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Chiral cyclodextrin-modified micellar electrokinetic chromatography and chemometric techniques for green tea samples origin discrimination



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ABSTRACT

Catechins and methylxanthines were determined in 92 green tea (GT) samples originating from Japan and China by using micellar electrokinetic chromatography with the addition of (2-hydroxypropyl)- β -cyclodextrin. GT samples showed high concentrations of (–)-epigallocatechin gallate and caffeine, with (–)-epigallocatechin, (–)-epicatechin gallate and (–)-epicatechin in relevant content and (+)-catechin, (–)-catechin and theobromine in much lower amounts. The amount of all the considered compounds was higher for Chinese GTs, with the exception of (–)-epicatechin gallate. Pattern recognition methods were applied to discriminate GTs according to geographical origin, which is an important factor to determine quality and reputation of a commercial tea product. Data analysis was performed by principal component analysis and hierarchical cluster analysis as exploratory techniques. Linear discriminant analysis and quadratic discriminant analysis were utilized as discrimination techniques, obtaining a very good rate of correct classification and prediction.

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1. Introduction

Tea is made from the leaves of *Camellia sinensis* Theaceae and is one of the world's most consumed beverages because of its attractive aroma, taste and health effects. Many kinds of tea are produced, although they can be mainly classified into three different types: the nonfermented green and white teas, the partially fermented oolong and pauchong teas, and the fully fermented black and pu-erh (red) teas [1]. Green tea (GT) is the second most traditional style of tea product, representing 20–22% of world tea production, and is manufactured by steaming or drying fresh tea leaves and inactivating the enzymes to prevent the oxidation of the tea polyphenols [2]. GT polyphenolic antioxidants are mostly represented by catechins, a group of compounds having a flavan-3-olic structure, which are easily extracted into water. Catechins may constitute up to 30% of the dry mass of tea leaves and are the major constituents of GT excluding carbohydrates, proteins and

lignin [3].

GT has been extensively studied for its antioxidant activities and potential for reducing lifestyle-related diseases. The principal components of tea having biological effects have been identified as catechins and xanthines. Catechins show a strong antioxidant activity and exert antiinflammatory, antiarthritic, antiangiogenic, neuroprotective, anticancer, antiobesity, antiatherosclerotic, anti-diabetic, antibacterial, antiviral and antidental caries effects. Xanthines are responsible for the stimulating effects; caffeine (CF) is a central nervous system and cardiac stimulant and has a diuretic effect, while theobromine (TB), which is present in lower amounts, has also a diuretic effect [4–10]. Catechins are mainly responsible for the astringent and bitter taste of tea infusion, while CF can enhance observably tea flavor [11]. The composition of tea varies according to the species, season, climate, age of the leaf (plucking position) and cultivation and also to the technologies applied during extraction, concentration and preservation process [12]. The most abundant catechins in GT are (+)-catechin ((+)-C), (–)-catechin ((–)-C), (–)-epicatechin (EC), (–)-epigallocatechin (EGC), (–)-epicatechin gallate (ECG), (–)-epigallocatechin gallate (EGCG), whose structures, together with those of CF and TB, are reported in Supplementary Fig. S1.

Owing to the beneficial effects on the human health of

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catechins and to their role together with that of CF on the tea quality grade, there is an increasing interest in studying the levels of these compounds in different types of tea products. Moreover, the market value is increasingly associated to the geographical origin since the reputation of the country as well as of a specific cultivation area has become one of the attributes recognized by the consumers/sellers as an important factor to determine the price of a commercial product. As a consequence, one of the most important issues in tea quality evaluation is the origin authentication of the products, because false use of geographical origins damages the interests of both consumers and sellers. Traditionally, GT quality is assessed by skillful tasters based on appearance, flavor, and aroma; anyway, the results of this type of assessment may be imprecise or rather subjective. Thus, methods for the analysis and verification of origin of GT are still focused on at present, and it is necessary to have at disposal fast and accurate analytical methods that replace the sensorial evaluation during the routine analysis and furnish more reliable quality parameters.

Different analytical procedures have been used for classification of varieties or geographical origin of teas including GTs: HPLC with UV detector set between 275 and 280 nm [1,13–15], elemental analysis [16–18], UV–vis spectroscopy [19], Near-Infrared spectroscopy [11,20,21], electronic tongue [22], electronic nose [22–24], multispectral or hyperspectral imaging [25,26], HPLC-DAD-MS [27], UPLC-TOF MS [28], UPLC-DAD-MS [29].

In the field of classification according to chemical composition, having at disposal a rapid and accurate analytical method is essential. Capillary Electrophoresis (CE) can be suitable for this purpose, offering many advantages in analytical laboratories with respect to chromatographic techniques due to its low cost, small sample volume, high efficiency, high separation power, eco-friendly characteristics and possibility of performing chiral separations directly dissolving the chiral selector in the background electrolyte. In particular, micellar electrokinetic chromatography (MEKC) has been used for geographical classification of green teas in the producing areas in Zhejiang province [30] and from Henan and Hubei province [31]. Cyclodextrin modified MEKC (CD-MEKC) was employed to differentiate Yabukita green tea samples according to growing areas in Japan [32] and in combination with electronic nose analysis for the quality control of GT samples subjected to a long-term storage [24].

In this paper, the content of catechins and methylxanthines (shown in Supplementary Fig. S1) in GTs has been determined by a previously developed chiral MEKC method with the addition of hydroxypropyl- β -cyclodextrin (CD-MEKC) [33], with an analysis time of about 8 min. With respect to other previous MEKC methods for the analysis of similar mixtures [3,12,32–38], the proposed procedure showed a shorter analysis time and/or allowed the enantioseparation of some of the catechins because of the presence of the chiral selector as a component of the electrophoretic buffer. In Supplementary Table S1 a detailed comparison among previously developed HPLC and MEKC methods is reported. In particular, in Refs. [1,3,13,14,26,30,31] the analysis time was higher with respect to the present method, ranging from 16 to 50 minutes. The methods presented in Refs. [35,38] showed a comparable analysis time, but separated a lower number of compounds. In Refs. [12,34,37], good performances were obtained in terms of number of resolved compounds and analysis time, anyway the chiral separation was not obtained. Only the method by Kodama et al. [32] was able to obtain the chiral separation of (\pm)C, but the analysis time was 20 min and TB was not considered among the analytes. Thus, the CD-MEKC method here used presented good performances in selectivity, analysis time and possibility of chiral analysis, representing a very good compromise in terms of all these aspects.

The method was applied to the quantitation of catechins and

methylxanthines in 92 GT samples of different geographical origin (Japan and China), having undergone different storage conditions and manufacturing processes, reported in Supplementary Table S2. The GT samples were directly supplied by specialized stores. The study was aimed to evaluate the possibility of using catechins and methylxanthines content in GTs as phytomarkers of the geographical origin. As above reported, several chemometric studies have been conducted on tea, but in general they consider only small number of samples or limited classifications regarding tea categories made by different processes, or they refer to not only GTs samples but also black and oolong teas. The novelty of the present study was to apply a chemometric approach to a wide number of GT samples analyzed by CD-MEKC, with the aim of evaluating the existence of data patterns and the relationship between the geographical origin of tea and selected parameters. Unsupervised methods such as principal component analysis (PCA) and hierarchical cluster analysis (HCA) were used as exploratory techniques, while discrimination models were built using supervised methods such as linear discriminant analysis (LDA) and quadratic discriminant analysis (QDA) in order to obtain discrimination rules.

2. Materials and methods

2.1. Chemicals and reagents

The reference standards of (+)C, (–)C, EC, EGC, ECG, EGCG, CF, TB and syringic acid (SY) used as internal standard were from Sigma-Aldrich (St. Louis, MO, USA). Boric acid, 86.1% phosphoric acid, sodium dodecyl sulfate (SDS), (2-hydroxypropyl)- β -CD (HP β CD, degree of substitution 0.6), were purchased from Sigma-Aldrich. Commercial GT samples were collected locally in specialized stores located in the cities of Florence, Prato and Turin (Italy).

Ultrapure water used for the preparation of running buffers and tea infusions was purified by Elix and Simplicity 185 Systems (Millipore, Billerica, MA, USA).

2.2. CE analysis

The CD-MEKC method used for the determination of the compounds was basically derived from a previous study [33]. The analyses were carried out using a 3D CE instrument from Agilent Technologies (Waldbronn, Germany). The instrument was controlled and the data were collected by the software 3D CE ChemStation (Agilent Technologies). Fused-silica capillaries (Unifibre, Settimo Milanese, Italy) of 33.0 total length, 8.5 cm effective length and 50 μ m inner diameter were used. Voltage and temperature were set at 15 kV and 25 $^{\circ}$ C, respectively. The CE sampling was performed by short end hydrodynamic injection at 25 mbar for 2 s; the detection wavelength was at 200 nm. The background electrolyte was made by 25 mM borate–phosphate buffer pH 2.5 supplemented with 90 mM SDS and 25 mM HP β CD. Further information on the CE method may be found in the paper by Gotti et al. [33].

2.3. Solutions and preparation of tea samples

Standard stock solutions of catechins, methylxanthines and SY (1 mg mL $^{-1}$) were prepared in a mixture of methanol/water in 15:85 ratio %v/v. All prepared stock solutions were stored at -20 $^{\circ}$ C for one week. Working standard solutions were prepared daily by appropriate dilution with water in a vial to 500 μ L in order to obtain the desired final concentration values of the different compounds.

The tea samples were extracted according to the following

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