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Original

Quantification of catechins and caffeine from green tea (*Camellia sinensis)* infusions, extract, and ready-to-drink beverages

Quantificação de catequinas e cafeína do chá verde (Camellia sinensis) infusão, extrato e bebida pronta

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Abstract

This study aimed to quantify the levels of catechins and caffeine in various forms of presentation of green tea: infusion tea bags, extract, and ready-to-drink beverage and, based on their content, identify the most suitable for consumption. High Performance Liquid Chromatography (HPLC) analytical method was used for the quantification of catechins and caffeine. The tea bags had the highest concentration of total catechins with 5 to 9.5% followed by the extract with 3.64 to 4.88%, and ready-to-drink green tea beverage showed low levels of catechins, from 0.14 to 0.26%. As for caffeine content, green tea extract had higher concentration (1.96 to 3.54%) compared to the tea bags (1.39 to 1.57%). Tea bags were found the most suitable for consumption because it contains higher amounts of catechins and smaller amounts of caffeine.
Keywords: *green tea; catechins; caffeine; high performance liquid chromatography (HPLC).*

Resumo

O presente estudo teve como objetivo quantificar os teores de catequinas e cafeína em diferentes formas de apresentação do chá verde –infusão, extrato e bebida pronta – e, baseado no teor destes constituintes, identificar aquela mais apropriada para consumo. A cromatografia Líquida de Alta Eficiência (CLAE) foi a metodologia analítica utilizada para a quantificação das catequinas e cafeína. O chá verde sachê obteve maior concentração de catequinas totais com 5 a 9,5%, seguido do extrato com 3,64 a 4,88%, o chá verde bebida pronta apresentou baixos teores de catequinas, 0,14 a 0,26%. Quanto ao teor de cafeína, o chá verde na forma de extrato obteve maior concentração, 1,96 a 3,54%, quando comparado ao sachê, 1,39 a 1,57%. O chá verde na forma de sachê mostrou-se mais indicado para consumo por conter maiores quantidades de catequinas e menores quantidades de cafeína.
Palavras-chave: *chá verde; catequinas; cafeína; cromatografia líquida de alta eficiência (CLAE).*

1 Introduction

After water, green tea (*Camellia sinensis*) is the most consumed beverage worldwide due to the bioactive compounds associated with numerous health benefits (KHAN et al., 2007; SUMPIO et al., 2006; COOPER; MORRÉ; MORRÉ, 2005). Studies report on the benefits of green tea consumption for preventing cardiovascular diseases, cancer, diabetes, obesity, and even its potent antioxidant action (KHAN; MUKHTAR, 2007; PASTORE; FRATELLONE, 2006; KURIYAMA et al., 2006). Obtained from the plant *Camellia sinensis*, green tea contains higher amounts of bioactive compounds when compared to other types of teas. Due to processing, the oxidation of these compounds occurs to a lesser extent than in black and oolong tea (FERNÁNDEZ et al., 2000; HENNING et al., 2004). Among the biologically active compounds present in green tea, catechins stand out since some studies have indicated that these compounds are the main representative of the class of biologically active polyphenols with a wide range of functional activities, especially their antioxidant activity (PASTORE; FRATELLONE, 2006; VALENZUELA, 2004). The most important currently studied catechins are: (+)–catechin (C), (–)–epicatechin (EC), (–)–epicatechin gallate (ECG), (–)–epigallocatechin (EGC), and (–)–epigallocatechin gallate (EGCG); the latter being the most active and the most commonly studied. The concentrations of catechins contained in green tea range from 35-45%, and (–)–epigallocatechin gallate is found in higher amounts (FIGUEROA; RODRÍGUEZ-RODRÍGUEZ; MUNIZ, 2004; VALENZUELA, 2004; RIO et al., 2004). Catechins are responsible for bitterness and astringency of green tea and are colorless and water-soluble compounds (MATSUBARA; RODRIGUEZ-AMAYA, 2006). With regard to the toxicity of catechins, studies have shown no toxic effects in animals (CHENGELIS et al., 2008; VALENZUELA, 2004; TAKAMI et al., 2008; OGURA et al., 2008). Green tea contains caffeine; according to the literature its content is around 6 $mg.mL^{-1}$ or 2 to 3.8% (LAMARÃO; FIALHO, 2009; SAITO et al., 2006) Caffeine has been used as an ergogenic aid by athletes in order to decrease fatigue and improve performance (ALTIMARI; ZUCAS; BURINI, 2000). Caffeine consumption during pregnancy can cause undesirable effects to the fetus such as low birth weight, spontaneous abortion, intrauterine growth retardation, and increased risk of premature rupture of membranes (PACHECO et al., 2008). This study aimed to determine the levels of catechins and caffeine in green tea products available in the market for consumption such as herbal

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infusion, extract capsules, and ready-to-drink beverage and to identify the one with the most beneficial effects or most suitable for consumption due to its high amounts of catechins.

2 Methods

2.1 Samples

Six samples of green tea were from different brands analyzed. Two samples of green tea infusion tea bags, two of ready-to-drink green tea, and two of green tea extract capsules. The samples were purchased from markets in Brasilia and the analyses were performed the Cetal laboratory in the city of Mogi of Cruzes in the state of São Paulo. All samples were sent to the laboratory in their original packaging. All analyses were performed in triplicates for each sample.

2.2 Sample preparation

By means of a flowchart, the preparation of the samples is described in steps for each type of green tea.

The following reagents were used in the preparation of the samples: acetonitrile HPLC grade Tedia 903344R, 10 mM sodium phosphate (NaH2PO4. H2O), JT Baker B17158, phosphoric acid, F Maia 36 290, and methanol HPLC grade Tedia 90307R. The equipment used for sample preparation was an analytical balance (Mettler Toledo AB 204, Max 210 g = 1 mg/minute 10 g = 0.1 mg), an USC 2800 ultrasonic unit, and an IKA hot plate stirrer RH basic 2.

2.3 Analysis of the contents of catechins and caffeine

The determination of catechins and caffeine were performed by High Performance Liquid Chromatography (HPLC). The chromatographic separations were carried out using a Shimadzu on a ODS-C18 column (15 cm × 4.6 mm) with oven temperature at 40 °C, detector UV 270 nm and flow rate of 1 mL/minute. Solvent A, 10 mM sodium phosphate (pH 2.6), and the solvent B acetonitrile (93:7, v/v) were the mobile phase for the gradient elution.

For the analysis of caffeine, it was used the standard anhydrous caffeine, C8H10N4O2 (Wako), MW 194.19 25 g and 98.5% purity. For the analysis of catechins, it was used: (+)–Catechin, C15H14O16 (Wako), MW 290.27 mg and 98%; purity. For epigallocatechin: (–)–Epigallocatechin, C15H14O7, (Wako) MW 306.27 10 mg and 98% purity for epigallocatechin Gallate: (–)–Epigallocatechin Gallate, CEE3076, MW 458.38 100 mg and 90% purity; and for epicatechin gallate: (–)–Epicatechin Gallate, C22H18O11 (Wako), MW 442.37 10 mg and 98% purity.

All standards were prepared at a concentration of 100 mg.mL^{-1} diluted in methanol. The equipment used in this study was a Shimadzu liquid chromatograph consisting of: SPD 10 AV UV visible detector; CTO-20A column oven; DGU-20A degasser; and an IKA hot plate stirrer RH basic 2.

2.4 Quantification of areas for standardization

The quantification of catechins and caffeine were performed by quantifying the areas of standardization, where [Sample] g.mL^{-1} = Area standard × [default g.mL^{-1}] / sample area. The results obtained in g.mL^{-1} were expressed in%.

2.5 Statistical analysis

For the statistical analysis, the software StatSoft® 1.0 was used for the determination of the mean and standard deviation of the results obtained, and the t-test was applied (Tukey) for the comparison of three distinct forms of presentation of the green tea samples with a significance of $p < 0.05$.

3 Results

The concentrations of catechins in the tea bags, extract, and ready-to-drink green tea are presented in Table 1.

There was a variation in the concentration of catechins between the brands analyzed. The tea bags and extract showed a difference in the concentrations of EC, EGCG, ECG, and CAF; no significant difference was found for C. In the samples of ready-to-drink green tea, there was no difference between the concentrations of catechins and caffeine. Considerable variation

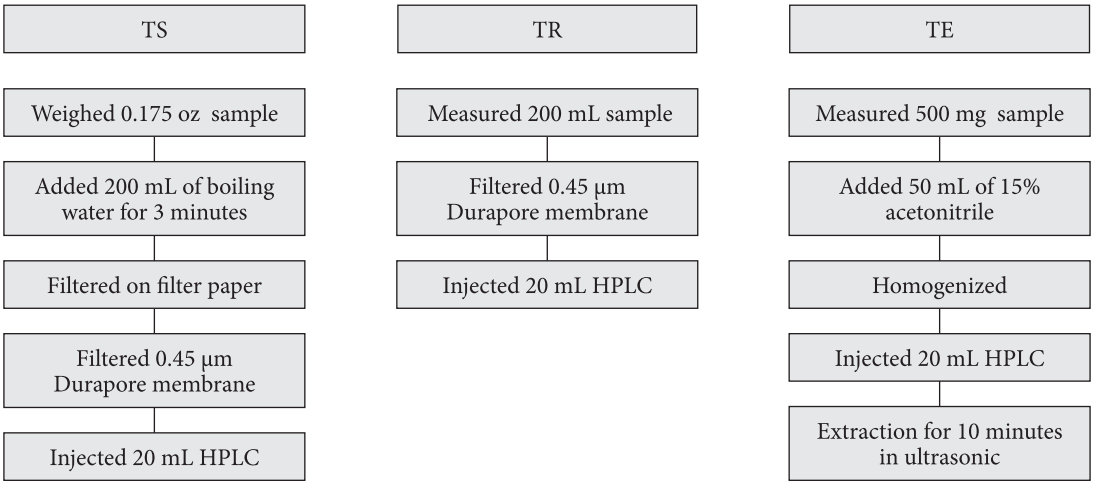

Figure 1. Flowchart - Green tea sample preparation. (TS) Tea bag, (TR) Ready-to-Drink Tea, (TE) Tea Extract.

in the levels of catechins between the samples has been reported in other studies (KHOKHAR; MAGNUSDOTTIR, 2002; MATSUBARA; RODRIGUEZ-AMAYA, 2006). Comparing the levels of catechins specifically as shown in Table 2, catechins in the tea bags ranked EC> EGCG> ECG> C, while the concentration of catechins in the green tea extract ranked EGCG> ECG> EC> C, and finally in the ready-to-drink green tea it ranked EGCG> EC> ECG> C. Table 2 shows the concentration of total catechins found in the samples. The samples of tea bags showed higher concentrations of catechins, about 40% more, when compared to green tea extract.

4 Discussion

Some studies found the following relative concentrations of catechins: ECGC> ECG> EC> C (DALLUGE et al., 1998; KHOKHAR; MAGNUSDOTTIR, 2002). The results found for the green tea extract followed this same sequence, but the catechin content was lower than that found in other studies, in which the total catechin content was 4-45% on average (DALLUGE et al., 1998; KHOKHAR; MAGNUSDOTTIR, 2002; MIZUKAMI; SAWAI; YAMAGUCHI, 2007; SAITO et al., 2006).

When comparing the levels of catechins in green tea infusion tea bags and extracts, the concentration of EC in the tea bags is much higher than that of the extract, as well as the total concentration of catechins in the forms of green tea analyzed. The preparation of infusion using tea bags, which were placed in boiling in water left to simmer for 3 minutes, may have facilitated the extraction of catechins; nevertheless, the fact that the processing of green tea extract may have caused catechin reduction should not be disregarded. When comparing the levels of ECG, there is a difference of more than 100% between the green tea extract in and the tea bags; and as for the EGCG, the difference is nearly 100%.

As for the tea bags, there was no statistical difference between the samples with respect to ECG, EC, EGCG and caffeine in comparison to the other forms of green tea presentation analyzed. Ready-to-drink green tea showed low levels of catechins, which suggests that its consumption is not effective if the aim is to get the health benefits attributed to the therapeutic properties of catechins. One explanation for the difference in the levels of catechins found in this study and others is the type of samples used. In comparative studies, leaves of *Camellia sinensis* were used in the preparation of infusion; in this study, however, herbal tea bags containing herb leaves and stems, which have lower concentrations of catechins, were used.

Nevertheless, there are considerable amounts of catechins in the tea bags. In order to meet the recommended intake, around 240 mg of catechins, (LAMARÃO; FIALHO, 2009), a consumption of about 7 cups of steeped tea would be necessary. It is important to note that there is no consensus on the specific dosage of catechins that could benefit human health. Many discrepant values are found in the literature.

Finally, some studies reported that green tea has a low oral bioavailability due to low concentrations of catechins found in the blood and urine suggesting that it should be consumed at regular intervals (FIGUEROA; RODRÍGUEZ-RODRÍGUEZ; MUNIZ, 2004; MATSUBARA; RODRIGUEZ-AMAYA, 2006; PASTORE; FRATELLONE, 2006). Very few studies have reported on the greatest bioavailability of cathecins in different forms of green tea presentation. With regard to caffeine, the green tea extract had a higher concentration than that of the tea bags, as shown in Table 2. One possible reason was the fact that the tea bag samples were prepared with boiling water, which could have destabilized the molecules of caffeine due to its thermal sensibility resulting in lower levels of this compound. Studies suggest that green tea has significant amounts of caffeine (LAMARÃO; FIALHO, 2009). However, this study did not find

Table 1. Levels of catechins and caffeine in tea bags, extract, and ready-to-drink green tea.

Tea	Brand	Concentration*				
		C	ECG	EC	EGCG	CAF
%						
TS	A	0.03 ± 0.00^a	0.67 ± 0.09^a	6.78 ± 0.09^a	2.29 ± 0.02^a	1.42 ± 0.00^a
	B	0.02 ± 0.00^a	0.35 ± 0.02^b	3.29 ± 0.27^b	1.09 ± 0.01^b	1.55 ± 0.04^b
TE	C	0.06 ± 0.00^a	2.32 ± 0.07^a	0.98 ± 0.03^a	2.74 ± 0.11^a	3.31 ± 0.04^a
	D	0.07 ± 0.00^a	0.72 ± 0.02^b	0.36 ± 0.00^b	1.21 ± 0.01^b	2.19 ± 0.02^b
TR	A	0.00 ± 0.00^a	0.00 ± 0.00^a	0.04 ± 0.00^a	0.02 ± 0.00^a	0.01 ± 0.01^a
	E	0.00 ± 0.00^a	0.00 ± 0.00^a	0.03 ± 0.00^a	0.01 ± 0.00^a	0.00 ± 0.01^a

(TS) Tea Sachet (TE) Tea Extract (TR) Tea Ready. (C) Catechin; (ECG) Epicatechin Gallate; (EC) Epicatechin; (EGCG) Epigallocatechin Gallate; and (CAF) Caffeine. *Means followed by the same letter in the same column do not differ significantly by Tukey test at the 5%. Probability level.

Table 2. Mean concentration of catechins and caffeine in the green tea samples analyzed.

Tea	Concentration*					
	C	ECG	EC	EGCG	CAF	CT
%						
TS	0.02 ± 0.00^a	0.51 ± 0.22^a	5.03 ± 2.46^a	1.69 ± 0.84^a	1.48 ± 0.09^a	7.25 ± 2.25^a
TE	0.06 ± 0.00^a	1.52 ± 1.13^b	0.68 ± 0.43^b	2.00 ± 1.08^b	2.75 ± 0.70^b	4.26 ± 0.62^b
TR	0.00 ± 0.00^a	0.00 ± 0.00^c	0.03 ± 0.00^c	0.15 ± 0.00^c	0.00 ± 0.00^c	0.20 ± 0.06^c

(TS) Tea Sachet (TE) Tea Extract (TR) Tea Ready. (C) Catechin; (ECG) Epicatechin Gallate; (EC) Epicatechin; (EGCG) Epigallocatechin Gallate; (CAF) Caffeine, and (TC) Total catechins. *Means followed by the same letter in the same column do not differ significantly by Tukey test at the 5%. Probability level.

such high levels; the findings revealed values around 3% of the total content of caffeine, which are very similar values to those of some studies (CHEN; GUO; ZHAO, 2008; FERNÁNDEZ et al., 2000). According to the ADA (American Dietetic Association), the caffeine consumption should be up to 300 mg/day to avoid possible adverse effects (AMERICAN…, 2004). In order to achieve such dosage, a consumption of 20 L of green tea infusion would be needed. Studies suggest the consumption of 4-7 cups of green tea per day, about 800 to 1400 L/day, in order to obtain the expected health benefits of catechins. Finally, due to variation in the quantification of these compounds, which in fact indicates an analytical variability, the present study highlights the need for better knowledge of the stability of these compounds and improved methods of extraction and quantification of catechins. It is also emphasized the importance of better knowledge of the availability of catechins to determine optimal quantities and forms of consumption more accurately.

5 Conclusion

Green tea infusion would be the best option due to its higher levels of catechins and lower levels of caffeine compared to the other forms of consumption analyzed.

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