

## Determination of Tea Components with Antioxidant Activity

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Levels of essential elements with antioxidant activity, as well as catechins, gallic acid, and caffeine levels, in a total of 45 samples of different teas commercialized in Spain have been evaluated. Chromium, manganese, selenium, and zinc were determined in the samples mineralized with HNO<sub>3</sub> and V<sub>2</sub>O<sub>5</sub>, using ETAAS as the analytical technique. The reliability of the procedure was checked by analysis of a certified reference material. Large variations in the trace element composition of teas were observed. The levels ranged from 50.6 to 371.4 ng/g for Cr, from 76.1 to 987.6 μg/g for Mn, from 48.5 to 114.6 ng/g for Se, and from 56.3 to 78.6 ng/g for Zn. The four major catechins [(–)-epigallocatechin gallate (EGCG), (–)-epigallocatechin (EGC), (–)-epicatechin gallate (ECG), and (–)-epicatechin (EC)], gallic acid (GA), and caffeine were simultaneously determined by a simple and fast HPLC method using a photodiode array detector. In all analyzed samples, EGCG ranged from 1.4 to 103.5 mg/g, EGC from 3.9 to 45.3 mg/g, ECG from 0.2 to 45.6 mg/g, and EC ranged from 0.6 to 21.2 mg/g. These results indicated that green tea has a higher content of catechins than both oolong and fermented teas (red and black teas); the fermentation process during tea manufacturing reduces the levels of catechins significantly. Gallic acid content ranged from 0.039 to 6.7 mg/g; the fermentation process also elevated remarkably gallic acid levels in black teas (mean level of 3.9 ± 1.5 mg/g). The amount of caffeine in the analyzed samples ranged from 7.5 to 86.6 mg/g, and the lower values were detected in green and oolong teas. This study will be useful for the appraisal of trace elements and antioxidant components in various teas, and it will also be of interest for people who like drinking this beverage.

**KEYWORDS:** Tea; trace elements; catechins; gallic acid; antioxidants; caffeine

### INTRODUCTION

Tea, a leaf extract of the plant *Camellia sinensis*, is the second most consumed beverage in the world, with an estimated 18–20 billion cups consumed daily and, for instance, an estimated average consumption of 1 L/person/day in the United Kingdom (1). Depending on the manufacturing process, teas are classified into three major types: nonfermented green tea (produced by drying and steaming the fresh leaves and thus, no fermentation, i.e., oxidation, occurs); semifermented oolong tea (produced when the fresh leaves are subjected to a partial fermentation stage before drying); and fermented black and red (pu-erh) teas (which undergo a full fermentation stage before drying and steaming, although the fermentation of black tea is oxidation and that of pu-erh tea is attained using microorganisms) (2).

Originating from China, tea has gained the world's taste in the past 2000 years. Initially, it was consumed only by Chinese monks, but its use spread to other regions, such as Great Britain, which allowed its effective diffusion to Western countries. Nowadays, consumption of tea is part of people's daily routine, as an everyday drink and as a therapeutic aid in many illnesses.

Worldwide, 80% of the tea consumed is black tea, which is also the most popular drink in Europe, North America, and North Africa (except Morocco), whereas green tea is drunk throughout Asia; oolong tea is popular in China and Taiwan (3). Approximately 76–78% of the tea produced and consumed worldwide is black tea, 20–22% is green tea, and <2% is oolong tea (1, 2).

The chemical composition of tea is complex: polyphenols, alkaloids (caffeine, theophylline, and theobromine), amino acids, carbohydrates, proteins, chlorophyll, volatile compounds, fluoride, minerals and trace elements, and other undefined compounds. Among these, the polyphenols constitute the most interesting group of tea leaf components and exhibit potent antioxidant activity in vitro and in vivo (3). Tea has been considered a medicine and a healthful beverage since ancient times, but recently it has received a great deal of attention because tea polyphenols are strong antioxidants. Oxidative stress has been shown to be involved in the pathogenesis of numerous diseases, including cancer (4, 5). Moreover, some epidemiological studies have associated the consumption of tea with a lower risk of several types of cancers, including stomach, oral cavity, esophagus, and lung cancers (6–8). Tea appears, therefore, to be an effective chemopreventive agent for toxic chemicals and

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carcinogens (5). Numerous studies have also demonstrated that the aqueous extract of the major tea polyphenols possesses antimutagenic, antidiabetic, antibacterial, anti-inflammatory, and hypocholesterolemic qualities (4, 6–8). Beneficial effects in oral diseases including dental caries, periodontal disease, and tooth loss, which may significantly affect a person's overall health, have been also described (3). Among all tea polyphenols, especially catechins and gallic acid have been considered to be the main players in these beneficial effects on the human health. The major tea catechins are (–)-epigallocatechin gallate (EGCG), (–)-epigallocatechin (EGC), (–)-epicatechin gallate (ECG), and (–)-epicatechin (EC) (9). Tea also contains a certain amount of caffeine, and caffeine has attracted much scientific and public attention in recent years due to its stimulatory effects (2).

Moreover, tea contains trace elements that are essential to human health. Chromium, manganese, selenium, and zinc play an important role in human metabolism, and interest in these elements is increasing together with reports relating trace element status and oxidative diseases (10, 11). Chromium is involved in carbohydrate and lipid metabolism; the most frequent sign of Cr deficiency is altered glucose tolerance. This nutrient has been associated with diabetes and cardiovascular diseases (12). Beneficial effects of dietary Cr supplementation, particularly in groups in which deficiencies are frequent, have been reported (13). An adequate intake (AI) has been set on the basis of estimated mean intakes; the AIs are 35 and 25  $\mu\text{g}/\text{day}$  for young men and women, respectively (14).

Manganese deficiency can cause abnormalities in the metabolism of carbohydrates, glycosaminoglycans, and cholesterol (15). Mn is a constituent of three metalloenzymes (i.e., arginase, pyruvate carboxylase, and manganese-superoxide dismutase) and activates a large number of enzymes, such as glycosyl transferases involved in mucopolysaccharide synthesis. Plant-based foods, especially whole grain cereals, legumes, and tea, are the richest food sources of Mn (16). The U.S. AI was set based on median intakes reported from the Food and Drug Administration total diet study. The AIs for adult men and women are 2.3 and 1.8 mg/day, respectively. A tolerable upper intake level (UL) of 11 mg/day was set for adults on the basis of a nonobserved adverse effect level for Western diets (14).

Selenium functions through selenoproteins, several of which are oxidant defense enzymes; Se acts as enzymatic cofactor of glutathione peroxidase in the elimination of peroxide radicals from the organism (15). Epidemiological studies have shown the possible effects of Se in the prevention and regression of cancer (12). Most Se is ingested in food, but foods derived from vegetables has a variable Se content depending on the zone where they have been cultivated (11). The tolerable upper intake level (UL) for adults is set at 400  $\mu\text{g}/\text{day}$  on the basis of selenosis as the adverse effect (17).

Zinc enzymes participate in a wide variety of metabolic processes including carbohydrate, lipid, and protein synthesis or degradation. This element is required for deoxyribonucleic and ribonucleic acid synthesis; it may also play a role in stabilizing plasma membranes (12). Zinc has been recognized as a cofactor of the superoxide dismutase enzyme, which is involved in protection against oxidative processes (11, 12). The net delivery of Zn to an organism is a function of the total amount of this element in foods and its bioavailability. Recently, the median intakes from food in the United States were  $\sim 9$  mg/day for women and 14 mg/day for men. The UL for adults is 40 mg/day, a value based on reduction in erythrocyte copper–zinc superoxide dismutase activity (14).

The composition of catechins, gallic acid, caffeine, and trace elements in commercial teas varied depending on species, season, and horticultural conditions and, particularly, with degree of fermentation during the manufacturing process (18, 19).

In this paper, we determined the presence of tea components with antioxidant activity. Trace elements (Cr, Mn, Se, and Zn) have been determined by electrothermal atomic absorption spectrometry (ETAAS). The four major catechins (EGCG, EGC, ECG, and EC), gallic acid, and caffeine have been determined by high-performance liquid chromatography (HPLC). To compare teas of different sorts, grades, and producing areas, a total of 45 tea samples commercialized in Spain have been analyzed. The growing popularity of tea in recent years on the basis of its beneficial health effects requires additional data and periodic control. In addition, the present findings are of potential use to food composition tables.

## MATERIALS AND METHODS

**Apparatus.** Cr, Mn, Se, and Zn were determined using a Perkin-Elmer 1100B double-beam atomic absorption spectrometer equipped with deuterium-arc-background correction (Perkin-Elmer, Norwalk, CT) and a Perkin-Elmer HGA-700 graphite furnace atomizer. Pyrolytic graphite platforms (ref. B012-1092) and pyrolytically coated graphite tubes (ref. B013-5653) were obtained from Perkin-Elmer. Argon of 99.999% purity (Sociedad Española de Oxígeno, Barcelona, Spain) at 300 mL/min flow was used as the internal gas during all stages except atomization, when the flow was stopped. Hollow cathode lamps (Perkin-Elmer) were used. A Selecta digestion block (Selecta SA, Barcelona, Spain) and Pyrex tubes were used for sample mineralization. A Moulinex blender (Moulinex F-75008 Paris, France) was used to homogenize the samples.

A high-performance liquid chromatograph system equipped with a binary LC pump model 250 (Perkin-Elmer), a Rheodyne injector model 7125, and photodiode array detector model 235 (Perkin-Elmer) was used for simultaneous determination of catechins, gallic acid, and caffeine in tea samples. A Nelson 1020 integrator (Perkin-Elmer) was used. A Spherisorb column ( $\text{C}_{18}$  reversed-phase) S10 ODS2 from Phase Sep (Deeside Ind. Est., Clwyd, U.K.) was used. A filtration system (Millipore Co., Bedford, MA) with a 0.45  $\mu\text{m}$  filter disk was used to obtain the filtered solutions of the samples and solvent, previously injected in the chromatograph.

**Reagents.** Bidistilled deionized water obtained with a Milli-Q system (Millipore, Milford, MA) was exclusively used. Standard solutions of Cr, Mn, Se, and Zn ( $1.00 \pm 0.002$  g) (Tritisol, Merck, Darmstadt, Germany) were used and diluted as necessary to obtain working standards. High-quality concentrated nitric acid 65% and vanadium pentoxide (analytical reagent, Merck) were used for sample mineralization. Magnesium nitrate and nickel nitrate (reagent grade, Merck) were used as chemical modifiers. Ammonium molybdate (reagent grade, Merck) was used to precondition the furnace tubes. Gallic acid was from Merck; EGCG, EGC, ECG, EC, and caffeine were from Sigma Chemical Co. (St. Louis, MO). Methanol, glacial acetic acid, and hydrochloric acid (37% v/v) used for the mobile phase and the tea extraction were from Merck (HPLC grade reagent).

**Material.** To eliminate the risk of contamination, all glassware and polyethylene material were washed with tap water after each use, soaked in a 6 N  $\text{HNO}_3$  solution (at least overnight), and rinsed several times with bidistilled deionized water.

**Sampling.** A set of 45 tea samples, including fermented (black and red), semifermented (oolong), and nonfermented (green) teas from different geographical origins (i.e., China, Japan, Sri Lanka, India, and South Africa) were analyzed in triplicate. All samples were commercially available in Spain, and they were supplied by a specialized shop. All samples are sold as loose leaf tea (not tea bags). Preliminary assays established the appropriate amount of sample for analysis to ensure homogeneity between samples and to ensure they were representative (20, 21).

**Sample Treatment for Trace Element Determination.** A portion of 0.250 g of homogenized sample was treated with 5 mL of 65%

**Table 1.** Instrumental Conditions for Cr, Mn, Se, and Zn Determination in Tea by ETAAS

element	wave-length (nm)	slit width (nm)	ashing temp (°C)	atomization temp (°C)	matrix modifier
Cr	357.9	0.7	1650	2500	Mg(NO <sub>3</sub> ) <sub>2</sub>
Mn	279.5	0.2	1400	2200	Mg(NO <sub>3</sub> ) <sub>2</sub>
Se	196.0	2.0	1000	2200	Ni(NO <sub>3</sub> ) <sub>2</sub>
Zn	213.9	0.7	600	1800	Mg(NO <sub>3</sub> ) <sub>2</sub>

**Table 2.** Analytical Characteristics for Cr, Mn, Se, and Zn Determination in Tea by ETAAS

element	detection limit <sup>a</sup> (pg)	sensitivity <sup>b</sup> (pg)	accuracy <sup>c</sup> (%)	precision <sup>d</sup> (RSD %)	slope ratio blank/addition <sup>e</sup>
Cr	1.0	3.0	97.95 ± 3.0	5.0–5.2	0.990–1.100
Mn	2.0	2.5	97.90 ± 1.0	4.5–4.8	0.998–1.000
Se	10.0	30.0	97.80 ± 2.0	5.5–6.0	0.997–0.999
Zn	0.5	0.8	98.70 ± 1.0	3.5–4.0	0.990–0.999

<sup>a</sup> Calculated according to IUPAC rules and corresponding to 3 times the SD of the blank ( $n = 10$ ). <sup>b</sup> Expressed as characteristic mass in pg/0.0044 A·s. <sup>c</sup> Results from recovery assays of five randomly chosen samples. <sup>d</sup> Relative standard deviation for 10 replicate determinations in each of five samples. <sup>e</sup> Application of the standard additions method in five randomly chosen samples.

HNO<sub>3</sub> and a few micrograms of V<sub>2</sub>O<sub>5</sub> (as a catalyst) in Pyrex tubes placed in the digestion block and then heated at 60 °C for 30 min and at 120 °C for 60 min. The solutions were cooled to room temperature, transferred to a calibrated flask, and diluted to a final volume of 25 mL with bidistilled deionized water. All analyses were done in triplicate. Cr, Mn, Se, and Zn were determined in this solution by ETAAS.

**Sample Treatment for Catechins, Gallic Acid, and Caffeine Determination.** A modification of the method proposed by Zuo et al. (2) was used. Tea samples of 2.5 g were minced, ground, and extracted three times with 20 mL of 80% (v/v) methanol for 3 h and then twice with 20 mL of 80% (v/v) methanol containing 0.15% HCl for 3 h. The extracts were combined and filtered through cotton to get rid of rough particles. The solution was further filtered through 0.45 μm of nylon membrane filter (Millipore). A 20 μL aliquot of this solution was injected onto HPLC.

**Trace Element Determination.** Cr, Mn, Se, and Zn were determined in the mineralized samples by ETAAS according to the optimized conditions shown in **Table 1**. For all elements, calibration was performed using aqueous standards. The heating program used temperatures set on HGA-700 power supply, and background-corrected peak areas caused by the analyte were obtained. Furnace conditions were optimized on the basis of time–temperature assays. The same procedure was used to run the blanks, which were prepared fresh daily.

The standard additions method was therefore unnecessary, and consequently the analysis was much simplified. To evaluate the analytical characteristics of the method for each element, the detection limits were calculated according to IUPAC rules (22), and the sensitivity expressed as characteristic mass (mass of analyte that produces 0.0044 absorbance unit) was evaluated. Accuracy was checked with recovery assays. Precision was checked in 10 replicate determinations on each of five different randomly chosen samples. The results are summarized in **Table 2**. The reliability of the method was further corroborated by using a certified reference material (**Table 3**). The paired *t* test showed good agreement (level of significance = 0.05) between the certified values and the results we obtained. Overall, it can be concluded that ETAAS is a precise and sensitive technique, which is easy to use and is thus a suitable method for the determination of a range of elements in tea samples.

**Catechins, Gallic Acid, and Caffeine Determination.** A modification of the method proposed by Zuo et al. (2) was used. This is a simple and fast HPLC method using a photodiode array detector for simultaneous determination of four major catechins, gallic acid, and caffeine. After multiple extractions with aqueous methanol and acidic methanol solutions, tea extract was separated within 33 min using a methanol–

**Table 3.** Analytical Method Validation against a Certified Reference Material (*Citrus Leaves* SRM 1572 NBS)

element	content <sup>a</sup> (μg/g)		accuracy (%)	precision (RSD %)
	measured <sup>b</sup>	certified <sup>b</sup>		
Cr	0.79 ± 0.08	0.80 ± 0.20	98.75	10.12
Mn	22.85 ± 1.50	23.00 ± 2.00	99.37	6.56
Se	0.0248 ± 0.003	0.025 ± 0.01	99.20	12.09
Zn	28.70 ± 1.00	29.00 ± 2.00	98.96	3.48

<sup>a</sup> Dry weight. <sup>b</sup> Mean ± SD, at 95% CI about the mean ( $n = 10$ ).

acetate–water buffer gradient elution system on the C<sub>18</sub> reversed-phase packaging column (4.5 mm × 25 cm, 5 μm). The wavelength was set in the range of 200–400 nm. A gradient elution was performed by varying the proportion of solvent A (water–acetic acid, 97:3 v/v) to solvent B (methanol), with a flow rate of 1 mL/min. The mobile phase composition started at 100% solvent A for 1 min, followed by a linear increase of solvent B to 63% in 27 min, and then the mobile phase composition was brought back to the initial conditions in 5 min for the next run. All of the prepared solutions were filtered through 0.45 μm membranes (Millipore), and the mobile phase was degassed before injection onto HPLC. Calibration curves were obtained at a detection wavelength of 280 nm for the four catechins, gallic acid, and caffeine using a series of standard solutions over the concentration range from 0.20 to 100.00 mg/L. All calibration curves were linear over the concentration ranges tested with correlation coefficients ≥ 0.998. **Figure 1** shows the chromatogram of a tea sample obtained under the chromatographic conditions described above. To check the performance of the method, the selectivity, linearity, detection, and quantification limits, accuracy and precision were evaluated. Resolutions of the peaks were calculated and shown to be > 1.5 in all cases. The limit of detection (LOD) and limit of quantification (LOQ) were calculated as the concentration giving a signal equal to 3 and 10 times, respectively, the signal/noise ratio. The accuracy of the method was evaluated from recovery assays, preparing spiked tea samples in triplicate at several levels of concentration. The average recoveries were calculated according to the method of Cuadros et al. (23). **Table 4** summarizes the linear range, LOD, LOQ, and recovery values. The precision of the method was evaluated by carrying out 10 replicate analyses of a standard solution on different days. The obtained relative standard deviation was always < 2% for all compounds. It should be noted that the critical step in the quantification of catechins, gallic acid, and caffeine in teas is sample extraction. The extraction method must enable complete extraction of the compounds of interest and must avoid chemical modification. Thus, a multiple extraction procedure is essential for the quantitative analysis of catechins, gallic acid, and caffeine in teas. Although isocratic elution could separate all catechins of interest, the separation time would have been generally long (~60 min), and several late-eluting components were broadened and tailed. A gradient elution program using methanol–acetic acid–water as solvent was finally chosen. The described technique can be used as an ideal analytical method in the quality control process during tea manufacturing. The statistical treatment was performed with the Statgraphic statistical software 6.0 package (STSG, Inc., Rockville, MD, 1991).

## RESULTS AND DISCUSSION

The levels ranged from 50.6 to 371.4 ng/g for Cr, from 76.1 to 987.6 μg/g for Mn, from 48.5 to 114.6 ng/g for Se, and from 56.3 to 78.6 ng/g for Zn. Results are summarized in **Table 5**. In addition, **Table 5** gives an overview of the tea samples with their name, type, and origin. The mean concentrations of Cr measured for the three typical kinds of tea (nonfermented, fermented, and semifermented teas) were 186.9, 156.2, and 166.5 ng/g. Analysis of variance of the data showed no statistically significant differences in mean Cr content among the different kinds of tea ( $p > 0.05$ ). Salvador et al. (24) determined Cr presence in black tea, although levels were not detectable. However, Onianwa et al. (25) reported values of Cr

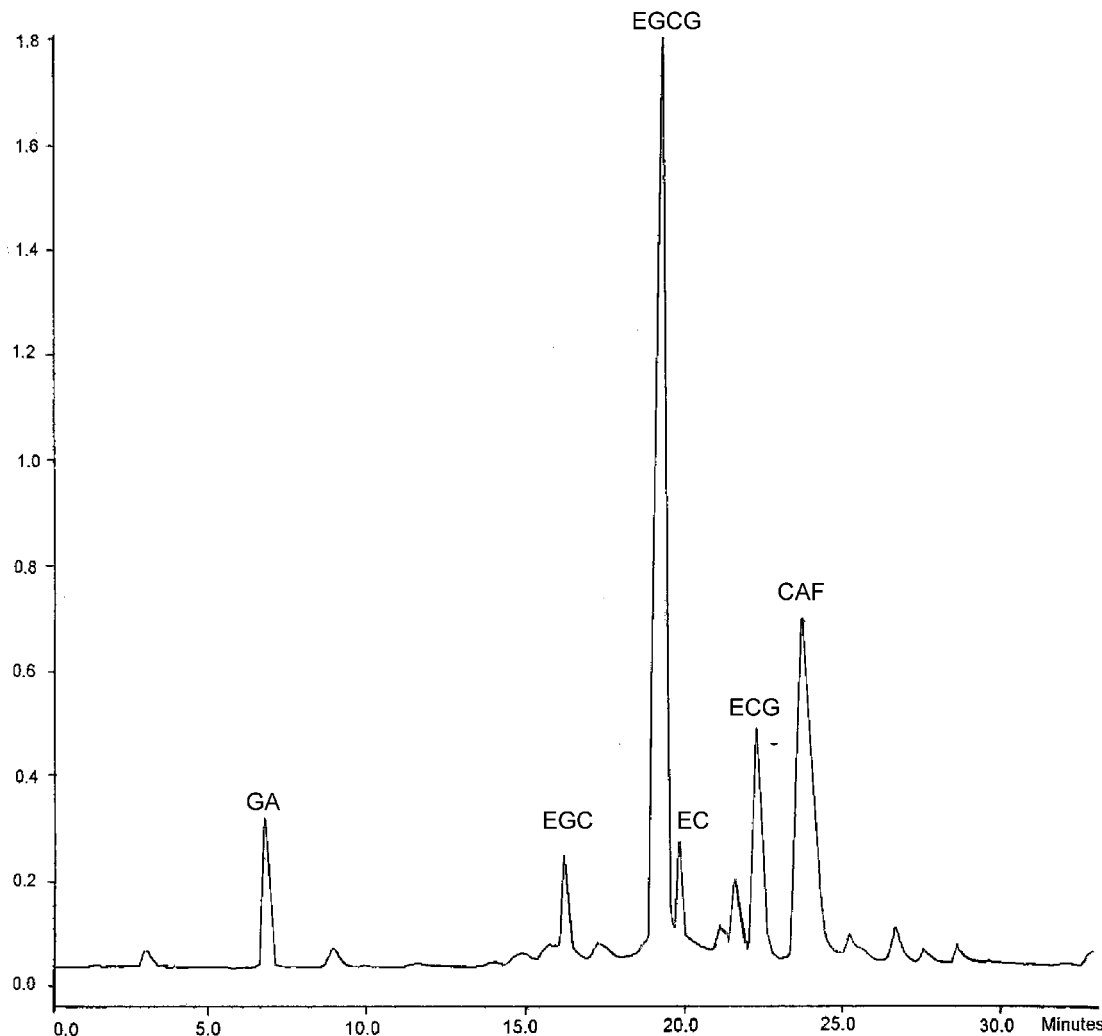


Figure 1. HPLC chromatogram of green tea at 280 nm. Peaks: GA, gallic acid; EGC, (-)-epigallocatechin; EGCG, (-)-epigallocatechin gallate; EC, (-)-epicatechin; ECG, (-)-epicatechin gallate; CAF, caffeine.

Table 4. Analytical Characteristics for the Determination of Catechins, Gallic Acid, and Caffeine by HPLC

compound <sup>a</sup>	linear range ( $\mu\text{g/mL}$ )	LOD <sup>b</sup> (ng/mL)	LOQ <sup>c</sup> (ng/mL)	accuracy (%)
EGCG	0.4–128.0	15.0	50.0	99.80 $\pm$ 0.70
EGC	0.58–56.0	90.0	300.0	98.50 $\pm$ 0.80
ECG	0.1–68.0	4.5	14.0	97.50 $\pm$ 1.00
EC	0.5–48.0	20.0	65.5	98.00 $\pm$ 1.00
GA	0.31–60.0	4.0	15.0	98.75 $\pm$ 0.50
caffeine	0.6–150.0	4.5	16.0	98.60 $\pm$ 0.80

<sup>a</sup> EGCG, (-)-epigallocatechin gallate; EGC, (-)-epigallocatechin; ECG, (-)-epicatechin gallate; EC, (-)-epicatechin; GA, gallic acid. <sup>b</sup> LOD, limit of detection. <sup>c</sup> LOQ, limit of quantification.

in tea ranging from 0.010 to 3.60 ppm (referred to solid samples). Lozak et al. (26) determined Cr in peppermint (*Mentha piperitae folium*) and nettle (*Urticae folium*) leaves in tea bags and in their infusions; in raw material the Cr content was 0.941  $\pm$  0.139 mg/kg, whereas the element content in infusion was 0.390  $\pm$  0.019 mg/kg. Bratakos et al. (27) indicated that meat, fish, and seafood, cereals, and pulses are rich sources of Cr (>0.100  $\mu\text{g/g}$ ). Garcia et al. (28) measured daily dietary Cr intake in the southern Spain by sampling duplicate diets in different population groups, and mean levels ranged from 9.39 to 205.16  $\mu\text{g/day}$ ; Cr intake correlated significantly with the

Table 5. Chromium, Manganese, Selenium, and Zinc Content in Tea

sample (origin)	n <sup>a</sup>	Cr (ng/g)	Mn ( $\mu\text{g/g}$ )	Se (ng/g)	Zn (ng/g)
green tea, sencha (Japan)	3	238.6	500.7	92.9	78.1
black tea, Assam (northeastern India)	3	371.3	338.2	48.8	65.0
semifermented tea, oolong (China)	3	177.5	354.0	52.6	62.4
black tea, Keemun (China)	3	155.1	295.0	87.2	64.5
red tea (South Africa)	3	100.1	76.1	114.6	69.7
black tea, Ceylan (Sri Lanka)	3	50.6	282.6	50.1	56.3
mixed tea, <sup>b</sup> Earl Grey (China)	3	79.0	91.5	95.7	74.1
black tea, English breakfast (Sri Lanka)	3	107.6	423.7	88.5	76.7
green tea, jasmine (Japan)	3	291.0	354.1	89.7	78.6
green tea, Kokaicha (Japan)	3	219.8	651.3	48.5	76.1
red tea, Pu-erh (China)	3	371.4	431.7	55.0	65.1
black tea, Darjeeling (India)	3	96.6	284.5	70.3	63.1
green tea, Bancha (Japan)	3	111.9	987.6	80.1	65.0
green tea, Paimutan (China)	3	141.7	236.6	75.1	75.1
green tea, gunpowder (China)	3	118.4	518.9	70.2	57.3

<sup>a</sup> n = number of samples, analyzed in triplicate. <sup>b</sup> Exclusive black tea mixture perfumed with bergamot essence.

intake of energy, protein, carbohydrate, Zn, Fe, Mg, K, Na, Ca, and nicotinic acid. Taking into account the levels of Cr we have detected in this study, tea could be considered a rich source of this element.

The mean Mn contents in green, black, and oolong teas were 541.6, 285.9, and 354.0  $\mu\text{g/g}$ , respectively. In red tea, the Mn levels ranged from 76.1 to 431.7  $\mu\text{g/g}$ . No statistically significant differences between these kinds of teas were observed ( $p > 0.05$ ). According to the bibliography, tea may be an important source of Mn, because leaf tea contains 350–900  $\mu\text{g/g}$  of this essential element (29). However, the leaching and bioavailability of Mn from tea have been little studied. Powell et al. (29) indicated that Mn is an element found in significant dietary amounts in tea and under simulated intestinal conditions was still 40% bioavailable. Fernández-Cáceres et al. (18) reported a mean Mn content in green, black, and instant teas of 824.8 mg/kg dry basis (levels ranged from 148 to 1595.4 mg/kg). Yasmeen et al. (30) found a 175 ppm mean content of this element in tea samples marketed by different companies throughout Pakistan. Ozdemir and Gucer (31) found that Mn levels in tea leaves ranged from 1107 to 2205  $\mu\text{g/g}$  on dry basis and noted that 30% of Mn was passed into the infusion in the form of Mn(II). Costa et al. (1) found Mn contents of  $110.0 \pm 2.7 \mu\text{g/g}$  in fermented tea,  $1782.3 \pm 130.1 \mu\text{g/g}$  in partially fermented tea, and 1486.6  $\mu\text{g/g}$  in green tea. Xie et al. (6) reported data on Mn levels ranged from 160 to 1500  $\mu\text{g/g}$  in 39 tea samples of different kinds and/or qualities produced in different regions of China.

Selenium mean levels were 76.3, 68.9, and 52.6 ng/g in green, fermented, and oolong teas, respectively. No statistically significant differences among tea kinds were observed ( $p > 0.05$ ). However, the most elevated Se levels were found in green tea samples. Xie et al. (6) analyzed 39 tea samples of different kinds and/or qualities in different regions of China and observed the influence of the origin, type, and quality of the tea on its mineral content; the concentration of Se was found to be very high (up to 7.5  $\mu\text{g/g}$ ) in some tea samples produced in a region with Se-rich soils, in contrast to an approximate level of 0.1  $\mu\text{g/g}$  found in most tea leaves. These authors reported that the Se content was much lower in black tea than in green and oolong teas but noted the influence of the Se soil presence. Both selenium and green tea have been reported to exhibit antitumorigenic and cancer chemopreventive properties. Amantana et al. (7) compared the antimutagenic activities of regular green tea and Se-enriched green tea obtained by foliar application of selenite; analytical studies revealed that the latter tea contained ~60-fold higher concentrations of Se compared with regular green tea, and an enhancing antimutagenic effect was observed.

The mean concentrations of zinc obtained in green, black, and oolong teas were 71.7, 66.6, and 62.4 ng/g, respectively. In red tea samples, the mean Zn content was 67.4 ng/g. No statistically significant differences between kinds of tea were observed ( $p > 0.05$ ). Salvador et al. (24) found Zn levels in tea from not detectable to 4.47  $\mu\text{g/g}$ . Xie et al. (6) reported data ranged from 20 to 60  $\mu\text{g}$  of Zn/g in a variety of 39 tea samples of different kinds and/or qualities produced in different regions of China (including green, black, and oolong teas). A wide variability of Se and Zn contents in tea has been observed in the literature (1, 18, 24). As has already been mentioned, climate and agricultural practices, including soil, water, and fertilizers, can be of great influence on the composition of teas. Thus, teas cultivated in different geographical areas will present significant differences in their chemical compositions. Total dietary Zn intakes are influenced greatly by food choices. Frequently, Zn intakes are correlated with protein intake, but the exact relationship is influenced by protein source. Diets with a rich Zn/protein ratio have liberal quantities of legumes, whole grains, and nuts. However, it has been reported that competition

between Zn and other elements for absorption binding sites can influence absorbability (12). Matsuura et al. (32) determined Mn and Zn levels in tea infusions (black tea) that were prepared as usual tea beverage by brewing black tea leaves in boiling water for 5 min; the extraction efficiency of each element was estimated according to the ratio of its concentration in tea infusions compared to that in tea leaves and ranged from 20 to 55%.

Han and Li (33) reported that because tea contains all kinds of minerals and trace elements such as K, Ca, Mg, Mn, P, Zn, and Fe, drinking tea is healthy for people. However, this content depends only on the sort of tea, which indeed does not connect directly with the grade of the tea. We sought correlations between trace elements that might affect their bioavailability and subsequent physiological effects; however, no statistically significant correlations between the concentrations of pairs of elements were detected. Costa et al. (1) observed large variations in the trace element composition (Al, Ca, Mg, and Mn) of teas. Fernández-Cáceres et al. (18) determined the metal content (Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, Sr, Ti, and Zn) of 46 tea samples, including green, black, and instant teas; no clear differences were found between metal contents of green and black teas. Pattern recognition methods such as principal component analysis (PCA), linear discriminant analysis (LDA), and artificial neural networks (ANN) were applied to differentiate the different tea types. These chemometric procedures were also useful for distinguishing between Asian and African teas and between the geographical origin of different Asian teas. Fernández-Cáceres et al. (18) indicated that clear differences appear in metal contents of tea leaves in comparison with instant teas; meanwhile, no apparent distinction appears between green and black teas. Xie et al. (6) noted that the concentration of an element in the leaves and in the infusion of the same kind of tea can differ widely for samples of various origins; this may be partly due to the geochemical conditions of the respective soils, and the pattern of elements found in plants reflects, in general, that of the soil in which they grow and, to some extent, their environment (34).

**Figure 2** represents the mean levels of Cr, Mn, Se, and Zn in each type of tea. We concluded that the regular consumption of tea can contribute to the daily dietary requirements of the above-mentioned elements.

In relation to the catechin content in the analyzed samples, levels of EGCG ranged from 1.4 to 103.5 mg/g. It is therefore the major polyphenolic constituent of green tea (mean  $\pm$  SD of  $85.9 \pm 11.3 \text{ mg/g}$ ). The high antioxidant activity of this catechin has been widely described (3, 35, 36). Sing et al. (36) reported that EGCG acts as an angiogenesis inhibitor by modulating protease activity during endothelial morphogenesis. Angiogenesis is a crucial step in the growth and metastasis of cancer. The levels of EGC in the tea samples we have analyzed varied between 3.9 and 45.3 mg/g, and the higher mean value was found in green tea ( $36.8 \pm 7.9 \text{ mg/g}$ ); the content of ECG ranged from 0.2 to 45.5 mg/g, and the higher values were detected in the green tea samples ( $20.3 \pm 13.1 \text{ mg/g}$ ); the levels of EC varied from 0.6 to 21.2 mg/g, and the higher values were found in the green tea samples, with a mean content of  $12.7 \pm 4.8 \text{ mg/g}$ . **Table 6** summarizes these results. We have found a direct correlation between the levels of EGCG–ECG ( $p < 0.01$ ), EGCG–EC ( $p < 0.001$ ), and ECG–EC ( $p < 0.001$ ). EGCG, ECG, and EC were identified as major components in all tea samples, and the presence of these catechins is modified in the same way. Clear differences among the profiles of fermented and nonfermented tea samples from different geographical

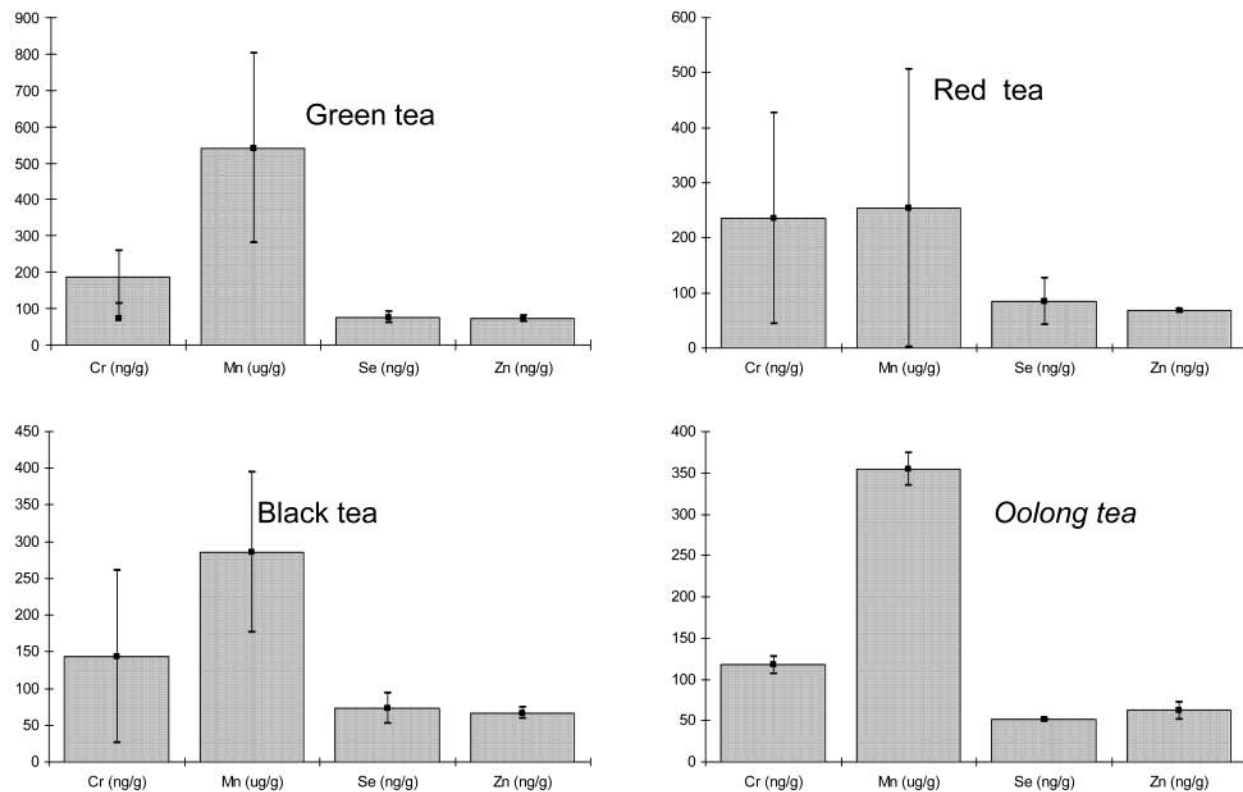


Figure 2. Levels of chromium, manganese, selenium, and zinc in each type of tea.

Table 6. Catechins, Gallic Acid, and Caffeine Contents in Tea (Results Are Expressed as Milligrams per Gram)

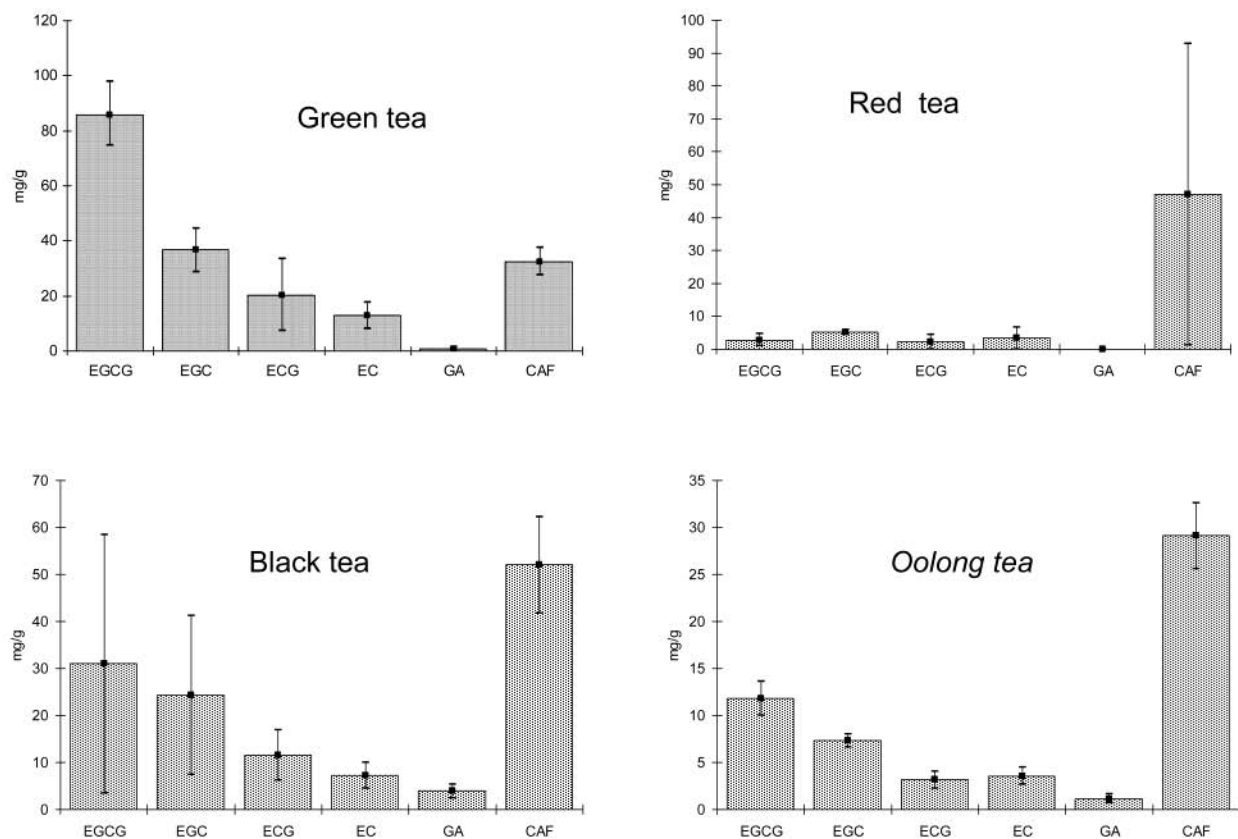
sample (origin)	<i>n</i> <sup>a</sup>	EGCG	EGC	ECG	EC	GA	caffeine
green tea, Sencha (Japan)	3	73.3	32.1	10.4	14.1	0.7	38.3
black tea, Assam (northeastern India)	3	27.9	3.9	11.5	7.4	2.5	47.4
semifermented tea, oolong (China)	3	11.8	7.3	3.1	3.5	1.2	29.1
black tea, Keemun (China)	3	12.3	20.1	4.4	4.0	3.1	41.5
red tea (South Africa)	3	4.1	4.8	4.4	6.1	0.04	86.6
black tea, Ceylan (Sri Lanka)	3	17.8	41.7	9.5	6.6	3.6	61.8
mixed tea, <sup>b</sup> Earl Grey (China)	3	12.5	40.7	9.6	4.9	6.7	48.9
black tea, English breakfast (Sri Lanka)	3	30.1	33.7	13.5	9.1	2.8	67.4
green tea, jasmine (Japan)	3	94.8	39.7	45.6	21.2	1.4	25.7
green tea, Kokaicha (Japan)	3	82.3	35.2	13.5	9.6	1.5	30.1
red tea, Pu-erh (China)	3	1.4	5.8	0.2	0.6	0.04	7.5
black tea, Darjeeling (India)	3	85.1	5.9	20.6	11.4	4.5	45.0
green tea, Bancha (Japan)	3	84.2	45.3	14.4	13.8	0.7	28.9
green tea, Paimutan (China)	3	103.5	44.1	23.0	8.1	0.3	34.4
green tea, gunpowder (China)	3	77.1	24.3	14.8	9.8	0.8	37.4

<sup>a</sup> *n* = number of samples, analyzed in triplicate. <sup>b</sup> Exclusive black tea mixture perfumed with bergamot essence.

origins can be noted. Nonfermented teas present higher peaks for all of the catechins, especially EC, EGCG, and ECG.

Fernández et al. (19) determined the content of catechins in a set of 45 tea samples, including fermented and nonfermented teas of different geographical origins. The amounts of catechins are always higher for nonfermented teas. EGCG and EGC are the major catechins present, with average contents of 7.358 and 3.955% w/w (dry basis), respectively. ECG presents values ranging between 0.910 and 3.556%. For fermented teas, EGCG and ECG are the catechins present in larger percentages, with average contents of 1.583 and 0.706%, respectively, and these values are less than those found in nonfermented teas. Zuo et al. (2) determined catechins in green, oolong, black, and pu-erh teas; the EGC content ranged between 5.71 and 37.6 mg/g, the EGCG content between 1.99 and 62.4 mg/g, the EC levels from 1.36 to 10.3 mg/g, and the ECG content between 1.32 and 21.8 mg/g. These authors reported that, in general, green

teas contain higher levels of catechins than oolong teas; in green tea, the levels of EGCG ranged from 51.1 to 52.7 mg/g, whereas in oolong teas, the EGCG content varied between 22.2 and 28.2 mg/g. The levels of EGC in green tea ranged from 27.7 to 30.8 mg/g, whereas in oolong teas EGC ranged from 10.0 to 15.9 mg/g; black tea presented a mean content of 5.71 mg/g. According to these authors, the catechin contents of pu-erh and black teas are very low, which is in agreement with the degree of fermentation during the manufacturing process. Black tea is obtained by a postharvest fermentation, an autoxidation catalyzed by polyphenol oxidase, whereas leaves for green tea are steamed to inactivate polyphenol oxidase prior to drying. Oolong tea is produced by a partial oxidation of the leaf, intermediate between the process used for green tea and that of black tea (34). The fermentation and heating of tea leaves can result in polymerization of monophenolic compounds, leading to conformational changes and thus contributing to the properties



**Figure 3.** Levels of catechins [EGCG, (–)-epigallocatechin gallate; EGC, (–)-epigallocatechin; ECG, (–)-epicatechin gallate; EC, (–)-epicatechin], gallic acid (GA), and caffeine (CAF) in each type of tea.

of various teas (1). During the fermentation, these compounds are oxidized or condensed to other large polyphenolic molecules such as theaflavins and thearubigins. The health effect of these oxidized products is not well understood yet (2, 3).

In the analyzed tea samples, gallic acid presents values that range between 0.04 and 6.7 mg/g, black tea being the one that presents the highest values of  $(3.9 \pm 1.5 \text{ mg/g})$  (see **Table 6**). Fernández et al. (19) analyzed the content of gallic acid in 45 tea samples, including fermented and nonfermented teas of different geographical areas; gallic acid presented values that ranged between 0.004 and 2.537%, and the lower percentages were obtained for the nonfermented teas. Zuo et al. (2) reported that the fermentation process also increased the liberation of gallic acids from CGs as indicated by the remarkably high levels of this acid in both pu-erh and black teas (means of 5.53 and 2.06 mg/g, respectively). These authors detected gallic acid values of 0.37–0.74 mg/g in green teas and of 1.42–1.67 mg/g in oolong teas. The amount of caffeine in the analyzed samples ranged from 7.5 to 86.6 mg/g (**Table 6**). Caffeine presence is higher in the case of black teas, showing values between 41.5 and 67.4 mg/g, whereas green and oolong teas show mean caffeine contents of 32.5 and 29.2 mg/g, respectively. Fernández et al. (19) also reported that caffeine content is higher in the case of fermented teas, showing values between 2.415 and 4.862%, whereas nonfermented teas show caffeine levels ranging between 1.468 and 3.863%. Zuo et al. (2) analyzed the caffeine content in green, oolong, black, and pu-erh teas, and the levels ranged from 7.44 to 29.6 mg/g. These authors observed a much reduced caffeine level in oolong teas (ranged 7.44–18.7 mg/g) and indicated that the causes of this reduction by biochemical mechanism or other factors are interesting and that further studies on this topic are warranted.

Fernández et al. (19) reported that many factors such as species, season, age of the leaves (plucking position), climate, and horticultural conditions (soil, water, minerals, fertilizers, etc.) can constitute important influences on tea composition. Catechins, together with phenolic acids such as gallic acid, are a group of polyphenols that constitute up to 30% of the dry weight of the tea leaf and are important factors in the taste of tea. The tea catechin, gallic acid, and alkaloid (caffeine, theophylline, and theobromine) contents have been considered as chemical descriptors to differentiate teas according to their geographical origins. In addition, McKay et al. (34) reported that the catechin concentration of any particular tea beverage depends on the type of tea (e.g., blended, decaffeinated, instant) and preparation (e.g., amount used, brew time, temperature); the highest concentration of catechins is found in brewed hot tea, less in instant preparations, and lower amounts in iced and ready-to-drink tea, and decaffeination reduces slightly the catechin content of black teas. Price and Spitzer (37) evaluated the temperature dependence of the rate of extraction of soluble constituents of black tea; the activation energy for the infusion of solubles was determined to be 41 kJ/mol; this value is higher than previous ones in the literature.

**Figure 3** represents the mean levels of EGCG, EGC, ECG, EC, gallic acid, and caffeine in the samples analyzed in this study. A wide variability could be observed not only between the different teas but also within the same type of tea. Different pattern recognition techniques were applied to the data set. PCA and LDA were applied as supervised learning methods to find classification rules. PCA was applied to visualize the data trends and provides a first evaluation of the discriminant efficiency of the selected features. LDA differs from data reduction methods such as PCA in that it is concerned with determining the so-

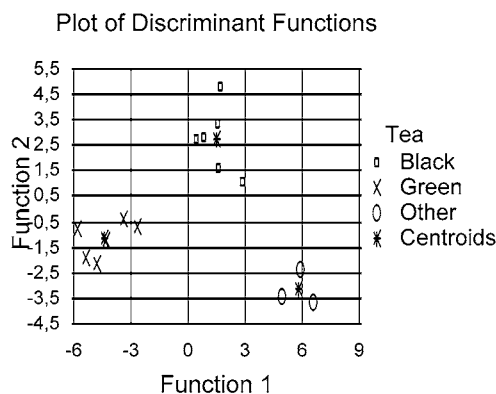


Figure 4. Plot of the green, black, and other tea samples in two of the discriminant functions.

called discriminant functions as linear combinations of the chemical descriptors which best separate the classes according to minimization of the ratio of within-class and between-class sum of squares. We have observed that certain trace elements (Cr), the total content of catechins (EC, EGC, and EGCG), gallic acid, and caffeine are adequate descriptors to distinguish among green tea, black tea, and other tea samples. Results are shown in Figure 4.

In conclusion, the levels of Cr, Mn, Se, and Zn in our samples were in the same range as found in other studies; however, a wide variability in results has been observed. Tea can be an important dietary source of Mn, which activates numerous essential enzymes; other foodstuffs contain relatively small amounts of Mn. Although tea is rich in minerals, the contribution of tea drinking as a mineral source is not clear because the contents of many of these elements are generally not known. In relation to tea catechin content, nonfermented teas present higher levels, especially of EGCG and EGC. Caffeine is present in higher amounts in the case of fermented teas, whereas the caffeine content in green and oolong teas is moderate. Tea catechins, gallic acid, and caffeine contents are related to the quality of tea leaves and to the degree of fermentation during tea manufacturing. In conclusion, tea could be an important dietary source of polyphenols and minerals with antioxidant activity, and future studies designed to accurately assess their presence and bioavailability are necessary.

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