (3, 5, 7, and 10 min). The resulting infusions were filtered through a 0.45-μm filter then diluted 1:99 with D.I. water, and the diluted samples were directly injected into the electrophoretic system.

2.5. Determination of tea infusion by MEKC

The separation performance under different conditions (buffer composition, and pH value) was investigated. The running buffer consisted of a mixture of sodium tetraborate (2–10 mmol l^{-1}), and sodium phosphate (2–10 mmol l^{-1}) at different pH values (7, 8, and 9). Phosphoric acid (1 mol l^{-1}) was used as an acidifying agent. SDS was used as the micelle agent with 10 mmol l^{-1} borate/4 mmol l^{-1} phosphate at pH 7. The concentration of SDS was varied from 25 to 35 mmol l^{-1}. Each sample was injected at the elevated anode end (30 cm above the cathode) into the capillary using hydrodynamic injection over 10 s. DMF was used as a marker since it does not interact with the micelles. The potential applied for the separation was +15 kV, resulting in a current of 15 μA. UV detection was performed at 200 nm for the catechins and caffeine. Between runs, the capillary was rinsed consecutively with water and the running buffer.

2.6. Method validation

The performance of the proposed MEKC method was evaluated by calibration equation, correlation coefficient, detection and quantification limits. The linearity of the MEKC method was tested by plotting the calibration curve for each compound against concentration. Each point of the calibration graph corresponds to the mean value obtained from three independent height measurements. Satisfactory results, in terms of linearity, were obtained for all the compounds ($R > 0.99$). Detection (LOD) and quantification (LOQ) limits were calculated, using the calibration graphs, where LOD and LOQ were the signal-to-noise ratio of three-times and ten-times, respectively.

3. Results and discussions

3.1. Optimisation of the MEKC method

Because the catechins are polyhydroxylated species and can form a complex with borate, a borate buffer at pH 7 with a concentration ranging from 2 to 10 mmol l^{-1} was tested. With increasing