JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 5471–5484 5471
DOI:10.1021/jf904537z

Profiling and Characterization by LC-MS^n of the Chlorogenic Acids and Hydroxycinnamoylshikimate Esters in Maté (*Ilex paraguariensis*)†

Rakesh Jaiswal, Tina Sovdat, Francesco Vivan, and Nikolai Kuhnert*

School of Engineering and Science, Chemistry, Jacobs University Bremen, 28759 Bremen, Germany

The chlorogenic acids of maté (*Ilex paraguariensis*) have been investigated qualitatively by LC-MS^n. Forty-two chlorogenic acids were detected and all characterized to regioisomeric level on the basis of their fragmentation pattern in tandem MS spectra, 24 of them for the first time from this source. Both chlorogenic acids based on *trans*- and *cis*-cinnamic acid substituents were identified. Assignment to the level of individual regioisomers was possible for eight caffeoylquinic acids (**1**−**8**), five dicaffeoylquinic acids (**20**−**24**), six feruloylquinic acids (**9**−**14**), two diferuloyl quinic acids (**25** and **26**), five *p*-coumaroylquinic acids (**15**−**19**), four caffeoyl-*p*-coumaroylquinic acids (**34**−**37**), seven caffeoyl-feruloylquinic acids (**27**−**33**), three caffeoyl-sinapoylquinic acids (**38**−**40**), one tricaffeoylquinic acid (**41**), and one dicaffeoyl-feruloylquinic acid (**42**). Furthermore, four caffeoylshikimates (**43**−**46**), three dicaffeoylshikimates (**47**−**49**), one tricaffeoylshikimate (**51**), and one feruloylshikimate (**50**) have been detected and shown to possess characteristic tandem MS spectra and were assigned by comparison to reference standards.

KEYWORDS: Caffeoylquinic acids; feruloylquinic acids; caffeoylshikimic acids; feruloylshikimic acids; chlorogenic acids; dicaffeoylquinic acids; diferuloylquinic acid; caffeoyl-feruloylquinic acids; caffeoyl-sinapoylquinic acids; tricaffeoylquinic acids; caffeoyl-*epi*-quinic acids; *Ilex paraguariensis*; maté; LC-MS; tandem MS

INTRODUCTION

Chlorogenic acids (CGAs) are a family of esters formed between quinic acid and certain *trans*-cinnamic acids, most commonly caffeic, *p*-coumaric and ferulic acid (*1*−*3*). Representative structures are shown in **Figure 1**. Using a more general definition, all esters of quinic acids and their diastereomers can be considered as chlorogenic acids. In the IUPAC system, used throughout this paper, (−)-quinic acid is defined as 1-L-1(OH),-3,4/5-tetrahydroxycyclohexane carboxylic acid, but Eliel and Ramirez (*4*) recommend 1α,3*R*,4α,5*R*-tetrahydroxycyclohexane carboxylic acid.

Structurally related, but much less common, are similar esters between shikimic acid and caffeic or ferulic acid. Formally, caffeoylshikimates (CSAs) are closely related to chlorogenic acids and could be chemically obtained from these through dehydration by loss of the 1-OH functionality. It is worth noting that in the literature, similar to quinic acid, a nomenclature issue exists with respect to the numbering of the ring carbon atoms. In this paper we use the definition of shikimic acid as (3*R*,4*S*,5*R*)-3,4,5-trihydroxy-1-cyclohexene carboxylic acid throughout. Cinnamoylshikimates have been previously reported in roasted maté, date palms, sweet basil, and carrots (*5*−*9*). On no occasion have the shikimate esters in dietary materials been analyzed by tandem MS and characterized to regioisomeric level. For galloylshikimates in *Quercus robur* shikimate esters have been characterized to regioisomeric level using purification followed by NMR characterization (*10*). In the case of roasted maté it was suggested that the shikimate derivatives were produced via the roasting process leading to loss of water at elevated temperatures (*5*).

Yerba maté is a popular drink prepared by aqueous infusion of the dried leaves of *Ilex paraguariensis* indigenous to South American countries such as Paraguay, Uruguay, Argentina, and Brazil. According to legend, the goddess of the moon and the cloud gave the Guarani Indians this "drink of friendship" as a reward for saving her from a jaguar (*11*). Maté leaves are directly infused to produce the desired tea beverage or roasted prior to infusion. Recently, yerba maté has become increasingly popular in other parts of the world with an estimated 5% of all tea products sold worldwide containing the leaves of *I. paraguariensis* (*11*). The commercial importance of this commodity is highlighted by the fact that the annual maté trade is currently worth around U.S. $1 billion, growing at an annual estimated rate of 50 t over the past decade (*11*). The consumption of maté has been associated with a range of health benefits connected to their high content of polyphenolic secondary metabolites (*12*, *13*). Additionally, it is worth noting that the organoleptic properties of maté have been compared to those of coffee, suggesting that the two popular drinks share some identical sensory components, most likely chlorogenic acids.

†This paper is dedicated to Professor W. A. Schenk on the occasion of his 65th birthday.

*Author to whom correspondence should be addressed (telephone 49 421 200 3120; fax 49 421 200 3229; e-mail n.kuhnert@jacobs-university.de).

Published on Web 04/20/2010

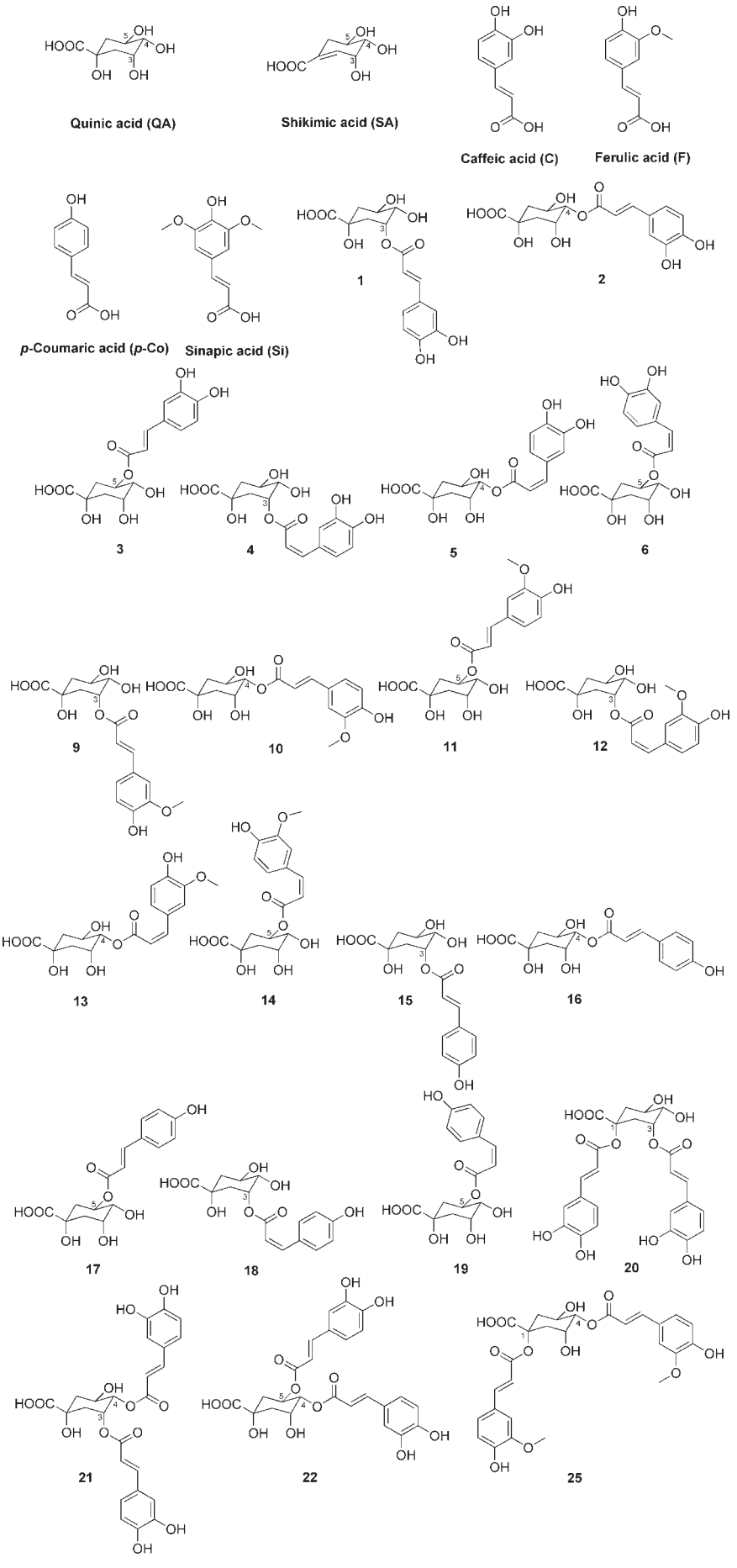
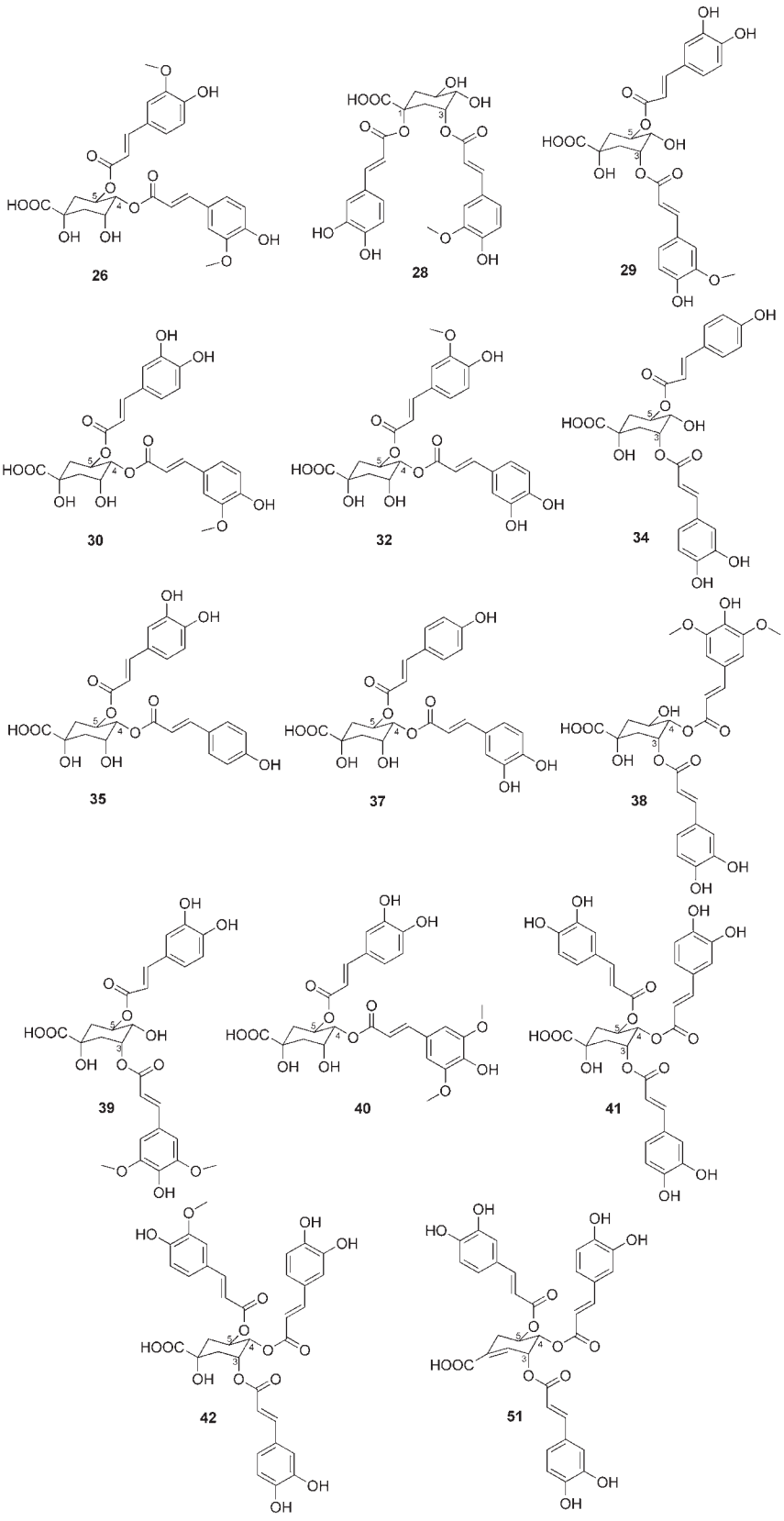


Figure 1. Continued



No.	Name	Abbreviation	Retention time (min)	R^1	R^3	R^4	R^5
1	3-*O*-caffeoylquinic acid	3-CQA	11.7	H	C	H	H
2	4-*O*-caffeoylquinic acid	4-CQA	19.5	H	H	C	H
3	5-*O*-caffeoylquinic acid	5-CQA	17.5	H	H	H	C
4	*cis*-3-*O*-caffeoylquinic acid	*cis*-3-CQA	12.4	H	C	H	H
5	*cis*-4-*O*-caffeoylquinic acid	*cis*-4-CQA	18.5	H	H	C	H

Figure 1. Continued

No.	Name	Abbreviation	Retention time (min)	R^1	R^3	R^4	R^5
6	*cis*-5-*O*-caffeoylquinic acid	*cis*-5-CQA	20.5	H	H	H	C
7	Caffeoyl-*epi*-quinic acid	C*e*QA*	26.2	-	-	-	-
8	Caffeoyl-*epi*-quinic acid	C*e*QA*	28.2	-	-	-	-
9	3-*O*-feruloylquinic acid	3-FQA	20.5	H	F	H	H
10	4-*O*-feruloylquinic acid	4-FQA	30.9	H	H	F	H
11	5-*O*-feruloylquinic acid	5-FQA	26.9	H	H	H	F
12	*cis*-3-*O*-feruloylquinic acid	*cis*-3-FQA	22.0	H	F	H	H
13	*cis*-4-*O*-feruloylquinic acid	*cis*-4-FQA	28.7	H	H	F	H
14	*cis*-5-*O*-feruloylquinic acid	*cis*-5-FQA	31.6	H	H	H	F
15	3-*O*-*p*-coumaroylquinic acid	3-*p*CoQA	17.1	H	*p*Co	H	H
16	4-*O*-*p*-coumaroylquinic acid	4-*p*CoQA	27.9	H	H	*p*Co	H
17	5-*O*-*p*-coumaroylquinic acid	5-*p*CoQA	24.4	H	H	H	*p*Co
18	*cis*-3-*O*-*p*-coumaroylquinic acid	*cis*-3-*p*CoQA	18.5	H	*p*Co	H	H
19	*cis*-5-*O*-*p*-coumaroylquinic acid	*cis*-5-*p*CoQA	28.5	H	H	H	*p*Co
20	1,3-di-*O*-caffeoylquinic acid	1,3-diCQA	34.5	C	H	C	H
21	3,4-di-*O*-caffeoylquinic acid	3,4-diCQA	38.1	H	C	H	C
22	4,5-di-*O*-caffeoylquinic acid	4,5-diCQA	40.7	H	H	C	C
23	A *cis*-4,5-di-*O*-caffeoylquinic acid	A *cis*-4,5-diCQA	42.2	H	H	C	C
24	A *cis*-4,5-di-*O*-caffeoylquinic acid	A *cis*-4,5-diCQA	48.0	H	H	C	C
25	1,4-di-*O*-feruloylquinic acid	1,4-diFQA	49.6	F	H	H	F
26	4,5-di-*O*-feruloylquinic acid	4,5-diFQA	51.2	H	H	F	F
27	Caffeoyl-feruloylquinic acid	C,FQA	40.0	-	-	-	-
28	1-*O*-caffeoyl-3-*O*-feruloylquinic acid	1C,3FQA	42.9	C	F	H	H
29	3-*O*-feruloyl-5-*O*-caffeoylquinic acid	3F,5CQA	44.5	H	F	H	C
30	4-*O*-feruloyl-5-*O*-caffeoyl-quinic acid	4F,5CQA	46.2	H	H	F	C
31	A *cis*-4-*O*-feruloyl-5-O-caffeoyl-quinic acid	A *cis*-4F,5CQA	48.0	H	H	F	C
32	4-*O*-caffeoyl-5-*O*-feruloylquinic acid	4C,5FQA	52.4	H	H	C	F
33	A *cis*-3-*O*-feruloyl-5-*O*-caffeoylquinic acid	A *cis*-3F,5CQA	53.5	H	F	H	C
34	3-*O*-caffeoyl-5-*O*-*p*-coumaroylquinic acid	3C,5*p*CoQA	41.9	H	C	H	*p*Co
35	4-*O*-*p*-coumaroyl-5-*O*-caffeoylquinic acid	4*p*Co,5CQA	43.2	H	H	*p*Co	C
36	A *cis*-4-*O*-*p*-coumaroyl-5-*O*-caffeoylquinic acid	A *cis*-4*p*Co,5CQA	45.4	H	H	*p*Co	C
37	4-*O*-caffeoyl-5-*O*-*p*-coumaroylquinic acid	4C,5*p*CoQA)	45.9	H	H	C	*p*Co
38	3-*O*-caffeoyl-4-*O*-sinapoylquinic acid	3C,4SiQA	43.7	H	C	Si	H
39	3-*O*-sinapoyl-5-*O*-caffeoylquinic acid	3Si,5CQA	46.9	H	Si	H	C
40	4-*O*-sinapoyl-5-*O*-caffeoylquinic acid	4Si,5CQA	54.0	H	H	Si	C
41	3,4,5-tri-*O*-caffeoylquinic acid	3,4,5-triCQA	49.1	H	C	C	C
42	3,4-di-*O*-caffeoyl-5-*O*-feruloylquinic acid	3,4-diC,5FQA	48.5	H	C	C	F
43	Caffeoylshikimic acid	CSA*	24.0		-	-	-
44	4-*O*-caffeoylshikimic acid	4-CSA	24.5		-	C	-
45	3-*O*-caffeoylshikimic acid	3-CSA	25.8		C	-	-
46	Caffeoylshikimic acid	CSA*	29.7		-	-	-
47	3,4-dicaffeoylshikimic acid	3,4-diCSA	45.6		C	C	-
48	3,4-dicaffeoylshikimic acid	3,4-diCSA	46.7		C	C	-
49	3,5-dicaffeoylshikimic acid	3,5-diCSA	51.8		-	C	C
50	4-feruloylshikimic acid	4-FSA	33.4		-	F	-
51	3,4,5-tri-*O*-caffeoylshikimic acid	3,4,5-triCSA	51.4		C	C	C

Figure 1. Structures, numbering, nomenclature, substituents, and chromatographic retention times for CGAs. *, acylation position is uncertain.

Studies investigating the biological activities of maté -based drinks identified saponins, purine alkaloids, and polyphenols as possible plant constituents responsible for health benefits (*14*−*18*). Chlorogenic acids, a subgroup of polyphenols, have been reported in maté on several occasions, with a total of 18 esters identified and reported so far. However, no attempt was made to characterize caffeoyl and feruloyl quinic acids to individual regioisomeric level using NMR or tandem mass spectrometry (*5*, *17*).

Plants that synthesize chlorogenic acids commonly produce many related compounds (over 45 in the coffee bean, for example) and by using LC-MS^n it has been possible to discriminate and carry out unambiguous structure elucidation between individual regioisomers of monoacyl and diacyl chlorogenic acids, without the need to isolate the pure compounds (*19*−*22*). This identification method is based on the fragmentation pattern observed in tandem mass spectra of chlorogenic acids. For example, all four regioisomers of caffeoylquinic acids or six regioisomers of dicaffeoylquinic acid show distinct MS^n spectra that can be rationalized by taking into account the unique hydrogen-bonding arrays found in each individual regioisomer. We believe hydrogen bonding to be the key stereochemical factor determining the fragmentation pattern of chlorogenic acids inducing one particular fragmentation pathway (*19*−*26*). It is worth noting that this tandem MS method represents one of the first examples whereby mass spectrometry allows a reliable and predictive assignment of isomeric compounds. If tandem MS spectra do not allow unambiguous structure information, additional parameters such as retention time, order of elution, and UV spectra can assist in the structure assignment. This investigation applies these methods to the qualitative profiling and characterization of chlorogenic acids in *I. paraguriensis*.

MATERIALS AND METHODS

All of the chemicals (analytical grade) were purchased from Sigma-Aldrich (Bremen, Germany). Green dry yerba maté leaves (Argentina) were purchased from two supermarkets in Bremen, Germany.

Sample Preparation. Green dry yerba maté leaves (5 g) were roasted in an oven (110 °C for 5 h), extracted with methanol (100 mL), homogenized with a blender, and ultrasonicated for 5 min. This extract was filtered through a Whatman no. 1 filter paper. The methanol was removed by vacuum evaporation, and the extract was stored at −20 °C. When required, it was thawed at room temperature, dissolved in methanol (120 mg/10 mL of methanol), filtered through a membrane filter, and used directly for LC-MS. Similar extraction and sample preparation methods have been used for the green dry yerba maté leaves without roasting.

UV Irradiation. The prepared samples of the green and roasted maté (1 mL of each) were placed in a photoreactor (Luzchem LZC-4 V, Ottawa, Canada) under a shortwave UV lamp and irradiated at 245 nm for 40 min.

LC-MS^n. The LC equipment (Agilent 1100 series, Bremen, Germany) comprised a binary pump, an autosampler with a 100 μL loop, and a diode array detector with a light-pipe flow cell (recording at 320 and 254 nm and scanning from 200 to 600 nm). This was interfaced with an ion-trap mass spectrometer fitted with an ESI source (Bruker Daltonics HCT Ultra, Bremen, Germany) operating in Auto-MS^n mode to obtain fragment ion m/z. As necessary, MS^2, MS^3, and MS^4 fragment-targeted experiments were performed to focus only on compounds producing a parent ion at m/z 335.1, 337.1, 349.1, 353.1, 367.1, 497.2, 499.2, 515.2, 529.2, 543.2, 559.2, 659.3, or 677.3. Tandem mass spectra were acquired in Auto-MS^n mode (smart fragmentation) using a ramping of the collision energy. Maximum fragmentation amplitude was set to 1 V, starting at 30% and ending at 200%. MS operating conditions (negative mode) had been optimized using 5-caffeoylquinic acid (**3**) with a capillary temperature of 365 °C, a dry gas flow rate of 10 L/min, and a nebulizer pressure of 10 psi.

High-resolution LC-MS was carried out using the same HPLC equipped with a MicrOTOF Focus mass spectrometer (Bruker Daltonics) fitted with an ESI source, and internal calibration was achieved with 10 mL of 0.1 M sodium formate solution injected through a six-port valve prior to each chromatographic run. Calibration was carried out using the enhanced quadratic calibration mode. It should be noted that in TOF calibration the intensities of the measured peaks have a significant influence on the magnitude of the mass error with high-intensity peaks resulting in detector saturation displaying larger mass errors. Usually this problem can be overcome by using spectra averaging on the side flanks of a chromatographic peak or using a more dilute sample as carried out in this case. All MS measurements were carried out in the negative ion mode.

HPLC. Separation was achieved on a 150 × 3 mm i.d. column containing diphenyl 5 μm with a 4 × 3 mm i.d. guard column of the same material (Varian, Darmstadt, Germany). Solvent A was water/formic acid (1000:0.05 v/v), and solvent B was methanol. Solvents were delivered at a total flow rate of 500 μL/min. The gradient profile was from 10% B to 70% B linearly in 60 min followed by 10 min isocratic and a return to 10% B at 80 min and 10 min isocratic to re-equilibrate.

Synthesis of Mixture of Regioisomers of Caffeoylshikimic Acid (***44***, ***45***, *and* ***52***). To a solution of shikimic acid (80 mg, 0.46 mmol) and DMAP (5 mg, 0.04 mmol) in CH_2Cl_2 (10 mL) were added pyridine (3 mL) and 3,4-diacetylcaffeic acid chloride (129 mg, 0.46 mmol) at room temperature. The reaction mixture was stirred for 12 h and acidified with 1 M HCl (pH ≈3). The layers were separated, and the aqueous phase was re-extracted with CH_2Cl_2 (2 × 15 mL). The combined organic layers were dried over Na_2SO_4 and filtered, and the solvents were dried in vacuo. The resulting ester was dissolved in a mixture of 20 mL of trifluoroacetic acid and water (7:3) at room temperature and stirred for 30 min. The solvents were removed in vacuo, and the resulting yellowish product was analyzed by HPLC-MS (see conditions above) and shown to contain a mixture of **45**, **44**, and **52**.

Synthesis of 3,4-Isopropylidene Shikimic Acid. A mixture of methyl shikimate **53** (500 mg, 2.66 mmol), *p*-toluenesulfonic acid monohydrate (5 mg, 0.029 mmol), 2,2-dimethoxypropane (2 g, 19.2 mmol), and acetone (10 g) was heated at reflux for 4 h. The reaction was cooled to room temperature and neutralized by the addition of sodium methoxide (16 mg, 0.30 mmol) in methanol (2 mL). Most of the solvent was removed in vacuo, and the gummy residue was dissolved in ethyl acetate (10 mL). This mixture was washed with water (20 mL), and water was back-extracted with ethyl acetate (2 × 15 mL). The organic layers were dried over sodium sulfate, and solvents were removed in vacuo. The resulting pale yellow, semisolid residue was purified by silica gel column chromatography (EtOAc/petroleum ether, 1:2), which yielded methyl-3,4-isopropylideneshikimate **55** as white crystals (515 mg, 2.25 mmol, 85%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.91 (1H, t, J = 4.0, 2.6 Hz, −C═CH), 4.73 (1H, t, J = 9.6, 9.4 Hz, −C—H), 4.10 (1H, t, J = 13.7 Hz, 6.4, −C—H), 3.90 (1H, m, −C—H), 3.76 (3H, s, −(CO)OCH$_3$), 2.79 (1H, dd, J = 17.4, 4.6 Hz, H-6eq), 2.55 (1H, s, OH), 2.23 (1H, ddt, J = 17.4, 8.2, 2.3 Hz, H-6 ax), 1.43 (3H, s, Me), 1.39 (3H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 133.7, 129.1, 111.1, 71.8, 69.3, 51.7, 30.9, 29.2, 26.2, 21.1, 21.1.

Synthesis of 5-(3,4-Diacetyl)-3,4-isopropylidene-caffeoylshikimic Acid (***56***). To a solution of 3,4-isopropylidene shikimic acid **55** (100 mg, 0.46 mmol) and DMAP (5 mg, 0.04 mmol) in CH_2Cl_2 (15 mL) were added pyridine (4 mL) and 3,4-diacetylcaffeic acid chloride **54** (129 mg, 0.46 mmol) at room temperature. The reaction mixture was stirred for 12 h and acidified with 1 M HCl (pH ≈3). The layers were separated, and the aqueous phase was re-extracted with CH_2Cl_2 (2 × 15 mL). The combined organic layers were dried over Na_2SO_4 and filtered, and the solvents were dried in vacuo. The crude product was purified by column chromatography (petroleum ether/EtOAc 3:1) to give the title compound **56** as a yellow oil (33.3%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (1H, d, J = 15.8 Hz, −C═CH), 7.36 (1H, dd, J = 7.5, 1.8 Hz, Ar−H), 7.32 (1H, d, J = 1.6 Hz, Ar−H), 6.90 (1H, t, J 2.5, 1.4, Ar−H), 6.34 (1H, d, J 16.1, −C═CH), 5.26 (1H, dd, J 11.0, 6.19, −C—H), 4.74 (1H, m, −C—H), 4.28 (1H, t, J = 6.19 Hz, −C—H), 3.75 (3H, s, −(CO)OCH$_3$), 2.82 (1H, dd, J = 17.8, 4.5 Hz, −C—H), 2.41 (1H, dd, J = 17.5, 6.2 Hz, −C—H), 2.3 (6H, s, −(CO)CH$_3$), 1.39 (3H, s, −CH$_3$), 1.37 (3H, s, −CH$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.1, 168.1, 166.5, 165.8, 143.6, 142.5, 134.3, 133.0, 129.5, 126.3, 123.9, 122.7, 118.9, 110.2, 74.0, 71.9, 70.1, 52.2, 27.9, 26.5, 25.9, 20.7, 20.7.

For the deprotection of **55**, a suspension of the methyl-3,4-isopropylideneshikimate ester **56** (515 mg, 2.25 mmol) in KOH solution (10 mL, 1M) was stirred at room temperature for 30 min, and the reaction mixture was neutralized by the addition of acidic ion-exchange resin (Amberlite IR 400, 2 g). The aqueous layer was extracted with ethyl acetate (2 × 20 mL), combined organic layers were dried over sodium sulfate, and solvent was evaporated in vacuo. The resulting ester was dissolved in a mixture of 20 mL of trifluoroacetic acid and water (7:3) at room temperature and stirred for 30 min. The solvents were removed in vacuo, and the resulting yellowish crude product that was analyzed by ^{1}H NMR and HPLC-MS and showed a single caffeoyl shikimic acid derivative, **52**, at m/z 335.1.

Synthesis of Bisacetal Protected Shikimic Acid ***57***. To a suspension of shikimic acid (500 mg, 2.87 mmol) in methanol (10 mL) were added 2,3-butanedione (0.48 mL, 5.5 mmol), trimethylorthoformate (1.1 mL, 10 mmol), and D-camphorsulfonic acid (50 mg, 0.01 mmol). The mixture was reflux for 16 h, then cooled to room temperature, and treated with sodium bicarbonate (80 mg, 0.80 mmol). The solvent was removed in vacuo to give a paste that was dissolved in ethyl acetate. Activated charcoal (2 g) was added, and the mixture was refluxed for 2 h and then left to cool to room temperature. The mixture was filtered over a thick pad of silica gel, which was further washed using ethyl acetate/methanol (9:1), and the resulting colorless filtrate was evaporated in vacuo to give a white solid. The crude was recrystallized from ethyl acetate to form the white shiny needles of bisacetal-protected methyl shikimate **57** (693 mg, 2.29 mmol, 80%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.86 (1H, dd, J = 5.5, 2.7 Hz, H-2), 4.34, (1H, t, J = 4.6 Hz, H-4), 4.07 (1H, ddd, J = 10.4, 5.9, 5.5 Hz, H-5), 3.72 (3H, s, OMe), 3.57 (1H, dd, J = 10.5, 4.6 Hz, H-4), 3.28 (3H, s, OMe), 3.24 (3H, s, OMe), 2.84 (1H, dd, J = 17.4, 5.9 Hz, H-6eq), 2.25 (1H, ddd, J = 17.4, 10.4, 2.7 Hz, H-6ax), 1.38 (3H, s, Me), 1.31 (3H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.6 (C═O), 135.1 (C═C), 132.2 (C═C), 100.1 (OCOMe), 99.3 (OCOMe), 70.6 (C3), 65.1 (C—O), 62.5 (C—O), 52.3 (COOMe), 48.1 (OMe), 48.0 (OMe), 30.1 (C6), 17.9 (Me), 17.7 (Me).

(*a*) *Synthesis of Bisacetal-Protected 3-(3,4-Diacetyl-caffeoyl)-5-shikimic Acid Methyl Ester* ***58***. To a solution of bisacetal-protected shikimic acid **57** (132 mg, 0.46 mmol) and DMAP (5 mg, 0.04 mmol) in CH_2Cl_2 (15 mL) were added pyridine (4 mL) and 3,4-diacetylcaffeic acid chloride **54** (129 mg, 0.46 mmol) at room temperature. The reaction mixture was stirred for 12 h and acidified with 1 M HCl (pH ≈3). The layers were separated, and the aqueous phase was re-extracted with

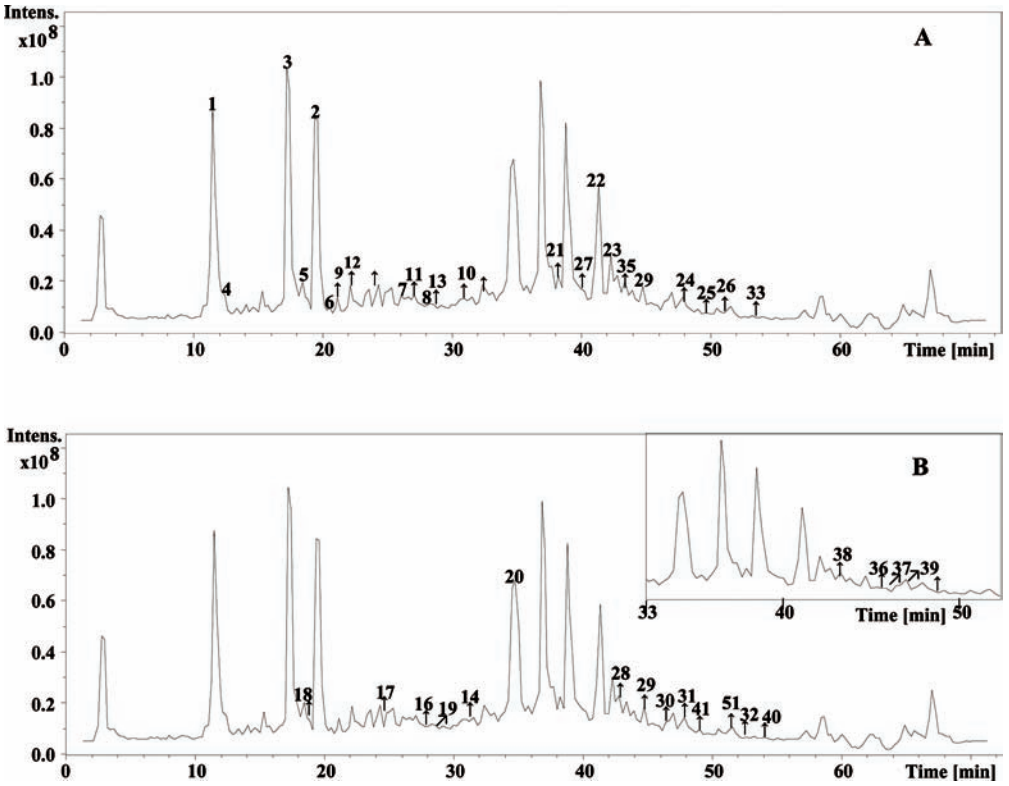


Figure 2. TICs of roasted maté extract: (**A**) full view; (**B**, inset) using ion trap MS in negative ion mode.

CH_2Cl_2 (2 × 15 mL). The combined organic layers were dried over Na_2SO_4 and filtered, the solvents were dried in vacuo, and the crude product was purified by column chromatography (petroleum ether/EtOAc 3:1) to give the title product **58** as a yellow oil (37.2%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.86 (1H, dd, J = 5.5, 2.7 Hz, H-2), 4.34, (1H, t, J = 4.6 Hz, H-4), 4.07 (1H, ddd, J = 10.4, 5.9, 5.5 Hz, H-5), 3.72 (3H, s, OMe), 3.57 (1H, dd, J = 10.5, 4.6 Hz, H-4), 3.28 (3H, s, OMe), 3.24 (3H, s, OMe), 2.84 (1H, dd, J = 17.4, 5.9 Hz, H-6eq), 2.25 (1H, ddd, J = 17.4, 10.4, 2.7 Hz, H-6ax), 1.38 (3H, s, Me), 1.31 (3H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2, 168.0, 166.4, 165.9, 143.6, 143.5, 142.5, 133.5, 133.4, 131.8, 126.6, 123.9, 122.7, 119.1, 99.9, 99.3, 68.9, 66.6, 63.1, 60.4, 52.1, 48.1, 29.7, 22.7, 21.1, 20.7, 14.3.

(*b*) *Deprotection of* ***58***. A suspension of the methyl-3,4-isopropylideneshikimate ester **56** (515 mg, 2.25 mmol) in KOH solution (10 mL, 1M) was stirred at room temperature for 30 min, and the reaction mixture was neutralized by the addition of acidic ion-exchange resin (Amberlite IR 400, 2 g). The aqueous layer was extracted with ethyl acetate (2 × 20 mL), the combined organic layers were dried over sodium sulfate, and solvent was evaporated in vacuo. The resulting ester was dissolved in a mixture of 20 mL of trifluoroacetic acid and water (7:3) at room temperature and stirred for 30 min. The solvents were removed in vacuo, and the resulting yellowish crude product that was analyzed by ^{1}H NMR and HPLC-MS (high-resolution TOF and tandem MS) showed a single caffeoyl shikimic acid derivative **45** at m/z 335.1

RESULTS AND DISCUSSION

A total of five different maté samples (samples I−V) were analyzed, three dried leaves samples (II−IV) and two roasted samples (I and V). One roasted sample was obtained as a commercial product (I), and the second sample was roasted under defined conditions (V) with a roasting time of 5 h at 110 °C, longer than that typically used in commercial products, to study whether dehydration of CGA derivatives to shikimates takes place. Maté leaves dried or roasted were extracted using methanol, and the extract ws directly used for LC-MS analysis. In comparison to isolation of CGAs from green coffee beans, no removal of proteins and peptides using Carrez reagent was required (*21*, *22*).

Preliminary Assessment of Data. All data for CGA presented use the recommended IUPAC numbering system (*1*), and specimen structures are presented in **Figure 1**. When necessary, previously published data have been amended to ensure consistency and avoid ambiguity. The roasted maté extract (sample I) and dry green leaf maté extract (sample II) were examined at least on five separate occasions. All other samples III−V were measured in duplicate. No appreciable differences in the LC-MS chromatograms between the different samples or different chromatographic runs were observed independent of whether they were obtained from dried leaves or roasted material. Additionally, specimen total ion chromatograms (TICs) for a representative maté extract are shown in **Figure 2**.

For the chromatographic analysis used in this contribution the HPLC method used was slightly modified from that reported previously (*19*). The phenylhexyl packing was replaced by a diphenyl packing, and acetonitrile was replaced by methanol as an eluent, resulting overall in a more economical method with slightly improved resolution.

When commercial standards were not available, peak identities were assigned on the basis of the structure-diagnostic hierarchical keys previously developed, supported by means of their parent ion, UV spectra, and sequence of elution/retention time relative to 5-CQA using methods validated in our laboratory (*23*−*25*).

In a first set of experiments the maté extracts were analyzed by high-resolution mass spectrometry using an ESI-TOF instrument in the negative ion mode. Following the strategy employed in previous studies (*2*, *3*, *23*), selected ion monitoring (SIM) located eight CQAs (**1**−**8**), six FQAs (**9**−**14**), five *p*-CoQAs (**15**−**19**), five diCQAs (**20**−**24**), two diFQAs (**25** and **26**), seven CFQAs (**27**−**33**), four CSAs (**43**−**46**), one FSA (**50**), three diCSAs (**47**−**49**), three CSiQAs (**38**−**40**), four *p*-CoCQAs (**34**−**37**), one diCFQA (**42**), one triCQA (**41**), and one triCSA (**51**). IUPAC names, abbreviations, numbering, and retention times are given in Figure **1** including unassigned derivatives. All structures are

Table 1. Negative Ion MS^3 Data for Monoacylchlorogenic Acids

		MS^1	MS^2							MS^3				
		parent ion	base peak	secondary peak						base peak	secondary peak			
no.	compd		*m/z*	*m/z*	int[a]	*m/z*	int	*m/z*	int	*m/z*	*m/z*	int	*m/z*	int
1	3-CQA	353.1	190.9	178.5	50			134.9	7	85.3	127.0	71	172.9	67
2	4-CQA	353.1	172.9	178.9	60	190.8	20	135.0	9	93.2	111.0	48		
3	5-CQA	353.2	190.0	178.5	5			135.0	15	85.2	126.9	66	172.9	27
4	*cis*-3-CQA	353.1	190.9	178.5	45			134.9	12	85.3	127.0	71	172.9	45
5	*cis*-4-CQA	353.1	172.9	178.9	74	190.8	90	135.0	9	93.2	111.0	67		
6	*cis*-5-CQA	353.2	190.0	178.5	28			135.0	13	85.2	126.9	70	172.9	64
7	CeQA	352.9	190.7	178.9	49	172.7	99	135.0	10	154.8	126.7	28	170.7	46
8	CeQA	352.9	178.7	190.7	51	160.7	10	134.8	20	134.8				
9	3-FQA	367.2	192.9	191.5	2	173.2	2			133.9	148.9	23		
10	4-FQA	367.2	172.9	192.9	16					93.1	111.5	44		
11	5-FQA	367.2	190.9	172.9	2					85.2	126.9	70		
12	*cis*-3-FQA	367.2	192.9	191.5	2	173.2	2			133.9	148.9	24		
13	*cis*-4-FQA	367.2	172.9	192.9	9					93.1	111.5	59		
14	*cis*-5-FQA	367.2	190.9	172.9	50					85.2	126.9	52		
15	3-*p*-CoQA	337.1	162.9	190.0	5					118.9				
16	4-*p*-CoQA	337.1	172.7							93.0	111.0	61		
17	5-p-CoQA	337.2	190.9	162.9	5					85.2				
18	*cis*-3-*p*-CoQA	337.1	162.9	190.0	12					118.9				
19	*cis*-5-*p*-CoQA	337.2	190.9	162.9	8					85.2				
43	CSA	335.2	160.9	178.9	12			134.9	50	132.9				
44	4-CSA	335.1	178.9	160.9	82			134.9	50	134.9				
45	3-CSA	335.1	178.9	160.9	80			135.0	25	134.9				
46	CSA	335.2	160.9	178.9	12			135.0	18	132.9				
50	4-FSA	349.1	174.7	192.7	41	148.7	78	133.7	27	159.7	145.8	5	110.9	4

[a] Intensity.

given in **Figure 1** excluding unassigned derivatives. For all compounds the high-resolution mass data were in good agreement with the theoretical molecular formulas, all displaying a mass error of below 5 ppm, confirming their elemental composition.

In a second set of experiments the maté extracts were subjected to LC-MS^n measurements in the negative ion mode using an ESI-ion trap mass spectrometer, allowing assignments of compounds to the regioisomeric level. Selected ion monitoring was carried out for all CGAs of known molecular weight already reported in the literature. Unknown compounds were readily identified by their characteristic fragment peaks at *m/z* 173 and 191 in an all MS^n extracted ion chromatogram searching for these fragments. **Table 1** contains the summarized MS^n data for the monoacyl CGAs and monoacyl shikimates, **Table 2** the equivalent data for diacyl CGAs, and **Table 3** the equivalent data for the triacyl CGA.

In general, peak identities were consistent both within and between analyses. However, when the mass spectrum for a particular substance included two ions of similar mean intensities, within-analysis experimental error dictated that in some individual MS scans one would be more intense and for other scans the reverse would be true. This phenomenon was encountered primarily when the signal intensity was lower, that is, with quantitatively minor components and/or higher order spectra. For example, the monoacyl CGA MS^3 ions at *m/z* 85.3 and at *m/z* 126.9 are essentially coequal in some spectra. However, in this particular case the lower mass ion has been assigned consistently as the base peak because in the spectra of several compounds this was clearly the case. Fragment ions with intensities of $< 10\%$ of the base peak have been reported only when they are needed for comparison.

Characterization of Caffeoylquinic Acids (M_r 354), Dicaffeoylquinic Acids (M_r 516), and Tricaffeoylquinic Acid (M_r 678). Eight caffeoylquinic acids (**1**−**8**) were located in the extract (**Figure 2**), and three of them were assigned using the hierarchical keys previously developed (*19*−*22*) as the well-known 3-*O*-caffeoylquinic acid (**1**), 4-*O*-caffeoylquinic acid (**2**), and 5-*O*-caffeoylquinic acid (**3**) (**Table 1**). A further three peaks present as minor components displayed fragmentation patterns identical to those of 3-, 4-, and 5-caffeoylquinic acid, respectively, and we suspected that they might be cis isomers of the corresponding caffeoylquinic acids. For confirmation of these isomers, extracts of roasted and green maté were irradiated with UV light at 245 nm for 40 min. After irradiation, we observed all three putative *cis* isomers in the chromatogram as peaks with significantly increased intensities if compared to their corresponding *trans* isomers from the original plant extract (**Figure 3**), which confirmed the presence of the three *cis*-caffeoylquinic acids (**4**−**6**).

We reported the presence of cis derivatives of chlorogenic acids earlier in coffee leaves (*26*). It appears as if chlorogenic acids present in plant tissue exposed to natural UV light undergo *trans*−*cis* isomerization, whereas in tissue unexposed to UV light, such as the seeds of coffee berries, they remain unchanged. Whether this *trans*−*cis* isomerization has any physiological significance such as protection from UV light, sensing of UV exposure, or even harvesting of UV photons must at this stage remain subject to speculation and requires further detailed investigations.

The remaining two caffeoylquinic acids (**7** and **8**) were tentatively assigned as diastereomers of CGAs, caffeoyl-epi-quinic acids, because they have different retention times and significantly different fragmentation patterns if compared to 1-, 3-, 4-, or 5-caffeoylquinic acid (**Table 1**). It is worth pointing out that diastereomers of chlorogenic acid display different tandem MS spectra if compared to the parent compound, allowing potential for a tandem MS based assignment of stereoisomers.

Similarly, five dicaffeoylquinic acid isomers were identified by their *m/z* 515 parent ion and assigned as 1,3-di-*O*-caffeoylquinic acid (**20**), 3,4-di-*O*-caffeoylquinic acid (**21**), and 4,5-di-*O*-caffeoylquinic acid (**22**), according to their characteristic fragmentation

Table 2. Negative Ion MS^4 Data for Diacyl Chlorogenic Acids

		MS^1	MS^2						MS^3							MS^4				
		parent ion	base peak	secondary peak					base peak	secondary peak						base peak	secondary peak			
no.	compd		*m/z*	*m/z*	int[a]	*m/z*	int	*m/z*	int	*m/z*	*m/z*	int	*m/z*	int	*m/z*	int	*m/z*	*m/z*	int	*m/z*	int
20	1,3-diCQA	515.2	353.1	335.1	2	173.0	4			190.9	179.0	60			135.1	6	85.1	111.1	86	172.9	60
21	3,4-diCQA	515.2	353.1	335.1	4	172.9	20			172.9	178.9	68	191.0	32	135.1	9	93.2	111.1	30		
22	4,5-diCQA	515.2	353.1	335.1	2	172.9	6			172.9	178.9	76	190.9	9	135.0	19	93.1	111.0	20		
23	A *cis*-4,5-diCQA	515.3	353.1	335.1	5	172.9	10			172.9	178.9	60	190.9	20	135.0	8	93.1	111.0	36		
24	A *cis*-4,5-diCQA	515.4	353.1	335.1	4	172.9	9			172.9	178.9	76	190.9	9	135.0	21	93.1	111.0	22		
25	1,4-diFQA	543.2	349.1	367.1	25	172.9	17	268.8	9	192.9	172.9	31	268.8	9	133.8	22					
26	4,5-diFQA	543.2	367.1	349.1	35					172.9	178.9	60	190.8	20	135.0	9	93.1	111.1	40		
27	C,FQA	529.1	367.1							160.8	192.7	14			134.8	18	132.7				
28	1C,3FQA	529.1	367.1	353.1	15					192.7	172.6	13	178.6	5	133.8	18	133.7	149.0	16	127.0	6
29	3F,5CQA	529.2	367.1	353.1	60	349.0	32	335.0	32	192.7	172.6	36			133.8	36	133.7	149.0	19		
30	A *cis*-4F,5CQA	529.1	367.1	335.0	3	172.7	14			172.9	192.9	62			133.8	7	93.2	111.1	22	127.0	14
31	4F,5CQA	529.2	367.1	335.0	4	172.7	21			172.9	192.9	71			133.8	8	93.2				
32	4C,5FQA	529.1	353.1	367.1	25					172.9	178.9	49	190.8	35	134.7	10	93.2			127.0	nd
33	A *cis*-3F,5CQA	529.1	367.1	334.8	4					192.7	172.6	52			133.8	15	133.7	149.0	22		
34	3C,5-*p*-CoQA	499.0	353.1	337.0	15					190.7							85.2	93.0	70	126.9	99
35	4-*p*-Co,5CQA	499.1	337.1	335.1	3	172.7	59			172.9	162.6	8					93.2	111.1	98		
36	A *cis*-4-*p*-Co,5CQA	499.2	337.1	335.1	3	172.7	30			172.9	162.6	21					93.2	111.1	90		
37	4C,5-*p*-CoQA	499.3	353.0			172.8	15			172.9	178.7	66	190.6	29	134.8	12	93.2				
38	3C,4SiQA	559.2	397.1	490.7	nd	233.0	10	172.9	14	172.9	222.9	76					93.1	111.0	81	71.4	25
39	3Si,5CQA	559.2	397.1	490.7	16	222.9	11	172.9	5	222.9	172.9	31					163.8	192.8	54	148.8	31
40	4Si,5CQA	559.2	397.1	490.8	23	222.9	nd	172.9	14	172.9	222.9	5					71.4				
47	3,4-diCSA	497.1	335.1							160.8	178.9	12			135.0	30	133.0	154.8	nd		
48	3,4-diCSA	497.1	335.1							172.9	178.9	75	160.9	47	135.0	47	111.0	154.8	45		
49	3,5-diCSA	497.1	335.1	449.1	95					178.7			160.6	25	134.8	58	134.7				

[a] Intensity.

Table 3. Negative Ion MS^4 Data for Triacyl Chlorogenic Acids

		MS^1	MS^2					MS^3							MS^4				
		parent ion	base peak	secondary peak				base peak	secondary peak						base peak	secondary peak			
no.	compd		*m/z*	*m/z*	int[a]	*m/z*	int	*m/z*	*m/z*	int	*m/z*	int	*m/z*	int	*m/z*	*m/z*	int	*m/z*	int
41	3,4,5-triCQA	677.3	515.1	497.3	4	353.1	22	353.1	335.1	14	190.8	14	172.9	27	172.9	178.8	73	190.8	59
42	3,4-diC, 5-FQA	691.3	529.2	353.1	12			353.1	367.2	47	335.1	36	192.9	48	172.8	178.8	73	191.0	48
51	3,4,5-triCSA	659.3	497.1	453.2	25	211.0	13	335.0	317.0	99					178.8				

[a] Intensity.

pattern as discussed previously (*20*). Two other isomers were assigned as A *cis*-4,5-di-*O*-caffeoylquinic acid (**23** and **24**) on the basis of their fragmentation pattern and increased intensity after UV irradiation (**Figure 3**). In these isomers only one caffeoyl part has cis geometry (A *cis*), whereby distinction between 4-*cis*,5-*trans*-diCQA and 4-*trans*,5-*cis*-diCQA was not possible.

A search for tricaffeoylquinic acids as previously reported in various Asteraceae plants (*27*−*32*) and maté (*5*) at *m/z* 677 in the extract resulted in the identification of one chromatographic peak. The presence of the MS^2 base peak at *m/z* 515 ($[M - \text{caffeoyl} - H^+]^-$) and MS^3 base peak at *m/z* 353 ($[M - \text{caffeoyl} - \text{caffeoyl} - H^+]^-$) with a secondary peak at *m/z* 335.1 (14% of base peak) and supported by MS^4 base peak at *m/z* 172.9 and a secondary ion at *m/z* 178.8 (73% of base peak) clearly suggests a 3,4-disubstitution pattern (**Figure 4**). A comparison of retention time and fragmentation pattern with those of 3,4,5-tri-*O*-caffeoylquinic acid present in green coffee extract (unpublished results) confirms the presence of 3,4,5-tri-*O*-caffeoylquinic acid (**41**) rather than the alternative 1,3,4-substitution, which is also consistent with the fragmentation data.

Prior to this investigation, only 1-, 3-, 4-, and 5-caffeoylquinic acid (*5*, *17*, *33*), one unassigned caffeoylquinic acid, 1,5-, 3,4-, 3,5-, and 4,5-dicaffeoylquinic acid, one unassigned dicaffeoylquinic acid, and one unassigned tricaffeoylquinic acid have been reported in maté (*5*, *33*).

Characterization of Feruloylquinic Acids (M_r 368) and *p*-Coumaroylquinic Acids (M_r 338). A targeted MS^n experiment at *m/z* 337 applied to the sample located five minor components, and three of them were identified by their fragmentation (*19*, *22*) as the well-characterized 3-*O*-*p*-coumaroylquinic acid (**15**), 4-*O*-*p*-coumaroylquinic acid (**16**), and 5-*O*-*p*-coumaroylquinic acid (**17**) (**Table 1**). Another two isomers were identified as the *cis*-3-*O*-*p*-coumaroylquinic acid (**18**) and *cis*-5-*O*-*p*-coumaroylquinic acid (**19**) on the basis of their fragmentation pattern (identical to corresponding trans isomers) and comparison with the UV-irradiated samples showing increased concentration of the cis isomers (**Figure 3**).

The analogous experiment at *m/z* 367 located 3-*O*-feruloylquinic acid (**9**), 5-*O*-feruloylquinic acid (**11**), 4-*O*-feruloylquinic acid (**10**), *cis*-3-*O*-feruloylquinic acid (**12**), *cis*-5-*O*-feruloylquinic acid (**14**), and *cis*-4-*O*-feruloylquinic acid (**13**) (**Table 1** and **Figure 3**). One *p*-coumaroylquinic acid was recently reported in maté without any further structure elucidation and assignment of stereochemistry (*5*, *33*).

Characterization of Caffeoyl-feruloylquinic Acids (M_r 530). Seven caffeoyl-feruloylquinic acid isomers were readily identified by their *m/z* 529 parent ion, and five of them were assigned as 3-*O*-feruloyl-5-*O*-caffeoylquinic acid (**29**), A *cis*-3-*O*-caffeoyl-5-*O*-feruloylquinic acid (**33**), 4-*O*-feruloyl-5-*O*-caffeoylquinic acid (**30**), A *cis*-4-*O*-feruloyl-5-*O*-caffeoylquinic acid (**31**), and

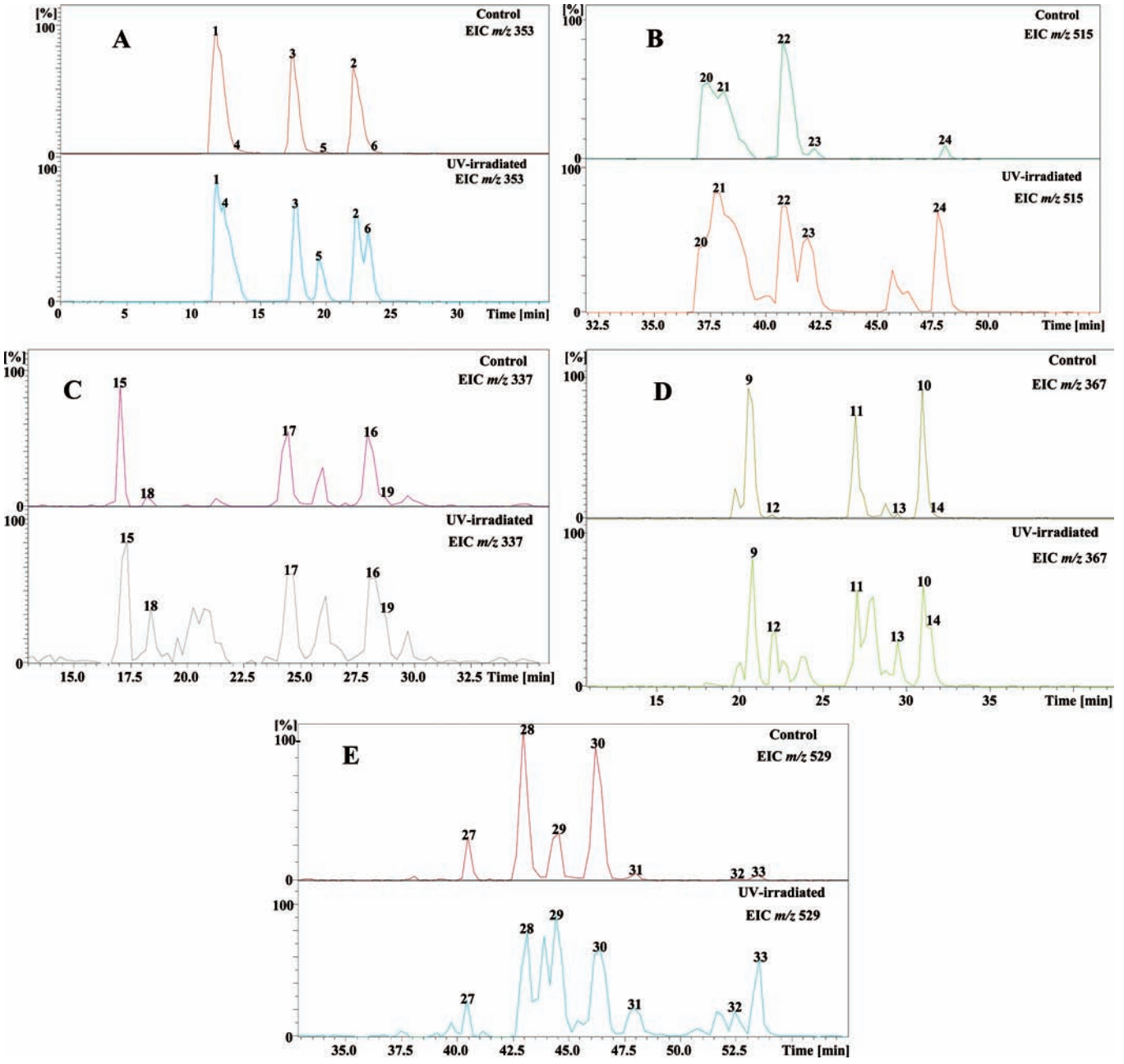


Figure 3. Extracted ion chromatograms (EICs) in negative ion mode before and after UV treatment: (**A**) at *m*/*z* 353 for caffeoylquinic acids (**1**−**6**); (**B**) at *m*/*z* 515 for dicaffeoylquinic acids (**20**−**24**); (**C**) at *m*/*z* 337 for *p*-coumaroylquinic acids (**15**−**19**); (**D**) at *m*/*z* 367 for feruloylquinic acids (**9**−**14**); (**E**) at *m*/*z* 529 for caffeoyl-feruloylquinic acids (**27**−**33**).

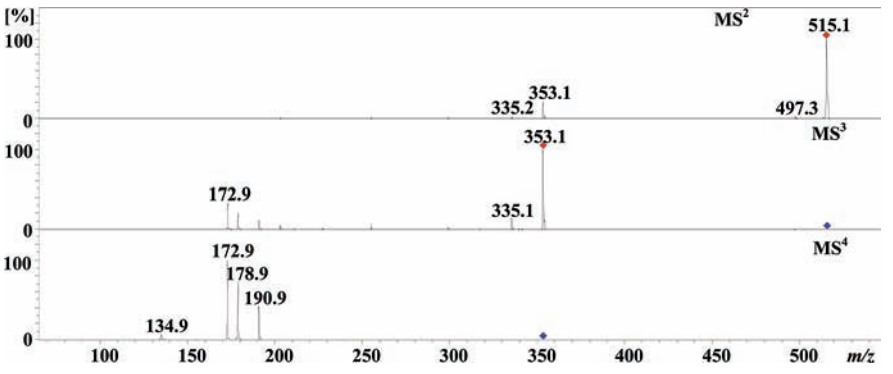


Figure 4. MS^2, MS^3, and MS^4 spectra of 3,4,5-tri-*O*-caffeoylquinic acid (**41**) (parent ion at *m*/*z* 677 in negative ion mode).

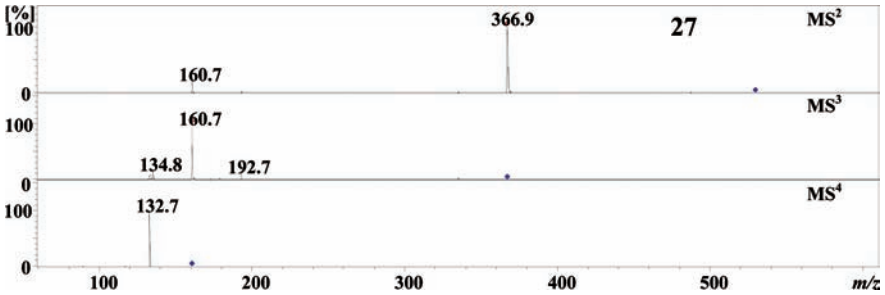


Figure 5. MS^2, MS^3, and MS^4 spectra of caffeoyl-feruloylquinic acid (**27**) (parent ion at *m*/*z* 529 in negative ion mode).

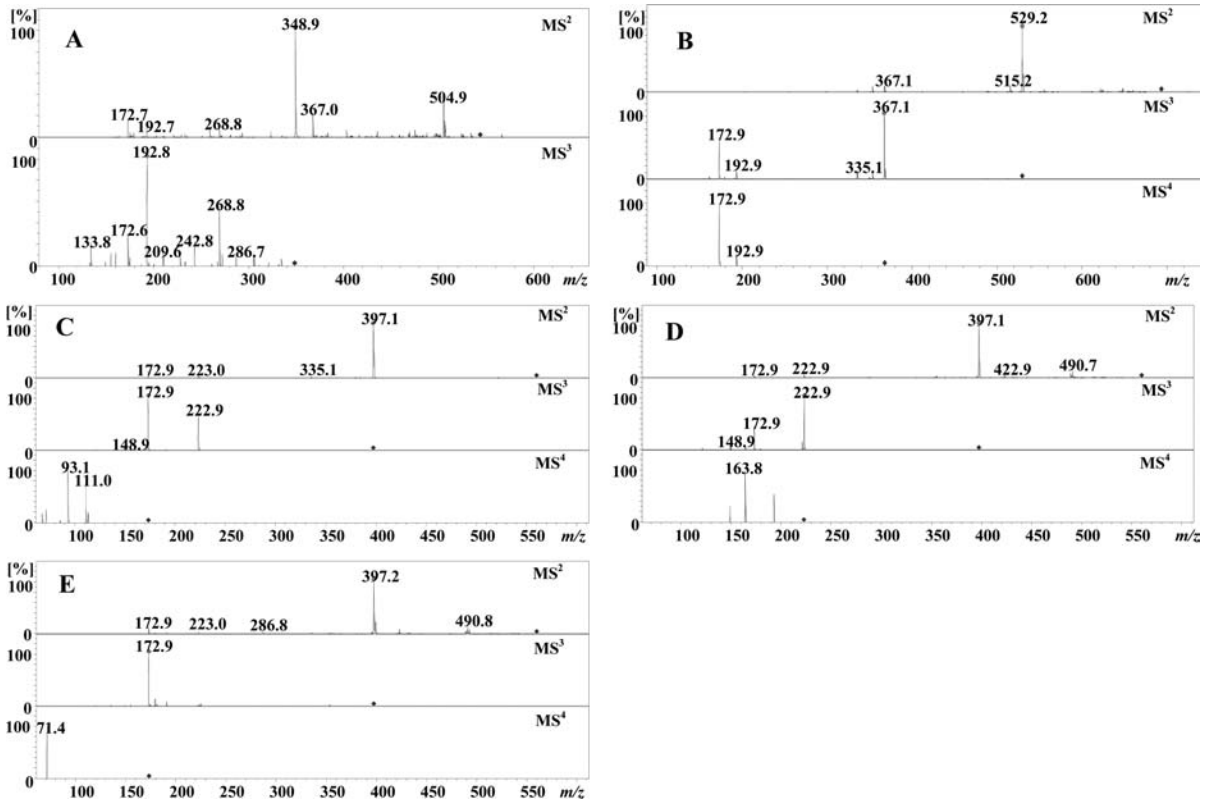


Figure 6. MS^2 MS^3, and MS^4 spectra in negative ion mode: (**A**) 1,4-diferuloylquinic acid (**25**) (parent ion at *m*/*z* 543); (**B**) 3,5,-di-*O*-caffeoyl-4-*O*-feruloylquinic acid (**42**) (parent ion at *m*/*z* 691); (**C**) 3-*O*-caffeoyl-4-*O*-sinapoylquinic acid (**38**) (parent ion at *m*/*z* 559); (**D**) 3-*O*-sinapoyl-5-*O*-caffeoylquinic acid (**39**) (parent ion at *m*/*z* 559); (**E**) 4-*O*-sinapoyl-5-*O*-caffeoylquinic acid (**40**) (parent ion at *m*/*z* 559).

4-*O*-caffeoyl-5-*O*-feruloylquinic acid (**32**) on the basis of their characteristic fragmentation pattern in the MS^2 and MS^3 spectra as described previously (*19*) and comparison with the UV-irradiated samples showing an increased concentration of the *cis* isomers (**Figure 3**).

The sixth caffeoyl-feruloylquinic acid produced a MS^2 base peak at *m*/*z* 367 ([feruloylquinic acid − H^+]$^-$) by the loss of caffeoyl residue and a secondary ion at *m*/*z* 353 (15% of base peak), and a MS^3 base peak at *m*/*z* 193 defines the presence of feruloyl residue at the 3-position (*19*). It loses its caffeoyl residue more preferentially than the feruloyl residue in MS^2 spectra, which suggests the 3-feruloyl-5-caffeoylquinic acid or 1-caffeoyl-3-feruloylquinic acid, and the lower intensity of the MS^3 secondary peak at *m*/*z* 173 (13%) and MS^4 secondary peak at *m*/*z* 149 (16%), if compared to 3-feruloyl-5-caffeoylquinic acid, that favors the assignment as 1-*O*-caffeoyl-3-*O*-feruloylquinic acid (**28**). It is unclear whether this compound is a genuine maté secondary metabolite or an artifact of the workup procedure resulting in acyl migration as observed for the known 1,5-diCQA to 1,3-diCQA (cynarin) isomerization in artichoke. From the overall data it appears as if *I. paraguariensis* does not produce quinic acid esters substituted in the 1-position, similar to coffee.

A further caffeoyl-feruloylquinic acid (**27**) was tentatively assigned as an epimer of caffeoyl-feruloylquinic acid because it has a different fragmentation pattern (**Table 2** and **Figure 5**) if compared to MS^2 and MS^3 spectra of all the possible reported isomers of caffeoylquinic acid and feruloylquinic acids (*19*).

Characterization of Diferuloylquinic Acids (M_r 544). Two diferuloylquinic acid isomers were positively identified by their parent ion at *m*/*z* 543, and one of them was assigned as 4,5-di-*O*-feruloylquinic acid (**26**) on the basis of their characteristic fragmentation pattern in the MS^2 and MS^3 spectra as described previously (*19*). The presence of the MS^2 base peak at *m*/*z* 348.9 ([feruloylquinic acid − H_2O − H^+]$^-$) supported by secondary peaks at *m*/*z* 367 ([feruloylquinic acid − H^+]$^-$) (23% of base peak), *m*/*z* 268.8 (9% of base peak) and, *m*/*z* 172.9 ([quinic acid − H_2O − H^+]$^-$) (18% of base peak) defines the 4-substitution (**Table 2** and **Figure 6**). A comparison to MS^2 and MS^3 spectra of 3,4-, 3,5-, and 4,5-diferuloylquinic acids (*19*) suggested the

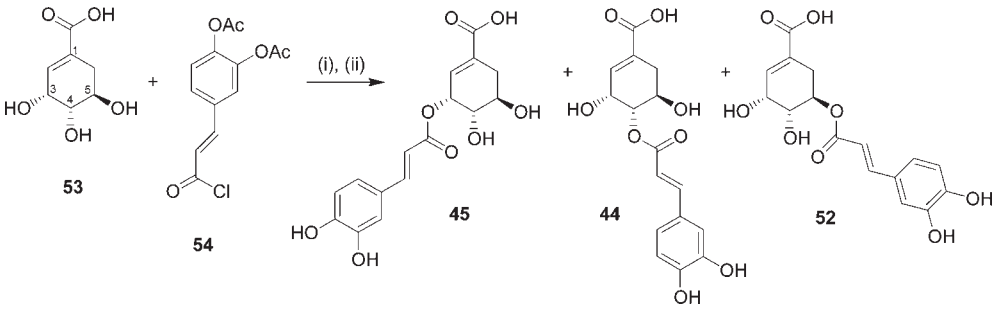


Figure 7. Synthesis of mixture of regioisomers of caffeoylshikimic acids.

presence of 1,4-substitution and assignment as 1,4-di-*O*-feruloylquinic acid (**25**).

All chlorogenic acids described in this contribution have been located in EIC (extracted ion chromatogram) searching for the known *m*/*z* values corresponding to their parent ions. Unknown chlorogenic acids can be readily identified and searched for in the chromatogram by searching in all MS^n chromatograms for signals of fragment ions at *m*/*z* 191 and 173 (e.g., in MS^3 for diacyl CGAs and in MS^4 for triacyl CGAs), which are characteristic of esters of quinic acid. By taking this approach, the following derivatives were located.

Characterization of Putative Dicaffeoyl-feruloylquinic Acid (M_r 692). Targeted MS^4 experiments located one parent ion at *m*/*z* 691 ($[M - H^+]^-$) with 320 nm λ_{max}. The presence of a MS^2 base peak at *m*/*z* 529 ($[M - \text{caffeoyl} - H^+]^-$), a MS^3 base peak at *m*/*z* 367 ($[M - \text{caffeoyl} - \text{feruloyl} - H^+]^-$), and a MS^4 base peak at *m*/*z* 172.9 with secondary peak at *m*/*z* 193 (20% of base peak) (**Table 2** and **Figure 6**) clearly defines the presence of a feruloyl group at position 4 and caffeoyl groups at positions 3 and 5 (*19*), and a comparison of retention time and fragmentation pattern with 3,5-di-*O*-caffeoyl-4-*O*-feruloylquinic acid present in green coffee extract (unpublished results) confirms the presence of 3,5-di-*O*-caffeoyl-4-*O*-feruloylquinic acid (**42**).

Characterization of Putative Caffeoyl-sinapoylquinic Acids (M_r 560). Targeted MS^4 experiments located three parent ions at *m*/*z* 559 (putative caffeoyl-sinapoylquinic acids) at retention times ranging from 43 to 54 min. These parent ions had UV spectra of typical chlorogenic acids. Previously, one caffeoyl-sinapoylquinic acid isomer has been reported in maté.

All three compounds show MS^2 base peaks of *m*/*z* 397.1−397.2 ($[SiQA - H^+]^-$) and a MS^3 base peak of *m*/*z* 172.9 ([quinic acid − H_2O − $H^+]^-$) or *m*/*z* 222.9 ([sinapic acid − $H^+]^-$) (**Table 2** and **Figure 6**). The presence of a MS^3 base peak of *m*/*z* 172.9 ([quinic acid − H_2O − $H^+]^-$) and *m*/*z* 222.9 ([sinapic acid − $H^+]^-$) clearly defines substitutions at positions 4 and 3, respectively (*19*). The presence of a MS^3 base peak of *m*/*z* 172.9 also shows the presence of vicinal diacyl groups, either 3,4- or 4,5-diacyl, and the absence of a MS^3 base peak of *m*/*z* 172.9 represents 3,5-diacyl groups (*19*). MS^2 and MS^3 patterns and orders of the elution for these putative caffeoyl-sinapoylquinic acids are similar to caffeoyl-feruloylquinic acids (3C,4FQA, 3F,5CQA, and 4F,5CQA), which strongly suggest the presence of 3C,4SiQA (**38**), 3Si,5CQA (**39**), and 4Si,5CQA (**40**). It should be noted that we have observed the presence of a further two regioisomers of sinapoyl-caffeoylquinic acid in another source, green Robusta coffee beans, assisting in the assignment (unpublished results).

Characterization of Caffeoylshikimic Acids (M_r 336) and Putative Dicaffeoylshikimic Acids (M_r 498), Feruloylshikimic Acids (M_r 350), and Tricaffeoylshikimic Acid (M_r 660). Four caffeoylshikimic acid esters (**43**−**46**), three dicaffeoylshikimic acid esters (**47**−**49**), one feruloylshikimic acid ester (**50**), and one tricaffeoylshikimic

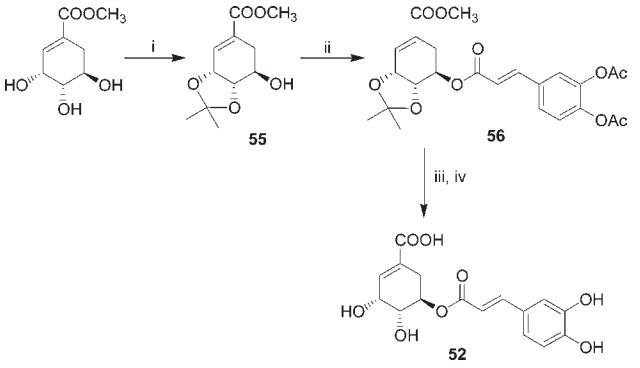


Figure 8. Synthesis of 5-caffeoylshikimic acid.

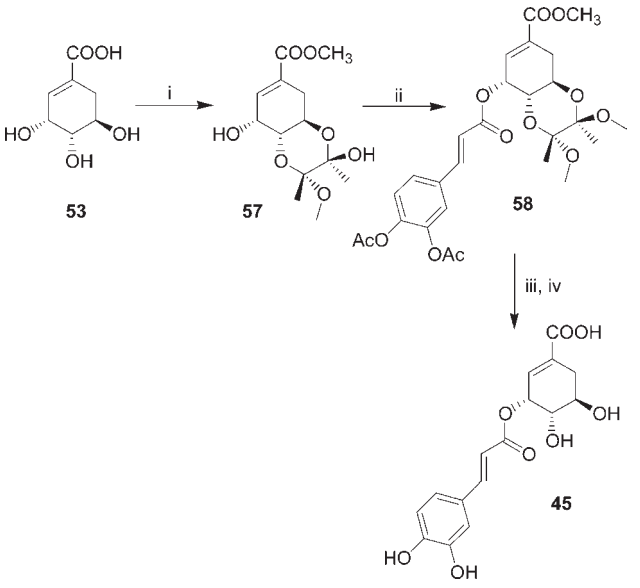


Figure 9. Synthesis of 3-caffeoylshikimic acid.

acid ester (**51**) were identified by their parent ions at *m*/*z* 335, 497, 349, and 659, respectively, and UV absorption at 320 nm (λ_{max}).

Further evidence for the assignment of the caffeoylshikimic acids came from an independent synthesis of a mixture of all three possible regioisomers of caffeoylshikimic acid (**Figure 7**). Unfortunately, preparative HPLC separation of the three regioisomers of caffeoylshikimic acid failed due to insufficient resolution and unfavorable solubilities. Hence, a selective synthesis of 3-caffeoylshikimic acid (3-CSA) and 5-caffeoylshikimic acid (5-CSA) was carried out (**Figures 8** and **9**). For 3-CSA, Leýs-diacetal protection was chosen to selectively protect the *trans* dihydroxy functionality, whereas for 5-CSA a di-isopropylidene protecting

group was chosen to selectively protect the *cis* dihydroxy moiety. Following coupling with an acetate-protected acid chloride of caffeic acid and final deprotection, both reference compounds were obtained in reasonable yield and purity.

Comparison of the chromatogram of the synthetic mixture of three regioisomers and the chromatogram of the two standards allows unambiguous assignment of all three regioisomers by comparison of retention time and tandem MS spectra (the third regioisomer 4-CSA follows automatically in this assignment). The order of elution of caffeoylshikimic acid regioisomers (5 > 4 > 3) was completely different compared to that reported in the literature (*7*) (3 > 4 > 5). The HPLC chromatogram of this synthetic mixture showed two regioisomers (**44** and **45**) with retention times and fragmentation patterns identical to the naturally occurring counterparts (**Figures 10** and **11**), which are assigned as 4-CSA (**44**) and 3-CSA (**45**). The two additional caffeoylshikimic ester derivatives found in the maté samples are by comparison of retention time and tandem MS spectra neither 5-CSA (**52**) nor a *cis*-cinnamate derivative of CSA (no identity of signals at *m*/*z* 335 after UV irradiation). Therefore, we tentatively assign them as diastereoisomers of caffeoylshikimic acid. All shikimic acid esters were found in both dried leaf maté and roasted maté, clearly indicating that they are not products obtained from the leaf processing but are genuine maté secondary metabolites.

Interestingly, the three regioisomeric shikimic acid esters show reproducible differences in their MS^n spectra, allowing their identification by tandem MS. Therefore, following our work on chlorogenic acids a second class of compounds, for which regiochemistry can be unambiguously identified using tandem MS, has been identified.

All three regioisomers show in their MS^2 spectra a base peak at *m*/*z* 178.9 corresponding to a caffeic acid fragment **A** ($C_9H_7O_4$) in **Figure 12**. In all MS^3 spectra a fragment of the transition *m*/*z* 335→178.8 shows a fragment **F** at *m*/*z* 134.9 corresponding to a decarboxylation of caffeic acid ($C_8H_7O_2$). 4-CSA (**44**) shows a strong fragment ion **C** at *m*/*z* 160.9 ($C_9H_5O_3$), which is absent for 3-CSA (**45**) and 5-CSA (**52**) and allows unambiguous identification of 4-CSA (**44**). This observation can be rationalized by taking into account the Hammond postulate. Any fragmentation is an endothermic process characterized by a product-like late transition state. For 3-CSA (**45**) and 5-CSA (**52**) the products of fragmentation are cyclohexadiene carboxylic acids **B** and **D** appearing as neutral losses with a conjugated diene moiety (**Figure 12**). For 4-CSA (**44**) no stabilization by forming a conjugated diene is possible, therefore allowing an alternative competing fragmentation pathway with a *p*-quino-methine ketene **C** as a fragment ion. It is worth noting that in comparison to quinic acid esters, where fragment spectra were rationalized in terms of hydrogen bonding and activation of leaving groups by the 1-COOH group, this pathway is absent in shikimic acid derivatives due to the stereochemical restriction of the sp^2 hybridized carbons.

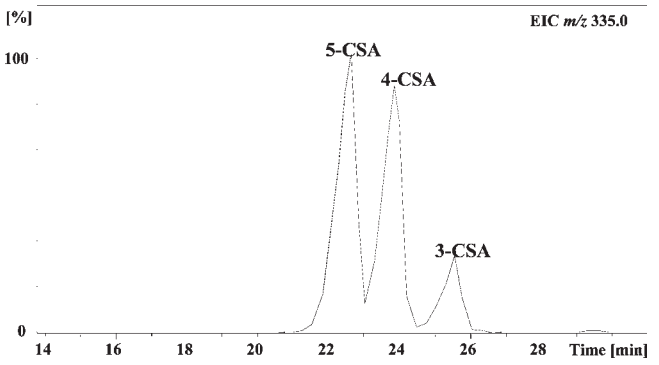


Figure 10. Extracted ion chromatogram (EIC) at *m*/*z* 335 (negative ion mode) for synthetic caffeoylshikimic acid regioisomers.

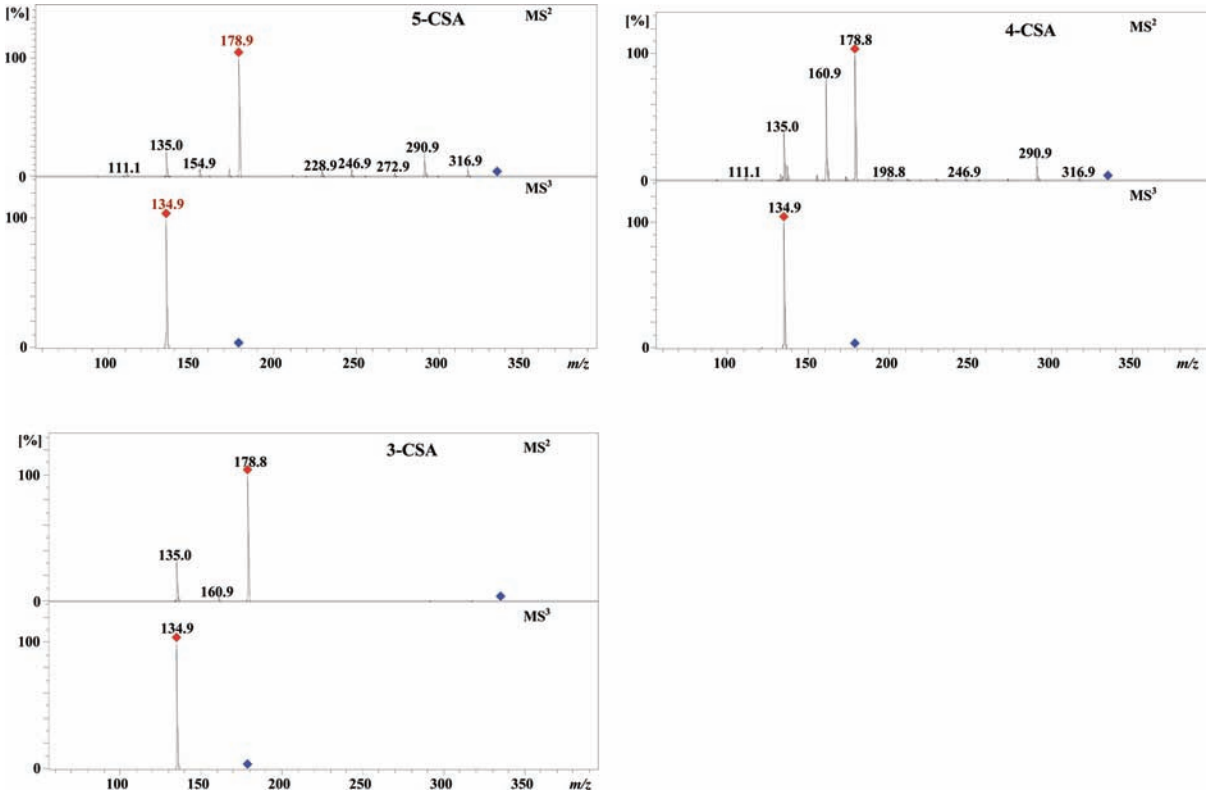


Figure 11. MS^2 and MS^3 spectra for synthetic 3-, 4-, and 5-caffeoylshikimic acid (parent ion at *m*/*z* 335 in negative ion mode).

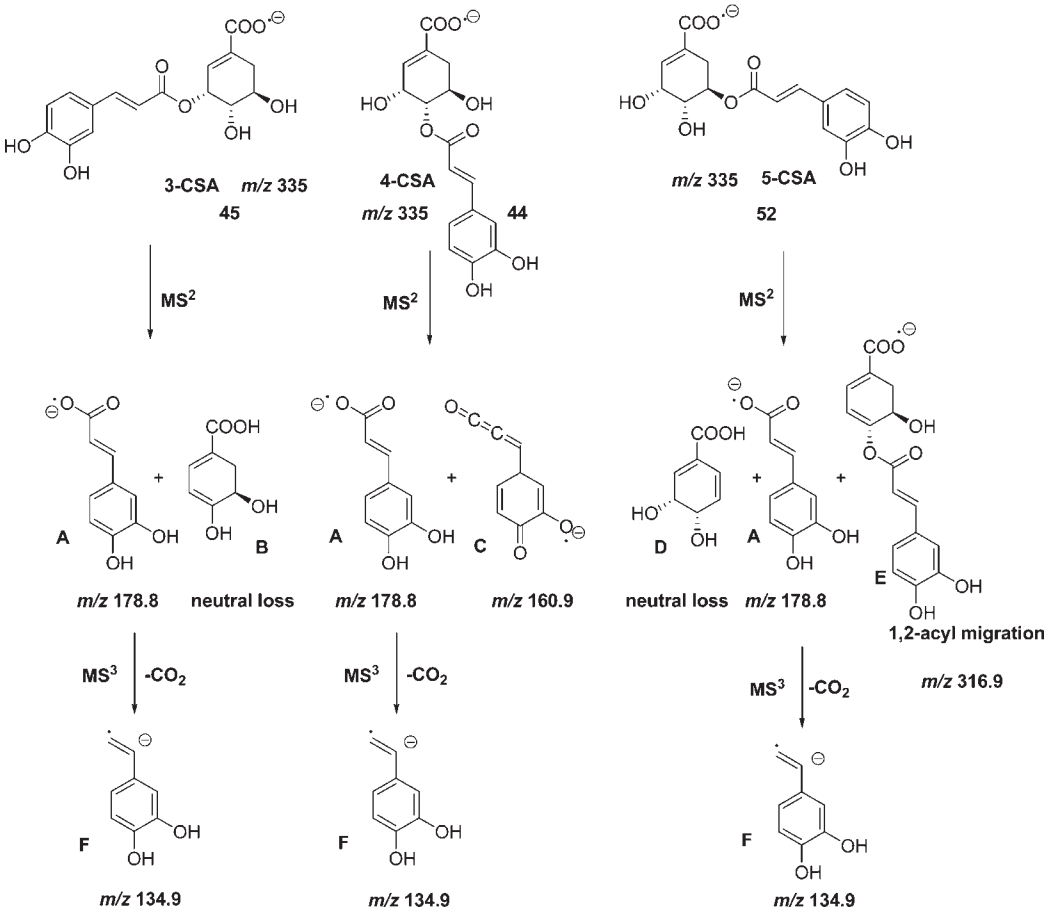


Figure 12. Fragmentation pathway of 3-, 4-, and 5-caffeoylshikimic acid.

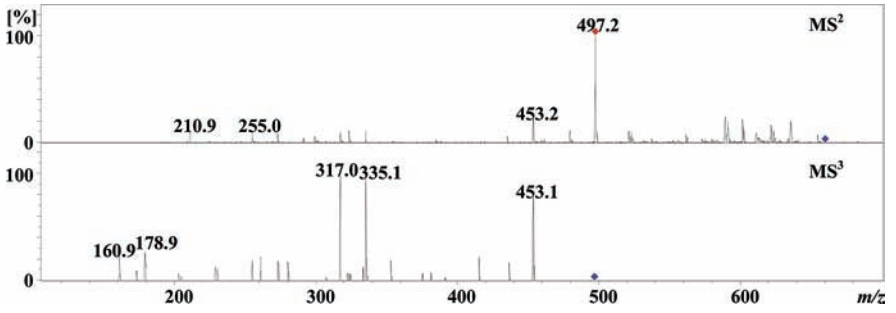


Figure 13. MS^2 and MS^3 spectra for 3,4,5-tri-*O*-caffeoylshikimic acid (**51**) (parent ion at *m*/*z* 659 in negative ion mode).

5-CSA (**52**) shows in MS^2 a fragment ion **E** at *m*/*z* 316.9 (M − H − H_2O, $C_{16}H_{14}O_7$), which is absent in 3-CSA (**45**), allowing differentiation between these two regioisomers. This observation can be rationalized by assuming a 1,2-acyl migration with loss of water, similar to 4-CQA (**44**) derivatives, only possible for the trans stereochemical relationship in the 4,5-disubstituted moiety (**Figure 12**).

All further shikimic acid esters were tentatively identified, due to the lack of authentic standards, due to similarities of tandem MS spectra if compared to the monocaffeoyl esters. Compounds **47** and **48** are 3,4-dicaffeoyl derivatives due to the absence of the fragment ion at *m*/*z* 316.9, suggesting 3-substitution and the appearance of the fragment ion at *m*/*z* 160.9 in MS^3, suggesting 4-substitution. Compound **49** is most likely 3,5-dicaffeoylshikimic acid and compound **50** 4-FSA following the same arguments.

Tricaffeoylshikimic acid was assigned as 3,4,5-tri-*O*-caffeoylshikimic acid (**51**) (**Figure 13**) because there is only one regioisomer. This compound was reported previously from this source (*34*). In our extracts no catechin derivatives were observed, reported by other groups (*34*). Interestingly, at least 12 isomeric monocaffeoyl-hexoses were observed additionally, on which we will report in due course.

ACKNOWLEDGMENT

Excellent technical support by Anja Mueller is acknowledged.

Supporting Information Available: Additional MS^2, MS^3, and MS^4 spectra and high-resolution mass data. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) IUPAC. Nomenclature of cyclitols. *Biochem. J.* **1976**, *153*, 23−31.
(2) Clifford, M. N. Chlorogenic acids and other cinnamates − nature, occurrence, dietary burden, absorption and metabolism. *J. Sci. Food Agric.* **2000**, *80*, 1033–1043.

(3) Clifford, M. N. Chlorogenic acids and other cinnamates − nature, occurrence and dietary burden. *J. Sci. Food Agric.* **1999**, *79*, 362–372.
(4) Eliel, E. L.; Ramirez, M. B. (−)-quinic acid: configurational (stereochemical) descriptors. *Tetrahedron: Asymmetry* **1997**, *8*, 3551–3554.
(5) Bravo, L.; Goya, L.; Lecumberri, E. LC/MS characterization of phenolic constituents of mate (*Ilex paraguariensis*, St. Hil.) and its antioxidant activity compared to commonly consumed beverages. *Food Res. Int.* **2007**, *40*, 393–405.
(6) Mansouri, A.; Embarek, G.; Kokkalou, E.; Kefalas, P. Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (*Phoenix dactylifera*). *Food Chem.* **2005**, *89*, 411–420.
(7) Ziouti, A.; ElModafar, C.; ElMandili, A.; ElBoustani, E.; Macheix, J. J. Identification of the caffeoylshikimic acids in the roots of the date palm, principle fungitoxic compounds vis-a-vis *Fusarium oxysporum* f sp albedinis. *J. Phytopathol.* **1996**, *144*, 197–202.
(8) Gang, D. R.; Beuerle, T.; Ullmann, P.; Werck-Reichhart, D.; Pichersky, E. Differential production of *meta* hydroxylated phenylpropanoids in sweet basil peltate glandular trichomes and leaves is controlled by the activities of specific acyltransferases and hydroxylases. *Plant Physiol.* **2002**, *130*, 1536–1544.
(9) Kuhnl, T.; Koch, U.; Heller, W.; Wellmann, E. Chlorogenic acid biosynthesis − characterization of a light-induced microsomal 5-*O*-(4-coumaoyl)-D-quinate/shikimate 3′-hydroxylase from carrot (*Daucus carota* L) cell-suspension cultures. *Arch. Biochem. Biophys.* **1987**, *258*, 226–232.
(10) Ishimaru, K.; Nonaka, G.; Nishioka, I. Tannins and related-compounds. 57. Gallic acid-esters of proto-quercitol, quinic acid and (−)-shikimic acid from *Quercus mongolica* and *Quercus myrsinaefolia*. *Phytochemistry* **1987**, *26* (5), 1501–1504.
(11) Folch, C. Stimulating consumption: yerba mate myths, markets and meanings from conquest to present. *Comp. Studies Soc. Hist.* **2010**, *52*, 6–36.
(12) Schinella, G. R.; Troiani, G.; Davila, V.; de Buschiazzo, P. M.; Tournier, H. A. Antioxidant effects of an aqueous extract of *Ilex paraguariensis*. *Biochem. Biophys. Res. Commun.* **2000**, *269*, 357–360.
(13) Schubert, A.; Pereira, D. F.; Zanin, F. F.; Alves, S. H.; Beck, R. C. R.; Athayde, M. L. Comparison of antioxidant activities and total polyphenolic and methylxanthine contents between the unripe fruit and leaves of *llex paraguariensis* A. St. Hil. *Pharmazie* **2007**, *62*, 876–880.
(14) Gnoatto, S. C. B.; Schenkel, E. P.; Bassani, V. L. HPLC method to assay total saponins in *Ilex paraguariensis* aqueous extract. *J. Brazil. Chem. Soc.* **2005**, *16* (4), 723–726.
(15) Gosmann, G.; Schenkel, E. P. A new saponin from maté, *Ilex paraguariensis*. *J. Nat. Prod.* **1989**, *52*, 1367–1370.
(16) Mazzafera, P. Caffeine, theobromine and theophylline distribution in *Ilex paraguariensis*. *Braz. J. Plant Physiol.* **1994**, *6*, 149–151.
(17) Carini, M.; Facino, R. M.; Aldini, G.; Calloni, M.; Colombo, L. Characterization of phenolic antioxidants from maté (*Ilex paraguayensis*) by liquid chromatography mass spectrometry and liquid chromatography tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **1998**, *12*, 1813–1819.
(18) Chandra, S.; de Mejia, E. G. Polyphenolic compounds, antioxidant capacity, and quinone reductase activity of an aqueous extract of *Ardisia compressa* in comparison to maté (*Ilex paraguariensis*) and green (*Camellia sinensis*) teas. *J. Agric. Food Chem.* **2004**, *52*, 3583–3589.
(19) Clifford, M. N.; Johnston, K. L.; Knight, S.; Kuhnert, N. Hierarchical scheme for LC-MS^n identification of chlorogenic acids. *J. Agric. Food Chem.* **2003**, *51*, 2900–2911.
(20) Clifford, M. N.; Knight, S.; Kuhnert, N. Discriminating between the six isomers of dicaffeoylquinic acid by LC-MS^n. *J. Agric. Food Chem.* **2005**, *53*, 3821–3832.
(21) Clifford, M. N.; Knight, S.; Surucu, B.; Kuhnert, N. Characterization by LC-MS^n of four new classes of chlorogenic acids in green coffee beans: Dimethoxycinnamoylquinic acids, diferuloylquinic acids, caffeoyl-dimethoxycinnamoylquinic acids, and feruloyl-dimethoxycinnamoylquinic acids. *J. Agric. Food Chem.* **2006**, *54*, 1957–1969.
(22) Clifford, M. N.; Marks, S.; Knight, S.; Kuhnert, N. Characterization by LC-MS^n of four new classes of *p*-coumaric acid-containing diacyl chlorogenic acids in green coffee beans. *J. Agric. Food Chem.* **2006**, *54*, 4095–4101.
(23) Clifford, M. N. The analysis and characterisation of chlorogenic acids and other cinnamates. In *Methods in Polyphenol Analysis*; Santos-Buelga, C., Williamson, G., Eds.; Royal Society of Chemistry: Cambridge, U.K., 2003; Vol. 14, pp 314−337.
(24) Clifford, M. N.; Kellard, B.; Birch, G. G. Characterisation of caffeoylferuloylquinic acids by simultaneous isomerisation and transesterification with tetramethylammonmium hydroxide. *Food Chem.* **1989**, *34*, 81–88.
(25) Clifford, M. N.; Zheng, W.; Kuhnert, N. Profiling the chlorogenic acids of aster by HPLC MSn. *Phytochem. Anal.* **2006**, *17*, 384–393.
(26) Clifford, M. N.; Zheng, W.; Kuhnert, N. Profiling the chlorogenic acids of aster by HPLC MSn. *Phytochem. Anal.* **2006**, *17*, 384–393.
(27) Agata, I.; Goto, S.; Hatano, T.; Ishibe, S.; Okuda, T. 1,3,5-Tri-*O*-caffeoylquinic acid from *Xanthium strumarium*. *Phytochemistry* **1997**, *33*, 508–509.
(28) dos Santos, M. D.; Gobbo-Neto, L.; Albarella, L.; de Souza, G. E.; Lopes, N. P. Analgesic activity of di-caffeoylquinic acids from roots of *Lychnophora ericoides* (*Arnica da serra*). *J. Ethnopharmacol.* **2005**, *96*, 545–549.
(29) Ma, Y. T.; Huang, M. C.; Hsu, F. L.; Chang, H. F. Thiazinedione from *Xanthium strumarium*. *Phytochemistry* **1998**, *48*, 1083–1085.
(30) Merfort, I. Caffeoylquinic acids from flowers of *Arnica montana* and *Arnica chamissonis*. *Phytochemistry* **1992**, *31*, 2111–2113.
(31) Peluso, G.; de Feo, V.; de Simone, F.; Bresciano, E.; Vuotto, M. L. Studies on the inhibitory effects of caffeoylquinic acids on monocyte migration and superoxide ion production. *J. Nat. Prod.* **1995**, *58*, 639–646.
(32) Timmermann, B. N.; Hoffmann, J. J.; Jolad, S. D.; Schram, K. H.; Klenk, R. E.; Bates, R. B. Constituents of *Chrysothamnus paniculatus*. 3. 3,4,5-Tricaffeoylquinic acid (a new shikimate pre-aromatic) and 3,4-, 3,5- and 4,5-dicaffeoylquinic acids. *J. Nat. Prod.* **1983**, *46*, 365–368.
(33) Dugo, P.; Cacciola, F.; Donato, P.; Jacques, A. J.; Caramao, E. B.; Mondello, L. High efficiency liquid chromatography techniques coupled to mass spectrometry for the characterization of mate extracts. *J. Chromatogr., A* **2009**, *1216*, 7213–7221.
(34) Markowicz Bastos, D. H. M.; Saldanha, L. A.; Catharino, R. R.; Sawaya, A. C. H. F.; Cunha, I. B. S.; Carvalho, P. O.; Eberlin, M. N. Phenolic antioxidants identified by ESI-MS from yerba mate (*Ilex paraguayensis*) and green tea (*Camelia sinensis*) extracts. *Molecules* **2007**, *12*, 423–432.

Received for review December 24, 2009. Revised manuscript received March 24, 2010. Accepted March 27, 2010. Financial support from Jacobs University Bremen is gratefully acknowledged.