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Maté drinking: caffeine and phenolic acid intake

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Herbal maté tea, known locally as 'chimarrão', is a stimulating beverage traditionally consumed by the Gauchos of South America. Infusions were prepared according to the method used by the Gauchos, and caffeine, chlorogenic acids and caffeic acid were determined in unhydrolysed and hydrolysed samples. An intake of 260 mg of caffeine, 240 mg of chlorogenic acids and 170 mg of caffeic acid was estimated, exceeding by far the intake recorded in the literature for other beverages containing these compounds. The factors influencing the concentrations of these compounds in the infusions indicate that higher amounts could be ingested. © 1997 Elsevier Science Ltd.

INTRODUCTION

Maté tea, an infusion known for its stimulating properties, is prepared from the leaves of *Ilex paraguariensis*, a member of the holly family. The beverage is prepared by compacting a certain amount of maté, previously moistened with water, against the wall of a vessel made from a gourd or 'cuia'. The vessel is filled with hot, but not boiling, water, taking care not to disturb the laterally disposed cake of maté. The infusion is drunk by sucking through a silver pipe or 'bomba', which has a flattened perforated disc at the end immersed in the infusion to act as a filter. For the same batch of maté, hot water can be added more than twice. The Brazilian popular name for herbal maté tea is 'chimarrão'.

Maté tea was first used by South American indians as a tonic and stimulating drink. Today it is known that maté leaves contain caffeine, explaining at least part of the observed physiological effect. Nowadays, the beverage is traditionally consumed by the Gauchos of South America, mainly Argentina, Southern Brazil, Paraguay and Uruguay (Alikaridis, 1987; James, 1991).

Most of the maté that is produced is consumed in South America. However, in the last few years, the USA, Germany and Japan started to import maté at a rate of 1000 tons per year (Tormen, 1995).

Besides caffeine, investigations on the chemical composition have shown that significant amounts of phenolic compounds can also be found in maté leaves. Until 1935, several researchers believed that tannin was present in maté leaves. However, Woodard and Cowland (1935) showed that, instead of a genuine tannin, maté contained caffetannin (a pseudo-tannin) which when hydrolysed yielded caffeic acid. Descartes (1956), Descartes and Brooks (1953) and Roberts (1956) showed that chlorogenic acids were also present in significant quantities in maté leaves. More recently, Clifford and Ramirez-Martinez (1990) reported that maté samples contained up to 9.8% of total chlorogenic acids (mono- and di-caffeoylquinic acids). All samples analysed by Clifford & Ramirez-Martinez were purchased in the UK and Germany and, by their description, we believe that only one was a typical South American processed maté (herbal maté tea). This sample had 2.89% of total chlorogenic acids and 0.89% of caffeine, on a dry weight basis.

Although several reports have indicated the presence of caffeine and chlorogenic acids in maté leaves, no study has dealt with the intake of caffeine and soluble phenolics, in particular, caffeic and chlorogenic acids, when an infusion is prepared according to the South American habit.

MATERIALS AND METHODS

Maté samples

Samples of herbal maté were purchased from local markets or they were gifts from colleagues. All samples are produced by Brazilian companies.

Sample	Origin (city and Brazilian state)
1	Laranjal do Sul, Paraná
2	Catanduvas, Santa Catarina
3	Santa Rosa, Rio Grande do Sul
4	Catanduvas, Santa Catarina
5	Curitiba, Paraná
6	Clevelândia, Paraná

7	Prudentópolis, Paraná
8	Cascavel, Paraná
9	Xaxim, Santa Catarina
10	Marmeleiro, Paraná
11	Palmas, Paraná
12	Santa Maria D'Oeste, Paraná
13	Cascavel, Paraná
14	Campo Erê, Santa Catarina
15	Cascavel, Paraná
16	Clevelândia, Paraná
17	Curitiba, Paraná
18	Clevelândia, Santa Catarina

Preparation of the infusions

The infusions of the maté samples were prepared in an attempt to imitate (in the laboratory) the proportions and the way maté tea is prepared by the Gauchos in South America. Maté samples (1.5 g) were weighed into 50 ml Erlenmeyer flasks, moistened with 2 ml of tap water and pressed against the corner of the flask. Then, 30 ml of 85°C tap water were carefully added so as not to disturb the pressed maté and left to cool to 55°C. The water was carefully drained off and a new addition of 30 ml of 85°C tap water was made. After further cooling and draining off of the water, the first and second extracts were combined and filtered.

Acid hydrolysis of the extracts and paper chromatography

Aliquots (400 μl) of the combined extracts were hydrolysed with the same volume of 4 M HCl in a boiling water-bath for 40 min in cap-sealed tubes, and extracted with ethyl acetate (5×5 ml) (Harborne, 1989). After reduction of the volume, they were kept at −18°C.

Unhydrolysed and hydrolysed samples were applied to Whatman 3MM chromatography paper (PC) and developed with Forestal solvent. Visualization of the compounds separated by PC was done under UV light (254 nm) with or without NH_4 vapour, and spraying with Folin–Ciocalteau reagent.

Caffeine and phenol analysis

Hydrolysed and unhydrolysed extracts were analysed for soluble phenols with the Folin–Ciocalteau reagent (Swain & Hillis, 1959) using phenol (phenic acid) as a standard.

For caffeine analyses, 100 mg of MgO was added to 1 ml aliquots of unhydrolysed extracts in Eppendorf tubes, heated on a water-bath at 80°C for 30 min, centrifuged, and the supernatant filtered through 0.45 μm filters. The caffeine concentration was estimated by high-performance liquid chromatography (HPLC) using a Shimadzu HPLC system (Shimadzu Corp., Japan). The solvent was delivered by a pump (Model LC-10AS) and the caffeine eluting from the column was monitored using a UV detector (Model SPD-10A) operating at 280 nm. Caffeine was isocratically separated on a reversed-phase Supelcosil-C18 column, 4.5 mm×150 mm, 5 μm (Supelco Inc., USA), using 15% methanol in 0.5% aqueous acetic acid at a flow rate of 1 ml min^{-1}. The signals from the UV detector were integrated using a Shimadzu C-R6A Chromatopac recorder and were compared to those of pure caffeine (Sigma grade).

Aliquots (40 μl) of the hydrolysed samples were dried under a steam of nitrogen, dissolved in methanol (50 μl) and the volume raised to 400 μl with distilled water. Chlorogenic acids and caffeic acid were analysed by HPLC, with 20% methanol in 0.5% aqueous acetic acid (pH adjusted to 2.5 with HCl), at a flow rate of 1 ml min^{-1}. The UV detector was set at 313 nm and pure (Sigma grade) 5-caffeoylquinic acid (5CQA) and caffeic acid were used as standards.

RESULTS AND DISCUSSION

Despite its large consumption by South Americans, most of the herbal maté tea is still produced from wild growing plants. *I. paraguariensis* is the main source of leaves; however, small percentages of leaves from other indigenous species are also used (Alikaridis, 1987). Therefore, genetic variability, environmental conditions and even different maté species being mixed together would affect the caffeine and phenol content in the samples analysed here.

Great variation in caffeine and soluble phenols was found in the infusions (Table 1), and it was not even possible to group them according to the locality (Brazilian state) where they were produced.

Table 1. Concentrations of soluble phenols and caffeine, and the quantities of wood in the samples of herbal maté tea used to prepare the infusions

Sample number	Soluble phenols (mg ml^{-1})	Caffeine (mg ml^{-1})	Wood (g per 1.5 g)
1	0.81	0.42	1.28
2	1.19	0.57	0.57
3	0.94	0.38	0.65
4	1.60	0.49	0.45
5	0.78	0.33	1.11
6	1.49	0.37	0.49
7	1.00	0.56	0.66
8	1.39	0.52	0.64
9	1.37	0.68	0.50
10	1.11	0.54	0.65
11	1.07	0.62	0.52
12	1.36	0.54	0.57
13	0.80	0.64	0.65
14	1.18	0.63	0.57
15	0.70	0.49	0.58
16	1.60	0.79	0.46
17	1.06	0.50	0.42
18	0.90	0.29	0.65

Although it is said that the herbal maté is produced from leaves, it is common to find, in the Brazilian maté, more than 30% of wood (Tormen, 1995). These are small pieces of the stems that are not separated during the processing. We used a sieve (0.5 mm aperture) to quantify the wood in the samples (Table 1) and correlated these data with the caffeine and phenol concentrations in the infusion. The correlation established for caffeine was -0.44 ($P < 0.05$) and -0.58 ($P < 0.05$) for phenols. This was to be expected, at least for caffeine, since its content in the leaves is much higher (≈ 10 mg g^{-1}) than in the wood (0.6 mg g^{-1}) (Mazzafera, 1994).

Aliquots of the prepared infusions were hydrolysed and analysed by PC and HPLC (Fig. 1). For both hydrolysed and unhydrolysed samples, the paper chromatograms showed two main spots which co-chromatographed with 5CQA and caffeic acid (Fig. 1(E)

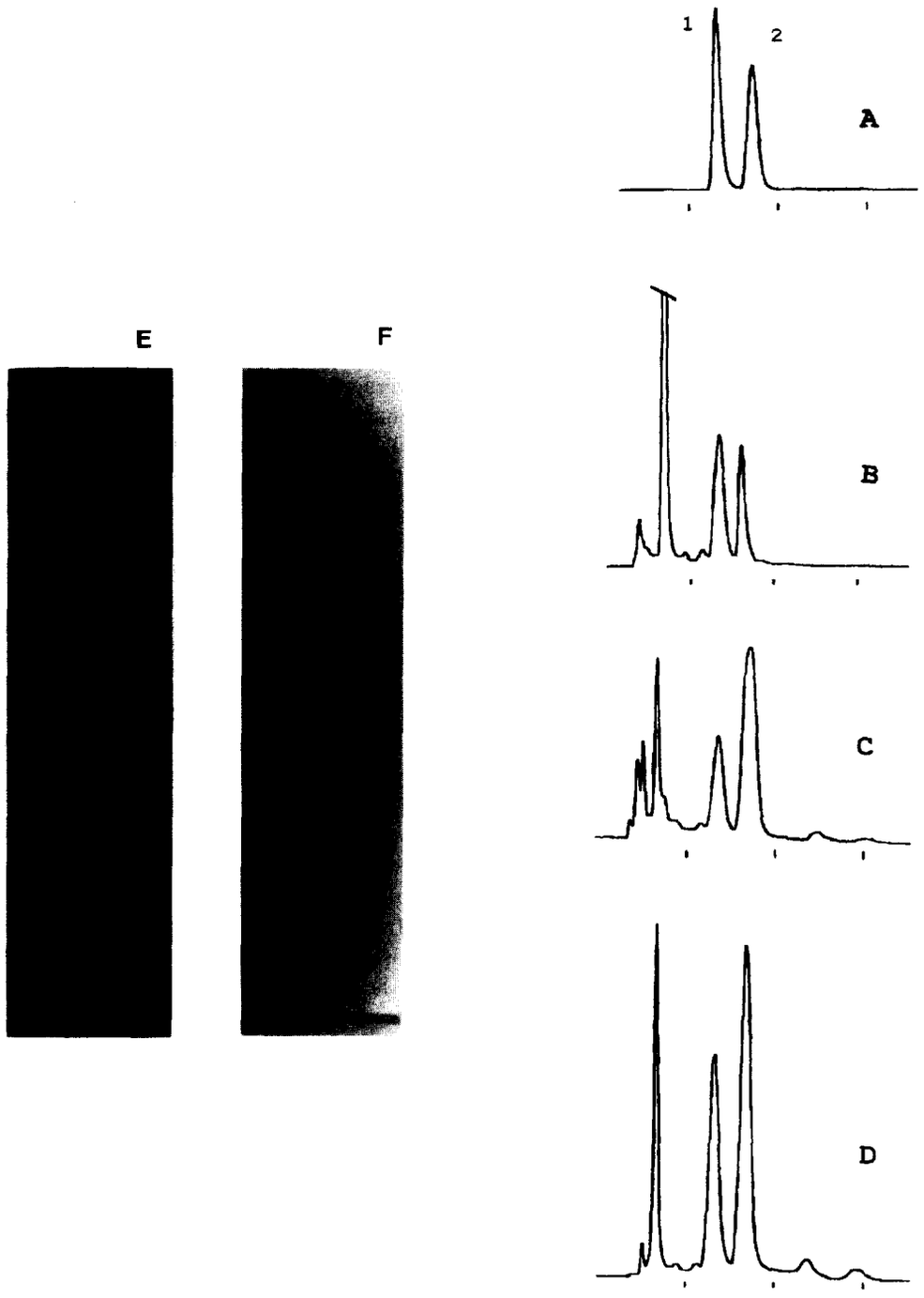

Fig. 1. (A) HPLC elution profile of standards (peak 1, 5CQA; peak 2, caffeic acid). Typical chromatography of unhydrolysed (B) and hydrolysed samples (C) with the UV monitor at 254 nm. (D) Hydrolysed sample with the UV monitor at 313 nm. Paper chromatography of hydrolysed and unhydrolysed samples visualised under UV (254 nm) with NH_4 vapour (E) and Folin–Ciocalteau reagent spraying (F). (From left to right: unhydrolysed sample, hydrolysed sample, 5CQA and caffeic acid standards.)

Table 2. Percentage of chlorogenic and caffeic acids in the hydrolysed infusions, phenol content of the unhydrolysed infusions, and calculated quantities of chlorogenic and caffeic acids in the unhydrolysed infusions

Sample number	CGA (%)	Caffeic acid (%)	Phenols (mg ml^{-1})	CGA (mg ml^{-1})	Caffeic acid (mg ml^{-1})
4	26.2	19.1	1.60	0.42	0.31
8	35.2	24.0	1.39	0.49	0.33
16	33.9	23.6	1.60	0.54	0.38

CGA, chlorogenic acid.

and (F)). When the same extracts were analysed by HPLC with the UV monitor operating at 254 nm, the chromatographic profiles were very similar (Fig. 1(C)). However, it could be observed that, proportionally, there was an increase of caffeic acid in the hydrolysed sample, and a decrease of a compound eluting earlier from the column. This peak might represent caffetannin, yielding caffeic acid under hydrolysis.

Using dilute formic acid with an acetonitrile gradient, Clifford and Ramirez-Martinez (1990) investigated the chlorogenic acids in maté, observing that, in the herbal maté sample, 5CQA was the main component. Probably because of the chromatographic conditions used here, it was not possible to separate mono- and di-caffeoylquinic acids (Fig. 1(D)). However, this also might be a result of the extraction conditions we used (85°C with cooling to 55°C). Clifford and Ramirez-Martinez (1990) analysed chlorogenic acids in samples extracted with boiling methanol (0.5 g, 2×50 ml) and in the beverage prepared according to the manufacturer (presumably boiling water). In the methanolic extract, caffeoylquinic acids account for 39% of the total chlorogenic acids. In the beverage, this value rose to 70%, with a sharp decrease in feruloylquinic acids and dicaffeoylquinic acids. Therefore, the calculations for chlorogenic acid content in the hydrolysed samples were based on the chromatography of the 5CQA standard (peak 1, Fig. 1(A)).

Since the PC and HPLC, using 254 nm detection, of the hydrolysed and unhydrolysed samples were similar, total phenols in the hydrolysed samples were estimated by the Folin–Ciocalteau method, using phenic acid as standard. From these data and the HPLC determinations for caffeic and chlorogenic acids, the percentages of these compounds in the hydrolysed samples were calculated and applied to the data of soluble phenols in the unhydrolysed samples to estimate their concentrations. Three samples, those containing the highest phenolic contents (Table 1), had caffeic and chlorogenic acids estimated in this way (Table 2).

There is not an established proportion between herbal maté and water for preparation of 'chimarrão'. Among the factors affecting the proportions, the most important are the size of the 'cuia' and regional habits. Also, for large amounts of herbal maté tea, addition of hot water to the 'cuia' can be more than three times. Consequently, it is presumed that the extraction rates of caffeine and soluble phenols are also affected. The proportion used in this work can be defined as a medium proportion, and it was based on two volumes of a small 'cuia' (2×250 ml) in which 25–30 g of herb is added. In addition, as shown in Table 1, the percentage of wood varied from 28% (sample 17) to 85% (sample 1), and this will certainly also influence the caffeine and phenol content of the beverage.

For the sample identified by Clifford and Ramirez-Martinez (1990) as herbal maté tea, and prepared according to the supplier's instructions, it was found that the caffeine and total chlorogenic acid contents per cup (assumed to be 150 ml) were 12 mg and 41 mg, i.e. 0.08 mg ml^{-1} and 0.27 mg ml^{-1}, respectively. Mean values from our data for caffeine (Table 1) and chlorogenic acids (Table 2) were 0.52 mg ml^{-1} and 0.48 mg ml^{-1}, respectively. Considering two volumes (500 ml) of the whole 'cuia', this might represent 260 mg and 240 mg. For caffeic acid, the intake might be 170 mg.

Therefore, compared to other caffeine-containing beverages, herbal maté tea prepared according to the Gauchos method provides a higher intake of this alkaloid. Burg (1975) estimated an intake of 85 mg and 60 mg of caffeine per cup of roasted and instant coffee, respectively. With respect to chlorogenic acids, Clifford and Walker (1987) reported an intake of 70–220 mg of total chlorogenic acids per cup (150 ml) of instant coffee.

Caffeine is one of the most studied drugs in terms of physiological effects on the human being, and several comprehensive reviews have been published (James, 1991; Milon *et al.*, 1988). Although chlorogenic acids are frequently found in foodstuffs, few data on physiological effects of chlorogenic acids are available (Viani, 1988). Oral ingestion of 200 mg of 5CQA stimulated stomach secretion with enhancement of hydrochloric acid production. Caffeic acid, at the same dose, had the same effect.

In conclusion, the data presented here indicate that consumption of the herbal maté tea provides a large intake of caffeine, chlorogenic and caffeic acids, greatly exceeding the intake recorded in the literature for other caffeine- and chlorogenic acid-containing beverages. The values observed here for such intake fully explain the stimulating and digestive properties attributed to herbal maté tea, so appreciated by the Gauchos.

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