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Chlorogenic Acids and Purine Alkaloids Contents of Maté (*Ilex paraguariensis*) Leaf and Beverage

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ABSTRACT

Five samples of Maté leaf have been analysed for chlorogenic acids, caffeine and theobromine, and found to comprise two distinct categories of product. Category 1 produced brown extracts and infusions with relatively small contents of alkaloids and total chlorogenic acids whereas Category 2 produced green extracts and infusions with larger total alkaloids contents and much larger total chlorogenic acids contents. The green Maté samples contained a quercetin glycoside, and were a convenient source from which to isolate 3-caffeoylquinic acid and 3,5-dicaffeoylquinic acid. Neither condensed nor hydrolysable tannins were detected.

INTRODUCTION

Maté, a stimulating beverage which superficially resembles green tea, has been consumed traditionally by the Gauchos of South America. The beverage is prepared by the infusion of green or dried leaves from various South American species of *Ilex* (Holly), in particular *Ilex paraguariensis* Saint Hilaire. In North America and Europe, similar, but less well known,

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beverages have been prepared from the leaves of *Ilex caroliniana* and *Ilex aquifolia*, respectively (Hegnauer, 1964; Bohinc & Korbar Smid, 1978).

Maté is now available from specialist retailers in the UK and elsewhere in Europe. Although no quantitative data could be found for the leaf as sold in Europe, or for the beverage prepared therefrom, early reports (Hauschild, 1935; Roberts, 1956; Badin *et al.*, 1962) suggested that Maté would have a significant content of several chlorogenic acids (CGA).

The CGA are a family of mono- and di-acyl quinic acids (Clifford, 1985*a*,*b*). Quinic acid is 1L-1(OH),3,4/5-tetrahydroxy cyclohexane carboxylic acid (IUPAC, 1976). The commoner acylating residues are caffeic acid (3,4-dihydroxycinnamic acid), ferulic acid (3-methoxy-4-hydroxycinnamic acid) and *p*-coumaric acid (4-hydroxycinnamic acid), thus producing caffeoylquinic acids (CQA), dicaffeoylquinic acids (diCQA), feruloylquinic acids (FQA), *p*-coumaroylquinic acids (*p*-CoQA) and the recently characterised (Clifford *et al.*, 1989*a*) caffeoylferuloylquinic acids (CFQA). Only one member of this family (5-CQA) is commercially available. This paper reports the results of screening Maté as a convenient source of CGA required for physico-chemical studies.

MATERIALS AND METHODS

Samples of Maté leaf, as described below, were purchased from specialist retailers in the UK and Germany:

—Sample 1. Dried Maté (German).
—Sample 2. Matte Herbal Tea. The authentic Brazilian reviver. Low tannin and caffeine.
—Sample 3. Green Maté (German).
—Sample 4. Maté Tea. Product of Brazil.
—Sample 5. Best Matte. Quality Tea.

Reference samples of purine alkaloids and of 5-CQA, and other reagents and solvents, were good quality materials from normal commercial sources. Authentic samples of other CGA were isolated from green coffee beans and characterised as previously described (Clifford *et al.*, 1989*a*,*b*).

The Maté preparations resembled small tea leaves, and grinding was not required. The moisture contents were determined on duplicate 1-g samples by drying to constant weight at 105°C.

For purine alkaloids and CGA analyses, extracts were prepared by refluxing triplicate 0·5-g samples in 70% methanol (5 × 50 ml volumes, 20 min each) using a Tecator Soxtec HT1043 extraction apparatus. The bulked extracts were treated with Carrez Reagent (1 ml A and 1 ml B),

diluted to 250 ml with 70% methanol, and filtered (Whatman No. 4). Carrez Reagent A was prepared by dissolving 21·9 g of zinc acetate dihydrate in water containing 3 g glacial acetic acid and diluting to 100 ml with water. Carrez Reagent B was prepared by dissolving 10·6 g potassium ferrocyanide trihydrate in 100 ml water.

For flavolans (condensed tannins) analyses, extracts were similarly prepared using 80% aqueous acetone. After bulking, the extracts were evaporated to dryness at reduced pressure, the residue dissolved in 70% methanol, and thenceforward treated as described above.

Aqueous infusions were prepared by following the supplier's instructions. The infusions were cleared with Carrez Reagents, filtered and diluted as necessary with distilled water.

All extracts and infusions were examined by reversed-phase HPLC (3μ C_{18} packing, dilute formic acid with acetonitrile gradient) as described previously (Clifford, 1986; Clifford & Jarvis, 1988). The eluates were monitored sequentially at 276 nm for purine alkaloids and flavolans, and at 313 nm for CGA. Preparative C_{18} HPLC was performed as described by Clifford *et al.* (1989*b*).

Proton NMR spectroscopy was performed using a Brucker AC 300. Samples were dissolved in CD_3OD and examined at room temperature against a tetramethylsilane standard.

UV spectra were recorded conventionally in 70% methanol using a Kontron Uvikon 860 recording spectrophotometer.

To test for flavolans, the autoxidative butanol–HCl reagent (butanol–conc. HCl 95:5 v/v; 6 ml) incorporating ferric ammonium sulphate (2% w/v; 0·2 ml) (Porter *et al.*, 1986) was applied to 1-ml aliquots of each extract and infusion, and to 200-mg samples of the Maté leaf. The samples were heated for 20 min in a boiling water bath.

RESULTS AND DISCUSSION

The primary objective of this study was to establish whether Maté was a convenient source from which to isolate CGA required for physico-chemical studies. Previous studies on Maté and leaves of other *Aquifoliaceae* indicated that purine alkaloids were likely to be present (Roberts, 1956; Badin *et al.*, 1962; Bohinc & Korbar Smid, 1978; Baltassat *et al.*, 1984), and since these coextract and coelute with CGA (Clifford *et al.*, 1989*b*), this study was designed to detect them also.

Moisture contents fell in the range 7–13% fresh weight. When alkaloids and CGA were present in the methanol extracts at levels exceeding the lower limit of integration, they have been reported as per cent dry basis (%db).

TABLE 1
The Contents of Chlorogenic Acids and Chlorogenic Acid-like Components in Maté Leaf (%db)[a]

Sample number	*CGA*				*5-FQA*[c]	*diCQA*				*Chlorogenic acid-like components*										*Total CGA*
	3	*4*	*5*	*Sub total*		*3,4*	*3,5*	*4,5*	*Sub total*	*I* 0·67[b]	*II* 0·87[b]	*III* 1·07[b]	*IV* 1·13[b]	*V* 1·19[b]	*VI* 1·43[b]	*VII* 1·74[b]	*VIII* 1·85[b]	*IX* 1·93[b]	*X* 2·03[b]	
1	0·33 (0·03)	0·37 (0·06)	0·57 (0·09)	1·27 (0·16)	0·39 (0·04)	0·14 (0·09)	0·17 (0·07)	0·21 (0·11)	0·62 (0·09)	+		0·04	0·04	0·08			+	+	+	2·88 (0·10)
2	0·27 (0·03)	0·29 (0·01)	0·60 (0·01)	1·11 (0·02)	0·42 (0·03)	0·10 (0·06)	0·48 (0·16)	0·55 (0·06)	1·12 (0·16)	+		0·05	+	0·10			+	0·06	+	2·89 (0·07)
3	2·15 (0·18)	0·89 (0·09)	1·56 (0·12)	4·53 (0·11)		0·65 (0·02)	2·82 (0·18)	1·10 (0·04)	4·56 (0·03)	0·38 (0·03)	0·07	0·03			0·15	0·04				9·76 (0·25)
4	2·32 (0·14)	0·90 (0·07)	1·43 (0·09)	4·65 (0·16)		0·60 (0·07)	2·60 (0·29)	0·97 (0·06)	4·16 (0·34)	0·34 (0·04)	0·08	+			0·19	0·07				9·40 (0·50)
5	1·75 (0·21)	0·79 (0·06)	2·02 (0·08)	4·71 (0·13)		0·64 (0·02)	2·64 (0·23)	0·95 (0·04)	4·03 (0·25)	0·24 (0·04)	0·06	+			0·13	+				9·16 (0·13)

[a] Mean (standard deviation) on three replicates. The standard deviation has not been calculated for minor components.
[b] Retention times relative to 5-CQA.
[c] Tentative assignment.

Components detected visually on the chromatograms at concentrations below this level (0·04% as 5-CQA, 0·02% as caffeine) have been recorded in the tables by the symbol (+).

Table 1 contains data for up to seven known CGA which are referred to using the IUPAC (1976) numbering system and the system of abbreviations (Clifford, 1985*a*,*b*) defined in the introduction. Some further components, which on the chromatograms were observed to absorb more strongly at 313 nm than at 276 nm, have been described tentatively as CGA-like components, and reported as 5-CQA equivalents. Each is identified in Table 1 by its retention time relative to 5-CQA and a number from the sequence I to X. Note that these roman numerals do not correspond to the arabic numerals used in recent publications to describe CGA-like components of coffee beans (Clifford & Jarvis, 1988; Clifford *et al.*, 1989*c*).

On the basis of CGA profiles, the Maté samples fall clearly into two groups. Whether these differences are due to the drying process to which the brown samples have been subjected or arise from the use of leaves of different species (Hegnauer, 1964; Bohinc & Korbar Smid, 1978), or both, is not clear. Samples 1 and 2 yielded brown extracts with relatively small CGA contents, whereas samples 3 to 5 yielded greenish extracts containing approximately three times as much CGA. While 5-CQA dominated the CGA fraction of the brown Maté samples, the green Maté samples were particularly rich in 3-CQA and 3,5-diCQA.

The presence of 3-CQA-1,5-lactone, reported in Maté by Hauschild (1935), could not be confirmed in the present study. However, Badin *et al.* (1962) concluded that this lactone was an artefact associated with a large 3-CQA content, and in this respect our results for the green samples are consistent.

A component which cochromatographed with authentic 5-FQA was observed in the brown extracts, but was not detected in the green. However, this assignment must remain tentative, since treating the crude brown extracts with tetramethylammonium hydroxide (Clifford *et al.*, 1989*b*) produced a complex mixture in which the expected methyl ferulate could not be demonstrated unequivocally.

Green samples differed from the brown samples also in their profiles of CGA-like components (Table 1). Component I cochromatographed with protocatechuic acid. Component VI was collected by preparative HPLC, and characterised by NMR and UV spectroscopy. It was identified tentatively as a quercetin glycoside, possibly a rhamnoglycoside. The arguments supporting this assignment are presented as the final section of the discussion.

Table 2 contains data for theobromine, caffeine and three unidentified components, XI to XIII, detected in the eluates at 276 nm only. Although not

TABLE 2
The Contents of Caffeine, Theobromine and other Purine Alkaloid-like Components in Maté Leaf (%db)[a]

Sample number	*Purine alkaloids*		*Purine alkaloid-like components*			*Total alkaloids*
	Theobromine	*Caffeine*	*XI* 0·42[b]	*XII* 0·56[b]	*XIII* 1·36[b]	
1	0·66 (0·05)	1·09 (0·15)	0·05	0·04		1·84 (0·10)
2	0·45 (0·06)	0·89 (0·08)	0·06	0·02		1·42 (0·13)
3	0·88 (0·12)	1·73 (0·21)	0·04		0·03	2·68 (0·20)
4	0·71 (0·14)	1·44 (0·15)	0·06			2·21 (0·27)
5	0·45 (0·04)	1·22 (0·13)	0·06			1·73 (0·15)

[a] Mean (standard deviation) on three replicates. The standard deviation has not been calculated for minor components.
[b] Retention times relative to 5-CQA.

necessarily purine alkaloids, the unknowns have been reported as caffeine equivalents.

The total purine alkaloids contents (1·42–2·68%) are not dissimilar to previous reports of 1·10–1·85% (Bohinc & Korbar Smid, 1978; Baltassat *et al.*, 1984). However, theobromine contents were larger, and theophylline was not detected. Cochromatography with authentic material indicated also that 1-methylxanthine, 3-methylxanthine, 7-methylxanthine, 1,7-dimethylxanthine (paraxanthine), uric acid, 1-methyluric acid, 1,3-dimethyluric acid and 1,7-dimethyluric acid could not be detected.

Porter's Reagent did not detect flavolans in the extracts, infusions or leaves. The lack of significant quantities of unidentified 276-nm-absorbing substances in the extracts and infusions indicated that little, if any, hydrolysable tannins were present. While these observations substantiate the 'low tannin' claim made for sample 2, it is not clear why this Maté alone is so described.

Beverage was prepared from the five Maté samples as instructed on the packets. No attempt was made to mimic the traditional South American brewing method, nor to investigate the effect of variables such as the ratio of leaf to water, brew temperature or brew time on the yield of alkaloids and CGA. Accordingly, the quantitative data presented in Table 3 must not be

TABLE 3
The CGA and Purine Alkaloid Contents of Maté Beverage (mg per cup)

Sample number	*Chlorogenic acids*				*Purine alkaloids*		
	Total CQA	*5-FQA*[a]	*Total diCQA*	*Total CGA*	*Caffeine*	*Theobromine*	*Total*
1	12	1·7	1·8	16	20	11	31
2	29	3·3	9·5	41	12	6	18
3	90		44	133	33	17	50
4	74		36	110	23	14	36
5	71		36	107	19	10	29

[a] Tentative assignment.

viewed as a definitive statement on the dietary burdens associated with Maté consumption. Comparison with previous surveys of beverage alkaloids (Burg, 1975; Bunker & Williams, 1979) suggests that the total alkaloid burden per cup of Maté will be similar to green or black tea. However, the theobromine content of Maté beverage will be several times greater than tea, but much less than cocoa.

There are comparatively few data on dietary CGA. Clifford and Walker (1987) indicated that intakes might range from 70 to 220 mg CGA per cup of instant coffee, which overlaps and exceeds the range suggested for Maté beverage. Green Maté beverage, however, could supply significantly more (perhaps $\times 10$) diCQA per cup.

Identification of component VI

Component VI was collected by preparative HPLC. After freeze-drying the bulked fractions, a sparingly water-soluble, green-tinged yellow solid was obtained. The UV spectrum in methanol had λ_{max} at 353 and 256 nm, with shoulders at 262–7 and 290–5 nm, and minima at 280 and 249 nm. Comparison with standard spectra (Mabry *et al.*, 1970; Markham, 1982; Harborne, 1984) suggested luteolin (flavone) glycosides, or certain quercetin (flavonol) glycosides.

The 300-MHz NMR spectrum of component VI was assigned as follows. Two protons, both singlets (6·22 and 6·41 ppm) with incompletely resolved *meta*-coupling, were assigned to a 5,7-disubstituted A ring. A one-proton singlet (7·67 ppm) and two *ortho*-coupled ($J = 8$ Hz) one-proton doublets (6·88 and 7·66 ppm) were assigned to a 3,4-disubstituted B ring, confirmed as having two free vicinal hydroxyls by a positive (yellow) response to the molybdate reagent (Clifford & Wight, 1976). This feature is common to

luteolin and quercetin, but the lack of a singlet assignable to a C3 proton excluded flavones.

A complex multiplet (3·2 to 3·9 ppm) indicates the presence of at least two sugar residues, but could not be assigned unequivocally. A one-proton doublet ($J = 7$ Hz) at 5·09 ppm was assigned tentatively to the C1 proton of a beta-*O*-glycoside; a one-proton singlet (4·52 ppm) and a three-proton doublet ($J = 6$ Hz) at 1·12 ppm were assigned, respectively, to protons at C1 and C6 (methyl) of a rhamnose residue. Collectively, this evidence indicates that component VI is a quercetin glycoside, possibly a rhamnoglycoside (Mabry *et al.*, 1970; Markham, 1982).

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