

## Caffeine in teas: levels, transference to infusion and estimated intake

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### Abstract

Caffeine is naturally present in several foods, being one of the most consumed dietary ingredients in the world; however, excessive intake may cause health concerns. This study evaluated caffeine levels in teas and their infusions, the transference rate during brewing, and estimated caffeine intake from tea infusion. Brands and batches of 4 types of teas were analyzed for caffeine content by high performance liquid chromatography with a diode array detector. Mate tea was the one that presented lowest levels (6.1 to 13.2 mg/g) while *Camellia sinensis* teas were from 14.3 to 34.8 mg/g. There were statistical differences between different types, brands and batches. Caffeine levels in infusions followed the same pattern of the leaves, with mate tea presenting lowest levels. Caffeine percentage of transference from leaves to infusion varied from 51.5 to 85.2%. Caffeine intake was estimated to be up to 191.4 mg/day. Tea may be considered an important source of caffeine intake for heavy tea drinkers.

**Keywords:** xanthine alkaloid; mate tea; *Camellia sinensis*; HPLC; brew.

**Practical Application:** Caffeine levels in tea are carried to the infusion, which may be an important source of caffeine intake.

### 1 Introduction

Tea is one of the most consumed beverages in the world because of its pleasant taste, aroma, flavor and potential health-promoting properties. It gained its popularity in recent years mostly by the increased range of new flavors in the market and the several studies of tea healthy properties (Sadowska-Rociek et al., 2014; Godoy et al., 2013).

Tea health benefits have been widely studied and there are scientific evidences that tea intake can reduce cholesterol levels; reduce the risk of developing hypertension, immune disorders and Parkinson's disease; and protect against cancer, cardiovascular and nervous systems diseases. Additionally, it presents antioxidant, antimicrobial, anti-inflammatory, anti-allergy and anti-obesity effects (Wheeler & Wheeler, 2004; Liu et al., 2000; Bae et al., 2015; Gramza-Michałowska et al., 2016; Rashid et al., 2016).

The composition of tea varies with the type of processing, leaves age, species, origin and climate. Moreover, each type of tea has its characteristic taste, health properties and different levels of caffeine. One of the main plants used as raw material for tea is *Camellia sinensis* from which green, white and black teas are obtained. The difference among them is how they are processed (Alonso, 1998; Sadowska-Rociek et al., 2014).

Green tea is not fermented and it is prepared from young leaves that are exposed to steam and high temperatures with the purpose to inactivate the oxidizing enzymes (Sajilata et al., 2008). Black tea is fully fermented and has a high level of caffeine, its preparation consists of the dehydration of the leaves and fresh stems, fermentation and drying through total evaporation of the

water present on the leaves and stems. After this stage, leaves are fermented by enzymatic oxidation process and are exposed to high temperatures until acquiring a hard and crumbly consistency (Alonso, 1998; Balentine et al., 1997). White tea is the purest and it is submitted to a minimum amount of processing. White tea is an unfermented tea, it is prepared from young leaves picked before the buds have fully opened, and that did not suffer oxidation. The process consists of drying, dehydration and heating (Silva et al., 2012).

Mate tea, also known as yerba mate, is a traditional South America tea made from leaves of *Ilex paraguariensis*, and its process involves three steps: crushing, drying and toasting of the leaves (Silva et al., 2012).

Caffeine is a methylated xanthine alkaloid derivative identified as 1,3,7-trimethylxanthine and it is naturally present in several foods. According to a caffeine consumption review, it is one of the most consumed dietary ingredients in the world for which tea and coffee are the main prominent sources (Heckman et al., 2010). Caffeine can affect the tea quality characteristics, such as taste, contributing to the acidity and conferring astringency and bitterness (Farah & Donangelo, 2006; Clifford, 1985). Several studies have reported the ability of caffeine to improve mood, attention, performance, alertness, speed at which information is processed and reaction time. In addition, a higher caffeine intake was associated with lower Parkinson's disease risk (Cysneiros et al., 2007; Dawkins et al., 2011; Liu et al., 2012; Peeling & Dawson, 2007).

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Moderate caffeine consumption may bring some health benefits; however, excessive intake of caffeine can increase the risk of tachycardia, dehydration, anxiety, headaches, sleep disorders, nervousness, tremors, indigestion and dizziness (Ruxton, 2008; Gramza-Michałowska et al., 2016; Tefera et al., 2016). Over the years, different studies have been raising concerns related to its ingestion by specific groups, especially pregnant women, children and heavy coffee consumers (Knight et al., 2004; Fitt et al., 2013; Crozier et al., 2012). Nawrot et al. (2003) reported that, in healthy adults, a daily caffeine intake of up to 400 mg day<sup>-1</sup> is not associated with adverse effects.

Since tea consumption is growing in Brazil and data regarding the quality of the product domestically marketed are still scarce, this study aims to evaluate the levels of caffeine in teas sold in Brazil and their infusions, the transference rate of this compound during brewing and estimate caffeine intake from tea infusion.

## 2 Materials and methods

### 2.1 Reagents and chemicals

Caffeine analytical standard was purchased from Sigma-Aldrich (St Louis, MO, USA). The following solvents (HPLC grade) and reagents were used in the analysis: methanol (J.T. Baker, Phillipsburg, NJ, USA), magnesium oxide, NaH<sub>2</sub>PO<sub>4</sub>, hydrochloric acid, ammonium hydroxide, zinc acetate and potassium ferrocyanide (Synth, Diadema, SP, Brazil), glacial acetic acid (J.T. Baker, Phillipsburg, NJ, USA) and phosphoric acid 85% (Ecibra, Santo, SP). Agilent 0.45 µm filters were used for filtering the extracts before the chromatograph injection. Water was purified on a Milli-Q system (Millipore Co., Milford, MA, USA).

### 2.2 Samples

Samples of industrialized teas leaves were purchased at stores located in the city of Campinas in São Paulo state. Samples analyzed were from 3 batches of tea types (mate, white, black and green) from 3 different brands, totalizing 36 samples of tea (leaves) as well as their respective infusions.

For the infusion sample preparation, 0.75 g of tea leaves was weighed and 100 mL of boiling water was added. The recipient remained covered for 3 minutes and subsequently the mixture was filtered.

### 2.3 Method

Method was based on the one described by Alves & Bragagnolo (2002). The samples were homogenized and 0.5 g of tea (leaves) or 5.0 mL of the infusion were transferred to an Erlenmeyer flask and 1 g of magnesium oxide and 50 mL of water were added. The mixture was heated on a hot plate for 15 minutes with agitation. After heating, the mixture was cooled in a water bath at room temperature, filtered in filter paper in a 100 mL volumetric flask and volume was completed with water. Solution was filtered with a 0.45 µm filter for further chromatographic analysis.

### 2.4 Chromatographic analysis

Analyses were carried out using a Shimadzu HPLC system equipped with a LC-20AT quaternary pump, in-line degasser, a Rheodyne 7725i injector (20 µL sample loop), and a diode array detector SPD-M20A (detection at 272 nm). LC solution software was used for data acquisition and processing. For separation, a C18 column (Merck Lichrosphere 100, 250 x 4 mm, 5 µm particle size) was used with a methanol:water (30:70) mobile phase at a flow rate of 1 mL/min. The compounds were quantified using external calibration curves. The analyses were performed in duplicate.

### 2.5 Method validation

Method was validated according to INMETRO (Instituto Nacional de Metrologia, Normalização e Qualidade Industrial, 2016) guidelines.

Triplicate HPLC injections of eight concentration levels (0.001 to 0.2 mg/mL) of caffeine standard solutions, in water, were used to construct linear regressions lines. Accuracy and precision data were obtained through recovery studies where a chamomile tea was used as blank sample. Tests were carried out by spiking the blank sample with caffeine standard solutions at three concentration levels (0.5, 2.0 and 3.0 g/100 g and 0.003, 0.02 and 0.04 g/100 mL for infusion) in six replicates. Precision of the method was evaluated through the relative standard deviation (RSD) of recovery analyses. The limits of detection (LOD) and quantification (LOQ) were estimated as 3.3 and 10 times, respectively, the ratio between the standard deviation of the blank response and the slope of the calibration curve.

### 2.6 Statistical analysis

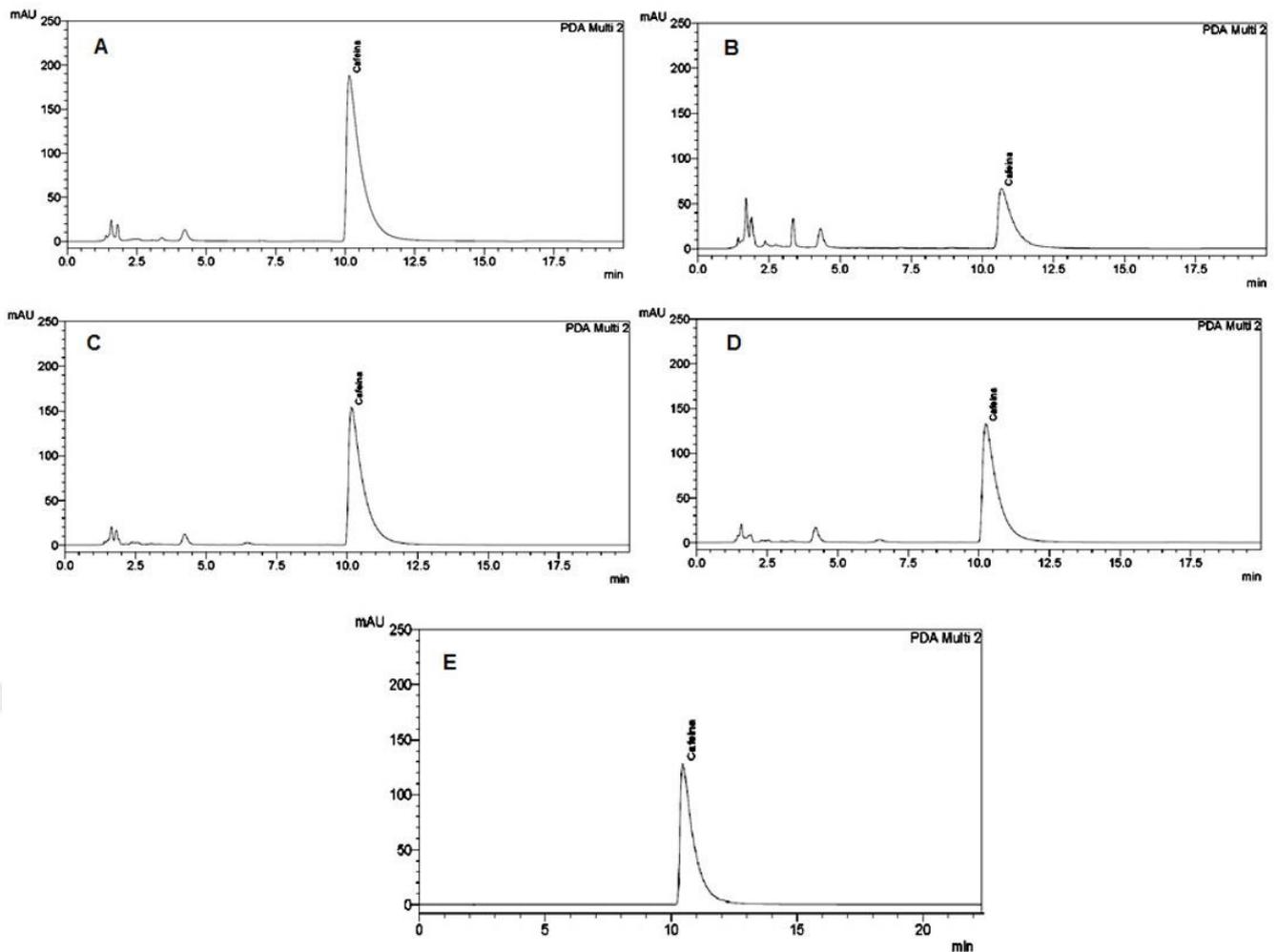
Data was processed by analysis of variance one-way ANOVA with means comparison (Tukey test) with 95% confidence using software Statistica (Statistica 5.5, Stat Soft Inc.).

### 2.7 Estimated intake

In order to evaluate the exposure of the population to caffeine through the consumption of tea, the *Survey on Household Budgets* (Instituto Brasileiro de Geografia e Estatística, 2010) was used as source of information on tea consumption. The *Survey on Household Budgets* is a survey conducted by the Instituto Brasileiro de Geografia e Estatística (IBGE) among selected households covering urban and rural areas of all 27 Brazilian states, during 12 months (May 2008-May 2009). The survey presents the amount of food and beverage acquired for household consumption (per capita/year) by the Brazilian population of different geographical regions.

## 3 Results and discussion

Figure 1 presents chromatograms of tea leaves samples illustrating peak separation. Calibration curve obtained was linear, with a correlation coefficient of 0.9998. LOD, LOQ, recovery and precision obtained for caffeine analysis in teas are shown in Table 1. LOD and LOQ were 0.001 and 0.002 g/100g



**Figure 1.** HPLC chromatograms of white (A), mate (B), black (C) and green (D) teas and standard solution (E). C18 column (Merck Lichrosphere). Detection by UV (272 nm). Mobile phase: methanol:water (30:70), flow rate: 1 mL/min.

**Table 1.** Validation parameters (accuracy, precision (RSD), limits of detection (LOD) and quantification (LOQ)) for caffeine analysis in teas and infusions.

	LOD <sup>1</sup>	LOQ <sup>1</sup>	Spike level <sup>1</sup>	Recovery (%) (n=6)	RSD (%)
<b>Tea leaves</b>	0.001	0.002	0.5	97	0
			2.0	97	1
			3.0	98	2
			0.003	87	8
<b>Infusion</b>	0.001	0.002	0.02	92	1
			0.04	96	1

<sup>1</sup>g/100g for tea leaves and g/100mL for infusion.

for tea leaves (g/100 mL for infusion), respectively. Recoveries ranged from 97% to 98% and 87% to 96%, for leaves and infusion, respectively, while RSDs varied from 0% to 8%. These results are considered satisfactory for determinations at the studied levels according to the criteria recommended by INMETRO (Instituto Nacional de Metrologia, Normalização e Qualidade Industrial, 2016).

Table 2 presents the average, minimum and maximum levels of caffeine in tea leaves samples. Mate tea was the one that presented lowest caffeine levels, from 6.1 to 13.2 mg/g. On the other hand, white, green and black teas, from *Camellia sinensis*, presented higher levels ranging from 21.5 to 31.0 mg/g, 18.3 to 25.3 mg/g and 14.3 to 34.8 mg/g, respectively. These values are similar to the ones reported for other potential sources of caffeine intake. In roasted coffee beans Tfouni et al. (2012) detected caffeine levels ranging from 11.10 to 18.84 mg/g. In guarana powder, which is usually used as a source of energy, caffeine levels may vary from 14.18 to 28.79 mg/g (Tfouni et al., 2007). Bae et al. (2015) reported similar results of caffeine levels in teas, 20.50 mg/g in green tea, 22.21 mg/g in black tea and 11.30 mg/g in mate tea.

By statistically analyzing the results obtained, one can verify that there are variations among different types of tea, different brands investigated and in some cases among different batches. According to the results obtained for leaves, mate tea showed statistical difference ( $P < 0.05$ ) in caffeine content, with levels being lower than the ones presented by the other teas. As for different brands from the same type of tea, there was no statistical difference ( $P < 0.05$ ) in caffeine levels between different brands

**Table 2.** Levels of caffeine in tea samples (leaves and infusion) of different brands and batches, caffeine percentage of transference from leaves to infusion and average caffeine intake.

Sample	Caffeine <sup>1</sup> (range)		Transference (%)	Caffeine intake (mg/day) <sup>2</sup>
	Leaves (mg/g)	Infusion (mg/100 mL)		
Mate A	6.2 (6.1-6.4)	4.0 (3.7-4.2)	85.2	7.3
Mate B	8.4 (7.2-9.4)	5.1 (4.1-6.2)	80.9	9.3
Mate C	10.8 (9.4-13.2)	6.8 (6.3-7.7)	84.9	12.4
Black A	22.4 (21.2-23.9)	12.3 (11.5-12.8)	72.9	22.5
Black B	31.7 (23.6-34.8)	14.9 (13.1-18.1)	64.4	27.2
Black C	18.7 (14.3-23.0)	9.6 (8.1-10.4)	69.7	17.5
Green A	21.8 (19.9-23.5)	11.8 (11.5-12.1)	72.7	21.5
Green B	24.1 (22.2-25.3)	9.5 (7.4-12.8)	51.5	17.3
Green C	20.4 (18.3-22.0)	10.9 (9.5-12.1)	71.9	19.9
White A	30.1 (29.1-31.0)	14.1 (12.4-15.4)	62.5	25.7
White B	26.4 (25.4-27.3)	12.5 (7.9-14.9)	75.6	22.8
White C	23.2 (21.5-26.2)	10.5 (9.2-12.7)	60.4	19.2

<sup>1</sup>Average of 3 batches in duplicate (n=6); <sup>2</sup>Considering a consumption of 182.6 mL day<sup>-1</sup> (Instituto Brasileiro de Geografia e Estatística, 2010).

of mate, green and black teas. White tea was the only one that showed significant difference among the evaluated brands. When considering different batches of the same brand, there was significant difference ( $P < 0.05$ ) in 42% of the cases. Difference were found in brands B and C of mate tea, brand B of black tea, brand A of green tea and brand C of white tea. These variations may occur because: a) teas commercialized in Brazil are usually a blend of teas from different origins and producers and b) caffeine occurs naturally in teas, and its content may vary according to the variety of plants, environmental growth conditions, seasonal variations and age of the leaves (Athayde et al., 2000; Heckman et al., 2010).

The caffeine levels in tea infusions and the percentage of transference from leaves to infusion during the preparation of the beverage were also evaluated. As can be seen on Table 1, the infusions followed the same pattern of the leaves, with mate tea presenting lower caffeine levels (3.7 to 7.7 mg/100 mL for infusion) than white (7.9 to 15.4 mg/100 mL), green (7.4 to 12.8 mg/100 mL) and black teas (8.1 to 18.1 mg/100 mL). Although caffeine levels in the tea leaves and in roasted coffee beans are similar, the teas infusions present a much lower concentration than the ones reported for coffee brew, ranging from 87.3 to 192.0 mg/100 mL (Tfouni et al., 2014). This can be explained by the fact that coffee brew is usually prepared with a ratio of 50g coffee to 100 mL of water, while for tea the ratio is of 0.75 g to 100 mL. Results obtained in the present study are in accordance with the ones that have been reported by Rostagno et al. (2011), with an average of caffeine levels in mate tea of 4.0 mg/100 mL, in white tea of 14.0 mg/100 mL, in green tea of 13.0 mg/100 mL and in black tea of 8.9 mg/100 mL.

As can be observed in Table 1, caffeine percentage of transference from leaves to the infusion varied from 51.5 to 85.2%, with the highest rates of transference occurring in the mate tea samples (80.9 to 85.2%). As in this study time and temperature of the brewing were controlled, one possible explanation for these differences could be different sizes of tea particles which can vary among types and brands of tea, where smaller particles would facilitate caffeine extraction during infusion preparation.

In order to evaluate the exposure of the population to caffeine through the consumption of tea, an estimate of the intake of this substance was carried out based on the obtained results and tea consumption data. For this purpose, it was considered the average values of caffeine detected in the infusions and tea consumption data from the *Survey on Household Budgets* (Instituto Brasileiro de Geografia e Estatística, 2010). The average tea leaf consumption in Brazil according to IBGE (Instituto Brasileiro de Geografia e Estatística, 2010) is 0.50 kg per capita a year, which results in 182.6 mL of infusion per person a day. Taking in account this daily tea intake, the caffeine intakes from the different tea infusions analyzed would range from 7.3 to 27.2 mg/person/day (Table 1). IBGE (Instituto Brasileiro de Geografia e Estatística, 2010) research also reports tea consumption by regions. Northeast is the region with lowest tea intake, 18 g/person/year, which is equivalent to a tea infusion intake of 6.6 mL/person/day. Considering the highest caffeine level detected (18.1 mg/100 mL), the Northeast population would have an estimated caffeine intake from tea of 1.2 mg/person/day. On the other hand, the South region of the country is shown to be the largest tea leaf consumer, 2.894 kg per capita a year, corresponding to an infusion daily intake of 1.1 L per capita. When building a worst case scenario considering this consumption and the highest caffeine content, caffeine intake would be of 191.4 mg/person/day.

The estimated intakes obtained are below the moderate daily intake of caffeine for healthy adults without adverse effects that is up to 400 mg/day (equivalent to 6 mg/kg body weight/day for a 65 kg person) (Nawrot et al., 2003). However, considering that teas represent only one of the many caffeine sources in the diet, this intake could be of concern, especially for heavy drinkers.

#### 4 Conclusions

The teas from *Camellia sinensis* presented higher levels of caffeine than mate tea. There were variations among types of tea, brands and batches; and a great part of caffeine present in the leaves was shown to be transferred to the infusion during preparation. The average daily intake of 263 mL of tea could contribute for an ingestion of up to 47.6 mg day<sup>-1</sup> of caffeine on

the diet. Although estimated daily intakes of caffeine from tea are lower than the ones estimated from coffee brew (198.2 to 435.8 mg) and guarana powder (551 mg) (Tfouni et al., 2007, 2014), tea may be considered an important source of caffeine intake for heavy tea drinkers, being responsible, in the worst case scenario, for 48% of the daily intake without adverse effects (400 mg day<sup>-1</sup>).

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