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The Chlorogenic Acid and Caffeine Content of Yerba Maté *(Ilex paraguariensis)* Beverages

Deborah H. Markowicz BASTOS *, Ana Claudia FORNARI, Yara S. de QUEIROZ,
Rosana Aparecida Manolio SOARES & Elizabeth A.F.S. TORRES

*Nutrition Department, School of Public Health, University of São Paulo,
Av. Dr. Arnaldo, 715. CEP 01246-904 São Paulo, Brasil*

SUMMARY. The contents of caffeine and 5-caffeoylquinic acid (5 cqa) in three yerba maté beverages (chimarrão, tererê and maté tea) were determined in this study. Analyses were performed by HPLC. Yerba maté *(Ilex paraguariensis)* is widely consumed in South America. It is rich in phenolic acids, which are absorbed by the body and may act as antioxidants or as free radical scavengers. One "cuia" (the apparatus used to drink chimarrão and tererê, comprising 500 mL) of chimarrão contains 135 mg of caffeine and 226 mg of 5-cqa. One "cuia" of tererê contains 85 mg of caffeine and 163 mg of 5-cqa. One cup (182 mL) of maté tea contains 13 mg of caffeine and 16 mg of 5-cqa. These data can be used to establish the dietary intake of bioactive compounds in these beverages.

RESUMEN. "Contenido de Ácido 5-Cafepoilquínico y Cafeína en Bebidas de Yerba Mate *(Ilex paraguariensis)"*. El objetivo de este trabajo fue el de analizar el contenido de cafeína y ácido 5-cafeoilquínico (5-cqa) en bebidas tales como chimarrão, tererê y maté té. La determinación de estos compuestos se realizó por HPLC. Las bebidas a base de yerba mate son de gran consumo en América del Sur, contienen grande cantidades de compuestos bioactivos, entre ellos cafeína, que posee actividad estimulante, y los ácidos clorogénicos, que actúan como antioxidantes. Una "cuia" de chimarrão de 500 mL contiene 135 mg de cafeína y 226 mg de 5-cqa y una "cuia" de tererê contiene 85 mg de cafeína y 163 mg de 5-cqa. Una taza de182 mL de té ("mate cocido") contiene 13 mg de cafeína y 16 mg de 5-cqa. Estos datos pueden ser utilizados para determinar el consumo de compuestos bioactivos de estas bebidas.

INTRODUCTION

The role of food and nutrition in the maintenance of human health, providing nutrients as well as other substances that can reduce the incidence of non-transmissible diseases, is a contemporary concern [1-4]. To conciliate low cost and well-accepted food items which may provide antioxidant substances in a regional diet is a challenge in public health [4-6]. The availability of accurate and complete data on food composition is crucial to estimate the dietary intake of bioactive substances. It is the core of epidemiological studies aiming to establish the relationship between dietary habits and risk of cardiovascular diseases, cancer and other pathological conditions [7].

Yerba maté *(Ilex paraguariensis)* is widely consumed in South America. It is rich in several bioactive compounds such as phenolic acids, which are absorbed by the body and may act as antioxidants or as free radical scavengers [8-12]; caffeine, responsible for yerba maté's stimulating properties long known by the native Indians in South America; and saponins, which may explain yerba maté's popular use as both a choleretic and a digestant [13,14]. Yerba maté is the raw material of three different types of beverages: the *chimarrão,* widely consumed in the South of Brazil, Uruguay, Argentina and Paraguay; the *tererê*, consumed in the central west of Brazil and Paraguay and the *mate tea,* consumed in the south-east of Brazil, Argentina and Uruguay. Both chimarrão and tererê are prepared with dried green maté leaves. Chimarrão

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* Author to whom correspondence should be addressed. *E-mail:* dmbastos@usp.br

is prepared with hot water and tererê is prepared with cold water. The maté tea is prepared with roasted leaves and brewed as any other herbal tea.

Published data on the phenolic and caffeine content of yerba maté beverages are scarce and refer mainly to chimarrão [15-17]. Studies show a wide variation in this content, resulting from the exposure of plants to different climate, growing and processing conditions, as well as from different brewing methods [16-20]. The aim of this study is to obtain data on the caffeine and 5-caffeoylquinic acid content of three yerba maté beverages: chimarrão, tererê and maté tea. There are no available data on the 5-cqa content of maté tea. These data can be used to establish the dietary intake of bioactive compounds in these beverages.

MATERIAL AND METHODS

Material

The samples were purchased in two hypermarkets in São Paulo. Four different brands of maté tea were purchased in boxes with 10 teabags each. Two different lots of each brand were analyzed. Three different brands of yerba maté, purchased in 1 kg paper bags, were analyzed.

Methods

Preparation of the maté tea infusion

Infusions were prepared according to the manufacturer's instructions (one tea bag per cup of hot water, brewed for 5 minutes). The final volume of 182 mL, corresponding to one cup, was adopted, as recommended by Fisberg & Villar [21]. Water temperature was 85 °C.

Preparation of chimarrão and tererê

Chimarrão was prepared according to Mazzafera [16]. One and a half grams of dried yerba maté was extracted with 60 mL of ultrapure water. The extraction was carried out in two steps (30 mL/3 min each). Tererê was prepared following a similar procedure, except that cold water (10 °C) was used.

Determination of soluble solids

A measured volume of aqueous extract (10 mL) was transferred into a tared beaker and evaporated to dryness. The residue was dried to constant weight at 105 °C in an oven.

Simultaneous determination of 5-cafeoylquinic acid and caffeine

Infusions were analyzed with no other modification than the appropriate dilution to fit the standard curves, as described by several authors [15,19,22]. A Shimadzu HPLC chromatograph, equipped with an LC-10ATvp quaternary pump, a Rheodyne manual injection valve with a 20 µL sample loop and a diode array detector SPD M10AVP, was used for the determinations. All the modules were controlled by a personal computer equipped with the HPLC System Manager software CLASS-VP. A 4.6 x 250 mm, 5 µm C18 Microsorb column was used for the separation. The analytical determination of caffeine, caffeic acid and 5-cafeoylquinic acid was carried out by means of high-performance liquid chromatography using a two-solvent isocratic elution as previously described by Mazzafera [16]. The composition of the solvents was: (A) water/acetic acid (99.5:0.5 v/v) and (B) methanol. The mobile phase composition was 75% of solvent A and 25% of solvent B. The flow rate was 1 ml/min. Data were obtained at 272 nm for caffeine and 323 nm for phenolic acids. Identification was based on the comparison of the spectra obtained between 250-350 nm and the retention time of the unknown substances in relation to that of pure standards. Peak purity, which is determined using the average of similarity of the apex vs. the upslope spectrum and the apex vs. the download spectrum performed by the photodiode array detector, was higher than 0.99 for both compounds. Quantification was achieved by external calibration, using a five-point curve of different dilutions of a standard solution. Pearson's correlation coefficient (r) was always > 0.99. A typical chromatogram, obtained in the conditions described above, is shown in Fig. 1.

Data analysis

Data were analyzed by ANOVA and Tukey's test using the statistical package SPSS v. 11.0 for Windows.

RESULTS AND DISCUSSION

One cup (182 mL) of maté tea contains an average of 13 mg of caffeine and 16 mg of 5-cqa (Table 1). Among the four commercial brands studied, only one showed significant difference ($p < 0.05$) for the caffeine and 5-cqa content, which was almost 40-50% higher than that of the other brands (Table 2). Maté tea ingestion contributes to a lower caffeine intake when compared to percolated coffee (Camargo & Toledo [23]).

A "cuia" of chimarrão (500 mL) contains an average of 135 mg of caffeine and 226 mg of 5-cqa.

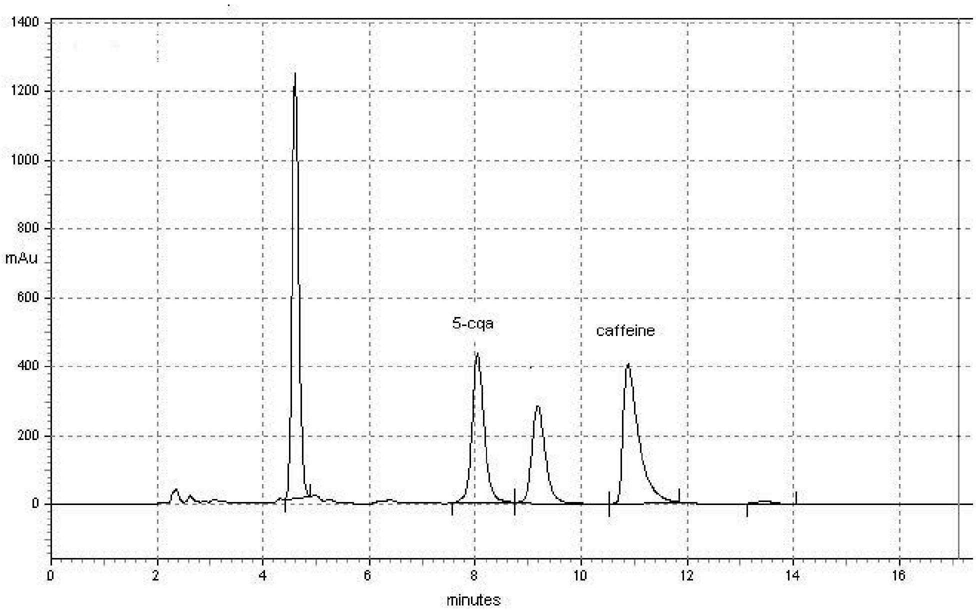

Figure 1. HPLC-Reverse Phase isocratic elution profile of chimarrão, tererê and maté tea.

	Caffeine intake (mg)			5-cqa intake (mg)		
	Maximum	**Minimum**	**Average**	**Maximum**	**Minimum**	**Average**
Chimarrão	165	101	135	232	214	226
Tererê	102	56	85	186	132	163
Maté tea	20	8	13	23	11	16

Table 1. Mean caffeine and 5-cqa content in one "cuia" of chimarrão (500 mL) and in one cup of maté tea (182 mL).

	caffeine µg/mL	5-cqa µg/mL	soluble solids mg/mL	Caffeine+ 5-CQA in soluble solids (%)
brand 1*	45.8a (18.3)	59.7a (13.8)	1.9a (0.3)	5.6
brand 2*	69.3a (32.5)	68.7a (34.2)	2.0a (0.4)	6.9
brand 3*	63.6a (17.0)	87.3a (12.8)	2.2a (0.2)	6.9
brand 4*	111.3b (5.8)	126.9b (15.6)	2.7b (0.1)	8.8
mean value (n =24)	72.5 (31.2)	86.1 (34.6)	2.2 (0.4)	7.2

Table 2. The caffeine, 5-cqa and soluble-solid content of the different maté tea brands.

*Average of six determinations. Standard deviation (n = 6) in parenthesis. In each column, different superscripts indicate significant difference ($p < 0.05$) among the different brands.

Tererê contains a lower amount of caffeine and 5-cqa when compared to chimarrão. One "cuia" of tererê contains 85 mg of caffeine and 163 mg of 5-cqa (Tables 1 and 3).

	Beverage	caffeine µg/mL	5-cqa µg/mL	soluble solids mg/Ml	Caffeine + 5-CQA in soluble solids (%)
brand 1 *	chimarrão	329.4a (13.6)	464.6a (17.1)	7.4a (0.1)	10.7
	tererê	204.2b (13.9)	339.4b (20.5)	5.7b (0.3)	9.5
brand 2 *	chimarrão	280.2a (22.4)	463.4a (37.4)	7.8a (0.1)	9.5
	tererê	195.7b (25.3)	370.7a (45.3)	7.1a (0.5)	8.0
brand 3 *	chimarrão	201.3a (13.4)	427.0a (40.4)	7.3a (0.2)	8.6
	tererê	111.2b (31.0)	264.9b (60.3)	4.8b (0.8)	7.8
mean value (n=9)	chimarrão	270.3 (57.9)	451.7 (34.3)	7.5 (0.3)	9.6
mean value (n=9)	tererê	170.4 (49.3)	325.1 (61.2)	5.9 (1.1)	8.4

Table 3. The caffeine, 5-cqa and soluble-solid content of chimarrão and tererê.
*Average of three determinations. Standard deviation (n = 3) in parenthesis. In each column, different superscripts indicate significant difference ($p < 0.05$) among the different brands of each beverage.

The habit of drinking chimarrão or tererê implies the consumption of large volumes of these beverages per day. According to Castellsagué *et al.* [25], heavy chimarrão drinkers may ingest more than 1.5 L of this beverage per day, which corresponds to the consumption of 405 mg of caffeine and 678 mg of 5-cqa.

There was no significant difference ($p > 0.05$) among the three brands of chimarrão or tererê when comparing either the caffeine or the 5-cqa content.

Phenolic compounds are responsible for many desirable physiological effects. Antioxidant activity is the most often reported one, but another range of activities whose mechanisms of action are not yet clear, is also described [3,4]. On the other hand, Olthof *et al.* [24] showed that an intake of 2 g of chlorogenic acid per day, corresponding to 1.5 L of strong coffee, raises plasma homocysteine concentrations in humans, which might cause cardiovascular risk. Coffee drinkers consume up to 1g of chlorogenic acid per day, and might be subject to this effect, indicating that chlorogenic acid is not the only homocysteine-raising factor in coffee.

Soluble solids in beverages are shown in Tables 1 and 2. Caffeine and 5-cqa represent 5.6-8.8% of the total of solids present in maté tea, 7.8-9.5% of the total of solids present in tererê and 8.6-10.7% of the solids present in chimarrão.

CONCLUSIONS

Yerba maté beverages are low cost sources of phenolic acids, representing an important phenolic source for the South American population that has the habit of consuming this product. One cup of maté tea contains an average of 16 mg of 5-caffeoylquinic acid; one “cuia” of chimarrão contains an average of 27 mg of 5-caffeoylquinic acid; and tererê contains an average of 20 mg of 5-caffeoylquinic acid (for the beverage prepared with 1.5 g of yerba maté brewed with 60 mL of water). These beverages contain a lower amount of caffeine if compared to other beverages rich in phenolic acids, such as coffee or black tea, and should be indicated in cases of caffeine sensitivity. The 5-caffeoylquinic acid content of these beverages is also lower than that of coffee or black tea. The physiological effects of the consumption of these beverages in the studied doses should be analyzed *in vivo*.

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