Improved LC-MS^n characterization of hydroxycinnamic acid derivatives and flavonols in different commercial mate (*Ilex paraguariensis*) brands. Quantification of polyphenols, methylxanthines, and antioxidant activity.

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Running title: Polyphenols, methylxanthines and antioxidant activity of yerba mate

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23 **Abstract**

24 Yerba mate is beverage rich in bioactive compounds popular in South America.
25 Polyphenols and methylxanthines were qualitatively and quantitatively analyzed in four
26 commercial brands of yerba mate, as well as the antioxidant capacity of the beverages.
27 Using LC/MS^n analysis, 58 polyphenols were identified of which 4-sinapoylquinic acid,
28 di- and tri-methoxycinnamoylquinic acids, two isomers of trimethoxycinnamoylshikimic
29 acid and four isomers of caffeoyl-2,7-anhydro-3-deoxy-2-octulopyranosonic acid were
30 identified for the first time in mate. Additionally, 46 polyphenols and 2 methylxanthines
31 were quantified by HPLC-DAD. Hydroxycinnamic acid derivatives and flavonols
32 comprised 90% and 10% of mate phenols, respectively, 3-caffeoylquinic (26.8-28.8%),
33 5-caffeoylquinic (21.1-22.4%), 4-caffeoylquinic (12.6-14.2%) and 3,5-dicaffeoylquinic
34 acids (9.5-11.3%%) along with rutin (7.1-7.8%) were the most abundant polyphenols,
35 whereas caffeine was the main methylxanthine (90%). *Ilex paraguariensis* is an
36 important source of polyphenols with moderate methylxanthines content; therefore its
37 high antioxidant capacity was mainly associated to its polyphenolic composition.

38

39 **Keywords:** yerba mate, hydroxycinnamic acid derivatives, methylxanthines, LC-MS^n
40 analysis, antioxidant capacity.

41 **Abbreviations:** AAPH, 2,2'-azobis(2-amidinopropane) dihydrochloride; ABTS, 2,2'-
42 azinobis-3-ethylbenzothiazoline-6-sulphonic acid; CDOA, caffeoyl-2,7-anhydro-3-deoxy-
43 2-octulopyranosonic acid; CF, caffeine; d.m., dry matter; DAD, diode-array-detector;
44 FRAP, ferric reducing antioxidant power; MX, methylxanthines; ORAC, oxygen radical
45 scavenging capacity; RA, relative abundance; RT, retention time; TB, theobromine; TE,
46 Trolox equivalents; TP, theophylline; TPP, total polyphenolic content; TPTZ, 2,4,6-
47 tripyridyl-*s*-triazine; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

48

1. Introduction

Ilex paraguariensis (St. Hil.) is a plant native to the subtropical regions of South America. Its leaves have been traditionally used to prepare yerba mate drink, which is the main alternative beverage to coffee or tea in countries such as Brazil, Argentina, Paraguay or Uruguay, where daily mate consumption has been estimated at more than 1 L/person. Popularity of yerba mate has increased worldwide due to its nutritional and medicinal properties, having been considered as a functional food (Bracesco, Sanchez, Contreras, Menini, & Gugliucci, 2011). In indigenous tribal medicine, yerba mate has been used for the treatment of numerous pathologies. Indeed, many recent studies documented the pharmacological activities of this beverage, demonstrating *in vitro* and *in vivo* antioxidant, anti-inflammatory, anti-obesity and anti-carcinogenic properties, among others (Arçari et al., 2009; de Mejía, Song, Heck, & Ramírez-Mares, 2010).

The nutritional and health benefits associated with the intake of yerba mate have been attributed to its high content in bioactive components, specifically methylxanthines and polyphenols. Methylxanthines are a group of purine alkaloids with a xanthine base that caffeine, theophylline, theobromine and paraxanthine have in common. Traditionally, negative health effects have been attributed to these compounds due to their stimulatory properties on the central nervous system. However, recent evidences support relevant health effects of methylxanthines, showing neuroprotective, hypoglycemic, anti-inflammatory or cardioprotective effects, among others (de Mejia & Ramirez-Mares, 2014; Martínez-Pinilla, Oñatibia-Astibia, & Franco, 2015). On the other hand, most therapeutic applications of yerba mate have been associated with its phenolic composition due to the well-known antioxidant, anti-inflammatory, anti-carcinogenic, anti-diabetic or neuroprotective capacities of dietary polyphenols (Del Rio et al., 2013). Hydroxycinnamate esters, commonly known as hydroxycinnamic acids or chlorogenic acids, are a family of esters in which a hydroxycinnamic acid moiety is linked to (-)-quinic acid. Caffeic, *p*-coumaric, ferulic, dimethoxycinnamic, and trimethoxycinnamic acids are

76 the hydroxycinnamic acids usually present in yerba mate, in which one or more hydroxyl
77 moieties of quinic acid are esterified forming a series of positional isomers (Clifford,
78 2000). Additionally, flavonols, hydroxycinnamic acids bonded to carbohydrates, and
79 hydroxycinnamoylshikimates have also been identified in mate. The latter, usually called
80 hydroxycinnamoylshikimic acids, are esters of hydroxycinnamic acids with shikimic acid,
81 which is chemically derived from the dehydratation of quinic acid (Jaiswal, Sovdat, Vivan,
82 & Kuhnert, 2010a).
83 Previous works have identified different hydroxycinnamoylquinic acids and
84 hydroxycinnamoylshikimates in yerba mate using HPLC-LC/MS^n, the technique most
85 commonly employed to characterize phenolic compounds due to its robustness,
86 reproducibility and sensitivity. In addition, fragmentation of the compounds depends on
87 the particular stereochemistry of the molecule, related to the substitution position on the
88 quinic acid, thus allowing to discriminate between individual isomers without previous
89 isolation of the pure compounds (Clifford, Johnston, Knight, & Kuhnert, 2003; Clifford,
90 Knight, & Kuhnert, 2005; Clifford, Knight, Surucu, & Kuhnert, 2006a; Clifford, Marks,
91 Knight, & Kuhnert, 2006b).
92 Plant variety and pathophysiology, in addition to environmental conditions and
93 processing, can induce important modifications in the main constituents of yerba mate
94 (Cardozo et al., 2007; Zielinski et al., 2014). Considering the variability of the
95 phytochemical composition, and thus the antioxidant capacity and biological activity of
96 yerba mate, which in turn influences the health effects of the beverage, the aim of the
97 present study was to qualitatively and quantitatively characterize by HPLC-LC/MS^n and
98 HPLC-DAD the phenolic and methylxanthine composition of four different commercial
99 brands of yerba mate, as well as their antioxidant capacity.
100
101
102 **2. Material and methods**

103 *2.1. Materials and reagents*

104 Four different commercial brands (A-D) of yerba mate (*Ilex paraguariensis*), selected
105 among the most widely consumed brands in Argentina, were purchased in a local
106 supermarket in Madrid (Spain). The four brands were elaborated from selected *Ilex*
107 *paraguariensis* leaves that had been slightly roasted for short time at temperatures under
108 100 °C, dried, minced, and stored at ambient temperature ("*estacionado natural*") during
109 12 months before packaging; the specific elaboration procedures are protected
110 proprietary information. The four mate brands contained minor amounts of stems ("*palo*")
111 and were produced in Argentina, two in the state of Misiones (brands A and B) and the
112 other two in Corrientes (brands C and D). 3,5-dicaffeoylquinic acid was acquired from
113 PhytoLab (Vestenbergsgreuth, Germany). 2,4,6-tripyridyl-*s*-triazine (TPTZ), caffeine and
114 potassium persulfate were from Fluka (Madrid, Spain). Caffeic acid, 5-caffeoylquinic
115 acid, ferulic acid, gallic acid, rutin, theobromine, theophylline, 2,2'-azinobis-3-
116 ethylbenzothiazoline-6-sulphonic acid (ABTS), 2,2'-azobis(2-amidinopropane)
117 dihydrochloride (AAPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
118 (Trolox), fluorescein sodium salt, were obtained from Sigma-Aldrich (Madrid, Spain).
119 Folin-Cicalteau reagent was from Panreac (Madrid, Spain). All other reagents were of
120 analytical or chromatographic grade.

121

122 *2.2. Extraction of phenolic compounds and methylxanthines from yerba mate*

123 The method previously described by Bravo, Goya, & Lecumberri (2007) was used to
124 extract the phenolic compounds of yerba mate. In addition, methylxanthines were also
125 extracted from yerba mate using the same procedure. Briefly, 1 g of mate from each
126 commercial brand was weighed in triplicate and extracted with 2 N hydrochloric acid in
127 aqueous methanol (50:50, v/v) for 1 h by constant shaking at room temperature. Samples
128 were centrifuged (10 min, 3000 *g*) and the supernatants were collected. Pellets were
129 extracted with acetone:water (70:30, v/v) for 1 h by constant shaking at room

130 temperature. After the second extraction, samples were centrifuged again (10 min, 3000
131 *g*) and the supernatants were removed, combined with the former and made up to 100
132 mL. An aliquot was concentrated using a vacuum centrifuge system (Speed Vac),
133 dissolved in 1% formic acid in deionized water, filtered through 0.45 µm and stored at -
134 20 ºC until chromatographic analysis, performed within the following 2 weeks.
135 In a preliminary test, we analyzed the extracts obtained from mate samples as present
136 in the packet, containing leaves and stems, without further mincing, with samples milled
137 to a particle size of 0.5 µm, not observing differences between both extracts (data not
138 shown). Therefore, for the present study we used the extracts obtained from the mate
139 samples as present in the packet ready for consumption, with no further mincing.
140

141 *2.3. Chromatographic characterization of phenolic compounds*

142 Phenolic composition of extracts obtained from yerba mate were analyzed using an
143 Agilent 1200 liquid chromatographic (LC) system equipped with an autosampler,
144 quaternary pump and diode-array detector (DAD). The 1200 LC-DAD system was
145 coupled to an Agilent 6530A Accurate-Mass Quadrupole Time-of-Flight (Q-ToF) detector
146 with electrospray ionization (ESI)-Jet Stream Technology (Agilent Technologies,
147 Waldrom, Germany). The sample (1 µL) was injected into the HPLC-ESI-QToF, and
148 separated on a Superspher RP18 column (4 x250 mm, 4 µm; Agilent Technologies)
149 protected with an ODS RP18 guard column. Elution was performed with a gradient
150 elution using a ternary system consisting in 1% formic acid in deionized water (solvent
151 A), acetonitrile (solvent B), and methanol (solvent C) at a constant flow rate of 1 mL/min
152 and 30 ºC. The solvent gradient changed according to the following conditions: from 90%
153 A – 5% B – 5% C to 80% A – 10% B – 10% C in 30 min, to 75% A – 13% B – 12% C in
154 10 min, to 65% A – 20% B – 15% C in 10 min, to 65% A – 17% B – 18% C in 5 min, and
155 returning to initial conditions in 10 min (90% A – 5% B – 5% C) followed by 5 min of
156 maintenance. Signals were registered at 280, 320 and 360 nm. The Q-ToF operating

157 conditions were as follows: negative ion mode, drying gas volume and temperature 12
158 L/min and 350 ºC, respectively, sheath gas volume and temperature 7 L/min and 325 °C,
159 respectively, nebulizer pressure 45 psig, capillary voltage 3500 V, nozzle voltage 0 V,
160 and fragmentor voltage 100 V. Mass Hunter Workstation Software was used to process
161 data.

162

163 *2.4. Determination of polyphenols and methylxanthines content in yerba mate*

164 Polyphenols and methylxanthines were simultaneously analyzed by HPLC in an
165 Agilent 1200 series system (Agilent Technologies) coupled to a thermostatic
166 autosampler, column oven and DAD detector to determine their content in the extracts
167 obtained from the four commercial brands of yerba mate, following the preparation
168 described in section 2.2. Separation was performed on a Superspher RP18 column (4 x
169 250 mm, 4 μm; Agilent Technologies) protected with an ODS RP18 guard column and
170 with the same gradient and conditions described above. Polyphenols were detected at
171 320 and 360 nm, which are the maximum of absorbance of hydroxycinnamic acid
172 derivatives and flavonols, respectively, whereas methylxanthines were registered at 272
173 nm. The identification of methylxanthines was carried out comparing retention time and
174 UV spectra with that of commercial standards. Polyphenols and methylxanthines were
175 quantified using pure standards. When they were not commercially available, other
176 chemically related compound were used; thus, 5-caffeoylquinic and 3,5-dicaffeoylquinic
177 acids were used to quantify mono- and diacyl derivatives of hydroxycinnamic acids,
178 respectively; caffeic acid was used to determine caffeic acid and caffeoyl-glycosides
179 content, and rutin to determine flavonols content.

180 Additionally, the total polyphenol (TPP) content was determined using Folin-Cicalteau
181 reagent (Bravo et al., 2007) and gallic acid as a standard. Absorbance at 750 nm was
182 measured after incubating test samples with the reagent, sodium carbonate solution

183 (75g/L) and distilled water (1:1:20:28, v/v) for 1 h (Beckman DU-640UV-Visible
184 Spectrophotometer, Fullerton, CA, USA).

185

186 *2.5. Antioxidant capacity*

187 The antioxidant capacity of mate extracts prepared by the procedure described in
188 section 2.2. was determined by three different methods.

189 *Ferric reducing antioxidant power* (FRAP) *assay*: the reducing capacity of yerba mate
190 was determined using the method modified by Pulido, Bravo, & Saura-Calixto (2000).
191 The extracts were mixed with FRAP reagent (0.3 M acetate buffer pH 3.6, 10 mM TPTZ
192 in 40 mM HCl and 20 mM $FeCl_3$ -3:1:1, v/v-), 0.3 M acetate buffer and dissolvent
193 (1:6:20:3, v/v) for 30 min, until the formation of a colored TPTZ-Fe^{2+} complex. Then, the
194 absorbance at 595 nm was measured in an automatized plate reader (Bio-Tek, Winooski,
195 VT, USA). Trolox was used as a standard and results were expressed as µmol Trolox
196 equivalent (TE) per gram of dry matter.

197 *ABTS assay*: the free radical cation $ABTS^{\bullet+}$, which was prepared by reaction of ABTS
198 with 2.45 mM potassium persulfate during 12-16 h at room temperature in the dark, was
199 used to evaluate the free radical scavenging capacity of the samples. This radical
200 decreases absorbance at 730 nm in the presence of an antioxidant (Re et al., 1999).
201 Mate extracts were mixed with the $ABTS^{\bullet+}$ radical in methanol (1:6:23; v/v) and, using a
202 Bio-Tek automatized plate reader, the absorbance was monitored for 30 min at 37ºC.
203 Results were expressed as µmol TE per gram of dry matter.

204 *Oxygen radical scavenging capacity* (ORAC) *assay*: this method is based on the
205 fluorescence decay of fluorescein in the presence of a peroxyl radical (AAPH) (Huang,
206 Ou, Hampsch-Woodill, Flanagan, & Prior, 2002). The samples were mixed with $8.5*10^{-5}$
207 mM fluorescein in 75 mM phosphate buffer pH 7.4, and 153 mM AAPH (1:6:1.2, v/v) on
208 a 96-multiwell plate. Fluorescence ($\lambda_{excitation}$ 485 nm, $\lambda_{emission}$ 528 nm) was measured for

209 90 min at 37ºC using an automatized plate reader (Bio-Tek). Trolox was also used as a
210 standard and results were expressed as µmol TE per gram of dry matter.
211

212 *2.6. Statistical analysis*

213 Statistical analysis were carried out using the program SPSS (version 19.0, SPSS,
214 Inc., IMB Company). Previously, homogeneity of variance of data was evaluated using
215 the Levene test. One-way ANOVA was used followed by Bonferroni test when variances
216 were homogeneous or when not the Games-Howell test was used. The significance level
217 was $p < 0.05$. Results were expressed as mean ± standard deviation (SD).
218

219 **3. Results and Discussion**

220 *3.1. Identification and characterization of polyphenols in yerba mate extracts*

221 Samples were analyzed by high-resolution mass spectrometry using an ESI-QToF
222 detector in the negative ion mode and selected ion monitoring (SIM) and subsequently
223 subjected to LC-MS^n analysis by collision-induced dissociation mass spectrometry,
224 allowing to assign compounds at regioisomeric level and, therefore, to characterize the
225 detected chromatographic peaks. UV-spectra evaluation was also considered to support
226 phenolic compounds' characterization. Most of the constituents displayed similar
227 spectral behavior with maximum absorption peak at 320-330 nm and a shoulder at 290-
228 300 nm, being characterized as hydroxycinnamic acid derivatives. A small group of
229 compounds showed the maximum of absorbance at 312 nm, corresponding to *p*-
230 coumaroylquinic acid derivatives (Alonso-Salces, Guillou, & Berrueta, 2009). The rest of
231 the minor constituents of yerba mate were characterized as flavonols attending to UV-
232 spectra characteristics with two maximum absorption peaks at 255-265 and 345-375 nm
233 (Dugo et al., 2009). A total of 58 polyphenols were identified in yerba mate, being
234 flavonols and the rest hydroxycinnamic acid derivatives. Table 1 lists the phenolic

235 compounds identified in yerba mate, ordered according to peak elution, and their
236 chemical characterization: retention time (RT), UV absorption maximum from DAD,
237 quasimolecular ion $[M-H]^-$, MS/MS fragment ions with relative abundance (RA) and
238 tentative nomenclature. No appreciable differences in the DAD and LC-MS
239 chromatograms were observed between the different commercial brands (A-D), except
240 for 4 additional compounds which only appeared in yerba mate A (Table 1). Typical
241 chromatograms of a mate extract at 320 and 360 nm are shown in Figure1 (b, c & d),
242 also showing the methylxanthine profile (Figure 1a). Elution/retention time of the
243 compounds in yerba mate is closely related to their hydrophobicity, which depends on
244 the number, position, and nature of the cinnamoyl moieties. The equatorial (C4 and C5)
245 hydroxyl groups in the quinic or shikimic acids give more hydrophobicity to the molecule
246 than free axial (C1 and C3) hydroxyl groups (Clifford et al., 2005), thus the
247 hydroxycinnamate esters with hydroxyl groups at position 3 were the most hydrophilic
248 derivatives. Considering the chromatographic gradient used, the elution order of the
249 different isomers in the present study was 3-acyl, 5-acyl and/or 4-acyl, since isomers 5-
250 acyl and 4-acyl do not follow a well-established elution order. Figure 2 shows the
251 chemical structures of the identified compounds.

252

253 *Hydroxycinnamic acid* (**peak 16**): One chromatographic peak showed a MS spectrum
254 with a quasimolecular ion at *m/z* 179 and a fragment ion at *m/z* 135 in the negative ion
255 mode. These ions were coincident with deprotonated caffeic acid and decarboxylated
256 cinnamic acid, respectively MS analysis of the corresponding standards allowed
257 unequivocal identification as caffeic acid, in accordance with previous studies that have
258 also identified caffeic acid in yerba mate (da Silveira, Meinhart, de Souza, Teixeira Filho,
259 & Godoy, 2016; Marques & Farah, 2009).

260

261 *Caffeoyl-glycosides* (**peaks 1-3, 5, 7-9, 11, 12, 18, 20 and 29**): Eight chromatographic
262 peaks (**1-3**, **5**, **7-9,** and **12**) showed the same $[M-H]^-$ ion at *m/z* 341 and UV spectra

263 compatible with hydroxycinnamic acid derivatives. Chromatographic peeks **3**, **9** and **12**
264 were identified on the basis of their fragmentation pattern (MS^2) and the hierarchical keys
265 previously developed by Jaiswal, Matei, Glembockyte, Patras, & Kuhnert (2014) as β-1-
266 caffeoylglucose, 6-caffeoylglucose and α-1-caffeoylglucose, respectively. The
267 fragmentation pattern of peaks **1**, **7,** and **8,** which was different to that of peaks **3**, **9** and
268 **12,** hindered to identify the regioisomers, being tentatively assigned as caffeoyl glucose.
269 Likewise, peaks **2** and **5,** which did not show fragmentation in MS^2 analysis, were also
270 tentatively identified as caffeoyl glucose based on their *m/z* values.
271 Additionally, four compounds (**peaks 11, 18, 20 and 29**) with parent ion at *m/z* 381
272 were detected. No structure with the molecular formula $C_{17}H_{18}O_{10}$ had been previously
273 reported in yerba mate and, on the basis of the MS^n spectrum and by matching with the
274 results obtained from SciFinder (March 2014), these compounds were tentatively
275 assigned ascaffeoyl-2,7-anhydro-3-deoxy-2-octulopyranosonic acid (CDOA) isomers.
276 CDOA is the result of the esterification of caffeic acid with a monosaccharide of eight
277 carbon atoms. To our knowledge, this compound has been identified in yerba mate for
278 the first time, having been only observed previously in *Erigeron breviscapus* (Zhang,
279 Zhao, Ma, Wu, & Zeng, 2010). Characterization of each regioisomer was not possible
280 due to the absence of their respective MS^2 spectra.
281
282 *Caffeoylquinic acids* (**peaks 4, 6, 13, 15 and 19**): The five peaks shared similar UV
283 spectrum with λmax at 321-326 nm and a shoulder at 296 nm. They were positively
284 identified as caffeoylquinic acids with a quasimolecular ion at *m/z* 353. Compounds **4**, **6**,
285 **13** and **19** showed a common MS^2 base peak ion at *m/z* 191, corresponding to
286 deprotonated quinic acid, while compound **15** provided the MS^2 base peak ion at *m/z*
287 173, which is originated from the dehydration of quinic acid. Fragment ion at *m/z* 173 is
288 clearly the substitution at position 4 (Clifford et al., 2003), allowing the identification of
289 peak **15** as 4-caffeoylquinic acid. Compound **13** was unequivocally identified as 5-
290 caffeoylquinic acid after comparison with the commercial standard whereas compound

291 **6**, which showed the secondary ions at *m/z* 179 and 135 corresponding to deprotanated
292 caffeic acid and decarboxylated caffeic acid, respectively, was assigned to 3-
293 caffeoylquinic acid isomer (Clifford et al., 2003; Dugo et al., 2009). Lastly, isomers **4** and
294 **19** were identified as *cis*-3- and *cis*-5-caffeoylquinic acids, respectively, taking as a
295 reference the identical fragmentation pattern (or lack of fragmentation) of their respective
296 *trans*-isomers (**6** and **13**, respectively) and the peak elution/retention time order,
297 considering the substituted carbon of quinic acid in *cis* (*cis*-5-acyl is more hydrophobic
298 that its corresponding *trans*-isomer, whereas *cis*-3-acyl elutes earlier that its
299 corresponding *trans*-isomer) (Clifford, Kirkpatrick, Kuhnert, Roozendaal, & Salgado,
300 2008).

301

302 *Feruloylquinic acids* (**peaks 14, 17, 24, 25 and 28**): These compounds showed a UV
303 spectrum similar to caffeoylquinic acid (λmax at 325-326 nm and shoulder at 296 nm)
304 and a common quasimolecular ion at *m/z* 367. Base peak of compound **14** at *m/z* 193
305 corresponding to deprotonated ferulic acid allowed its assignation as 3-feruloylquinic
306 acid. MS^2 spectra of compounds **24** and **25** presented as base peaks ions at *m/z* 173
307 and 191, respectively; in accordance to what has been described above for isomers 4-
308 and 5-caffeoylquinic acid and to the literature (Clifford et al., 2003), they were identified
309 as 4- and 5-feruloylquinic acid, respectively. Contrarily, peaks **17** and **28** showed a
310 different fragmentation pattern that the rest of feruloylquinic acids identified, ruling out
311 the possibility of being *cis*-isomers.

312

313 *p-Coumaroylquinic acids* (**peaks 10, 21 and 22**): Three *p*-coumaroylquinic acid
314 isomers were identified attending to their quasimolecular ion at *m/z* 337 and their
315 characteristic UV spectra, showing only one maximum at 312 nm. Base peak at *m/z* 163
316 derived from the deprotonated coumaric acid moiety was observed in MS^2 for peak **10**,
317 showing an analogous behavior to 3-feruloylquinic and 3-caffeoylquinic acids, thus
318 allowing its assignation as 3-*p*-coumaroylquinic acid (Clifford et al., 2003). The MS^2

319 spectrum of peak **21** was dominated by an intense fragment ion at *m/z* 173, confirming
320 its identity as 4-*p*-coumaroylquinic acid. Compound **22** had a pattern fragmentation
321 characteristic of a 5-acyl-hydroxycinnamoylquinic acid (MS^2 at *m/z* 191) (Clifford et al.,
322 2003), allowing its identification as 5-*p*-coumaroylquinic acid.

323

324 *Sinapoylquinic acid* (**peak 27**): Compound **27** showed a parent ion at *m/z* 397
325 suggesting that it was sinapoylquinic acid (Jaiswal, Patras, Eravuchira, & Kuhnert,
326 2010b). The presence of the ion at *m/z* 173 as MS^2 base peak allowed confirming, for
327 the first time, the presence of the isomer 4-sinapoylquinic acid in yerba mate.

328

329 *Caffeoylquinic lactones* (**peaks 23 and 26**): Peaks **23** and **26** showed *m/z* values for
330 the extracted MS chromatograms of 335 and a typical UV spectrum of
331 hydroxycinnamates (λmax at 325-326 nm and shoulder at 296 nm). The MS^n base ion at
332 *m/z* 161 was key to identify these compounds as caffeoylquinic acids, instead of
333 caffeoylshikimic acids, which with identical quasimolecular ion ($[M-H]^-$ at *m/z* 335)
334 present different MS^2 base ion, as it has recently been clarified in roasted coffee by
335 Jaiswal et al. (2014). The identification of caffeoylquinic lactones in yerba mate differ
336 from the usually characterized hydroxycinnamoylshikimic acids (Jaiswal et al., 2010a),
337 although both compounds derive from the dehydration of quinic acid.

338

339 *Trimethoxycinnamoylshikimic acid* (**peak 36**): MS^n analysis showed a peak with a
340 quasimolecular ion at *m/z* 393, with UV-spectrum characteristic of hydroxycinnamic acid
341 derivatives. The accurate mass and molecular formula provided by the Mass Hunter
342 software suggests its identification as trimethoxycinnamoylshikimic acid, although further
343 characterization is required to confirm this outcome.

344 *Dicaffeoylquinic acids* (**peaks 32, 33, 35, 39 and 52**): Five chromatographic peaks
345 with typical UV spectra of hydroxycinnamate esters (λmax at 324-327 nm and shoulder
346 at 296 nm) and quasimolecular ion at *m/z* 515, coincident with dicaffeoylquinic acid, were

347 identified in yerba mate extracts. All compounds shared the same MS^2 base peak at *m/z*
348 353 originated by the loss a caffeoyl residue. The presence of a secondary ion at *m/z*
349 173 in MS^2 spectrum of peaks **32** and **39** suggested the substitution of a caffeoyl group
350 at position 4. Moreover, according to previous studies (Alonso-Salces et al., 2009;
351 Clifford et al., 2005), peaks **32** and **39** were assigned to 3,4-dicaffeoylquinic acid and
352 4,5-dicaffeoylquinic acid, respectively. Peak **33** was identified as 3,5-dicaffeoylquinic
353 acid attending to the corresponding commercial standard. Compound **52** was identified
354 as*cis*-4,5-dicaffeoylquinic acid, since it showed the same fragmentation pattern as the
355 corresponding *trans*-isomer (peak **39**) (Clifford et al., 2008). In contrast, MS^2
356 fragmentation pattern of isomer **35** did not show coincidence with any of the isomers
357 reported by others authors (Clifford et al., 2005) hindering the identification of the specific
358 isomer of this dicaffeoylquinic acid.
359
360 *Caffeoyl-feruloylquinic acids* (**peaks 42, 44, 46, 47, 50, 53 and 54**): Seven isomers of
361 caffeoyl-feruloylquinic acid were tentatively identified in the yerba mate extracts when
362 the *m/z* value for the extracted MS chromatogram was set at 529 nm. In addition, all
363 compounds showed an UV spectra characteristic of hydroxycinnamate esters. In the
364 identification of these compounds it was considered that on the one hand the cinnamoyl
365 residue at C5 is linked by a highly labile bond, whereas the link at position 4 is the
366 strongest bond; also, the elution order was similar to that described for the
367 dicaffeoylquinic acids (3,4-, 3,5- and 4,5-diacyl) (Clifford et al., 2003).The MS^2 base peak
368 at *m/z* 367 of compound **42** after losing a dehydrated caffeoyl residue and the presence
369 of an ion at m/z 173, pointed to a substitution at position 4; this, along with its early elution
370 allowed its identification as 3-caffeoyl-4-feruloylquinic acid. The fragmentation patterns
371 of chromatographic peaks **44** and **46** were characterized by the absence of the
372 secondary ion at *m/z* 173, which suggested that both compounds were 3,5-diacyl
373 derivatives of hydroxycinnamic acids. The identification of peaks **44** and **46**, as 3-feruloyl-
374 5-caffeoylquinic acid and 3-caffeoyl-5-feruloylquinic acid, respectively, was possible

375 thanks to the intense fragment ions at *m/z* 367 and 353 in MS^2 spectra, respectively,
376 originated from the loss of dehydrated caffeic and ferulic acids substituted at position 5.
377 Compounds **50** and **53** were assigned as 4,5-diacyl derivatives, due to the presence of
378 the secondary ion at *m/z* 173 on both MS^2 spectra also showing a delayed retention time,
379 being tentatively assigned as 4-feruloyl-5-caffeoylquinic acid (**50**) and 4-caffeoyl-5-
380 feruloylquinic acid (**53**) (Alonso-Salces et al., 2009; Clifford et al., 2006a). Compound **47**
381 was tentatively assigned as *cis*-3-caffeoyl-4-feruloylquinic acid as a result of showing a
382 MS^2 fragmentation pattern similar to the corresponding *trans*-isomer (peak **42**).
383 Regarding compound **54,** it was not possible to assign the position of the substituents of
384 both hydroxycinnamic acids (caffeic and ferulic acids) esterified to quinic acid, with a
385 different MS^2 spectrum from other compounds reported in the literature (Clifford et al.,
386 2006a).
387
388 *Caffeoyl-p-coumaroylquinic acids* (**peaks 40, 43, 48 and 51**): Targeted MS^n analysis
389 at *m/z* 499 identified four minor peaks at the end of the chromatogram. Peaks **48** and **51**
390 showed a UV-spectrum with a maximum at 312 nm, suggesting to be *p*-coumaric acid
391 derivatives, while no UV-spectra of peaks **40** and **43** were obtained since they coeluted
392 with peaks **39** and **42**, respectively. The absence of an MS^2 secondary ion at *m/z* 173 for
393 peak **43**, corresponding with dehydrated quinic acid and indicative of substituent at
394 position 4, suggest to be the 3,5-diacyl isomer. Moreover, the MS^2 base peak at *m/z* 353
395 after losing a dehydrated *p*-coumaroyl residue, probably substituted at position 5 which
396 is the most labile bond of all, allowed the identification of peak **43** as 3-caffeoyl-5-*p*-
397 coumaroylquinic acid. Peaks **40** and **51** also showed an intense MS^2 peak at *m/z* 353 in
398 addition to a secondary ion at *m/z* 173 (indicative of a substituent at position 4), which
399 along with the well-established elution order of dicinnamoylquinic acids (3,4-, 3,5- and
400 4,5-diacyl) allowed the identification as 3-*p*-coumaroyl-4-caffeoylquinic acid (peak **40**)
401 and 4-caffeoyl-5-*p*-coumaroylquinic acid (peak **51**) (Clifford et al., 2006a). Lastly, peak
402 **48** showed a MS^2 base peak at *m/z* 337 generated after losing a dehydrated caffeoyl

403 residue, and a secondary ion at *m/z* 173 indicative of a substituent at position 4, which
404 allowed its identification as 4-*p*-comaroyl-5-caffeoylquinic acid (Clifford et al., 2006a;
405 Jaiswal et al., 2010a).
406
407 *Caffeoyl-sinapoylquinic acids* (**peaks 45 and 49**): Chromatographic peaks **45** and **49**,
408 which also presented a typical UV-spectrum of hydroxycinnamate esters (λmax at 326
409 nm and shoulder at 296 nm), were identified as caffeoyl-sinapoylquinic acids according
410 to the quasimolecular ion at *m/z* 559. Both peaks showed a common MS^2 base peak at
411 *m/z* 397 after losing a dehydrated caffeic acid. However, the secondary ion at *m/z* 173
412 identified in compound **49** together with its later elution compared to the compound **45**
413 allowed its identification as 4-sinapoyl-5-caffeoylquinic acid. On the contrary, the
414 absence of the fragment ion at *m/z* 173 in the MS^2 fragmentation pattern of compound
415 **45** made possible the identification as 3-sinapoyl-5-caffeoylquinic (Jaiswal et al., 2010b).
416
417 *Flavonols* (**peaks 30, 31, 34, 37, 38 and 41**): Six compounds in yerba mate extracts
418 had an UV spectrum compatible with a flavonoid structure, specifically of flavonols, which
419 is characterized by two absorption maxima at 255-265 and 345-367 nm. Compound **30**
420 was positively identified as rutin, supported by the quasimolecular ion at *m/z* 609 and the
421 use of the standard. The quasimolecular ion at *m/z* 463 and the fragment ion at *m/z* 301
422 as MS^2 base peak, which derives from the loss of a sugar moiety yielding deprotonated
423 quercetin, allowed confirming that peak **31** was quercetin-glycoside. Peaks **37** and **38**
424 had a similar maxima absorbance at 262-266 nm, characteristic of kaempferol moiety.
425 The MS^n analysis of compound **37** showed a quasimolecular ion at *m/z* 593 and fragment
426 ion at *m/z* 285, compatible with the loss of a rhamnoglucoside moiety, which allowed its
427 identification as kaempferol-rhamnoglucoside. Although no MS^2 spectrum was observed
428 in compound **38**, the parent ion at *m/z* 447.0930, matching with the molecular formula
429 $C_{21}H_{20}O_{11}$ provided by Mass Hunter software, made its identification as kaempferol-
430 glycoside possible (Bravo et al., 2007; Dugo et al., 2009). Lastly, chromatographic peaks

431 **34** and **41** shared the same quasimolecular ion at *m/z* 477, corresponding to the flavonol
432 isorhamnetin-glycoside. The lack of fragment ions in their respective MS^2 spectrum
433 avoided the differentiation between both compounds.
434
435 *Compounds exclusive to mate A* (**peaks 55-58**): Four hydroxycinnamic acid
436 derivatives were exclusively found in the commercial brand A of yerba mate. Compound
437 **57** showed the same parent ion than peak **36**, suggesting to be isomers, although the
438 absence of fragments ions in MS^2 spectra hindered the identification of each
439 stereoisomer. Compounds **55** and **56** showed a quasimolecular ion at *m/z* 411 and 381,
440 respectively, compatible with trimethoxycinnamoylquinic acid (**55**) and
441 dimethoxycinnamoylquinic acid (**56**), respectively; both compounds had been previously
442 described in other plants, such as coffee (Jaiswal et al., 2010b), but not in yerba mate.
443 Finally, MS^n analysis showed a peak (**58**) with a parent ion at *m/z* 543.1510 in negative
444 mode compatible with diferuloylquinic acid, dimethoxycinnamoyl-caffeoylquinic acid or
445 *p*-coumaroyl-sinapoylquinic acid previously described according to the literature (Clifford
446 et al., 2006a; Clifford, Wu, Kirkpatrick, Jaiswal, & Kuhnert, 2010; Jaiswal et al., 2010b).
447 The lack of fragment ions in its MS^2 analysis complicated its identification. However, the
448 high content of different forms of ferulic acid in yerba mate suggests that this compound
449 might be tentatively identified as diferuloylquinic acid.
450
451 *3.2. Quantification of polyphenols by HPLC in yerba mate extracts*

452 After the identification of the polyphenols present in the four commercial brands of
453 yerba mate, the content of hydroxycinnamate esters and flavonols were determined by
454 HPLC-DAD at 320 and 360 nm, respectively. Table 2 summarizes the content of phenolic
455 compounds, grouped by chemical structure (the content of individual compounds is
456 shown in Table 1, Supporting information). Some compounds overlapped in the same
457 chromatographic peak and thus were quantified jointly considering the most abundant;

458 peaks **13-14** were quantified as 5-caffeoylquinic acid, peaks **17-18** as feruloylquinic acid,
459 peaks **19-20** as *cis*-5-caffeoylquinic acid, peaks **26-27** as caffeoylquinic lactones, peaks
460 **35-36** and **56** as dicaffeoylquinic acid, peaks **39-41**as 4,5-dicaffeoylquinic acid, peaks
461 **42-43** as 3-caffeoyl-4-feruloylquinic acid, and peaks **47** and **57** as *cis*-3-caffeoyl-4-
462 feruloylquinic acid. Compounds **55** and **58** were at trace levels, below the limit of
463 quantification, and compound **34** overlapped with an unknown substance hindering its
464 quantification.

465 Results showed a high total phenolic content in all the commercial brands of yerba
466 mate, around 80 mg/g dry matter. These results are in accordance with values previously
467 reported ranging from 81 to 97 mg/g (Bravo et al., 2007; Marques & Farah, 2009),
468 highlighting the importance of yerba mate as a rich source of polyphenols compared with
469 other foods widely consumed such as cocoa powder (35 mg/g), black tea (10.2 mg/g),
470 orange juice (0.6 mg/mL) or red wine (10.1 mg/mL).

471 Caffeoylquinic acids were the major constituents of the phenolic fraction of yerba
472 mate. In particular, 3-caffeoylquinic acid was the major compound in all extracts,
473 accounting for over 26.8-28.8% of the total polyphenols, followed by 5-caffeoylquinic
474 (21.1-22.4%) and 4-caffeoylquinic acids (12.6-14.2%) (Table 1, supporting information).
475 These values were similar to the results previously reported by others authors (Bravo et
476 al., 2007; Heck, Schmalko, & Gonzalez de Mejia, 2008; Marques & Farah, 2009).
477 Dicaffeoylquinic acids were the second most abundant group of polyphenols, being 3,5-
478 dicaffeoylquinic acid the main isomer presents in yerba mate (9.5-11.3%). The high
479 content in caffeoylglycosides determined in all commercial brands (over 3-3.5%) is
480 noteworthy, particularly that of CDOA (up 1.4% of total polyphenols), which was identified
481 for the first time in yerba mate. The caffeoylglycoside content was higher than other
482 typical hydroxycinnamoylquinic acids associated to this plant, such as feruloylquinic or
483 *p*-coumaroylquinic acid isomers (2.5% and 0.5%, respectively). Minor amounts of some
484 dihydroxycinnamic acid derivatives, such as caffeoyl-feruloylquinic, caffeoyl-*p*-
485 coumaroylquinic or caffeoyl-sinapoylquinic acids were determined and ranging from

486 0.05% to 0.7% of the total polyphenols. Caffeic acid was mainly esterified with quinic
487 acid, although minimal amount of free caffeic acid was also quantified, accounting for
488 0.2% of total polyphenols. Flavonols were another important group of polyphenols
489 identified in yerba mate (up to 10% of total polyphenols), being rutin the most abundant
490 (80%), even more than other hydroxycinnamic acid derivatives, in agreement with others
491 authors (Bravo et al., 2007; da Silveira et al., 2016; Heck et al., 2008).

492 Although there are many studies on the qualitative and quantitative characterization
493 of yerba mate polyphenols (Bravo et al., 2007; da Silveira et al., 2016; Marques & Farah,
494 2009, among others), the number of polyphenols identified and quantified is substantially
495 lower than in the results here presented. In this sense, the inclusion of a third mobile
496 phase (ternary system) compared to the widely biphasic gradient used, enhanced both
497 resolution and peak symmetry, making feasible the quantification of 46 out of the 58
498 polyphenols identified, which represents a significant improvement over previously
499 published works (Bravo et al., 2007; da Silveira et al., 2016; Marques & Farah, 2009;
500 Jaiswal et al., 2010a).

501 Comparing the four commercial brands, the content of minor hydroxycinnamic acid
502 derivatives and flavonols was similar among them showing more differences in the
503 mono- and dicaffeoylquinic acids. Mate C and A presented the highest and lowest
504 polyphenolic content, respectively, with a contrary tendency regarding hydroxycinnamic
505 acid derivatives; while C showed the highest hydroxycinnamoylquinic acids content, A
506 was the richest in caffeoylquinic lactones. This tendency was extrapolated to samples B
507 and D. However, flavonols content *vs*. total polyphenol, showed a low correlation (R^2 =
508 0.56, polyphenol total *vs.* flavonols). The aforementioned differences among individual
509 constituents of yerba mate might be caused by genetic, environmental and production
510 factors, which can affect the biosynthetic pathways of polyphenols (Nakamura,
511 Donaduzzi, & Schuster, 2009). Nevertheless, in the present study the qualitative and
512 quantitative analysis was similar among the different commercial brands, in agreement
513 with previous studies that did not report important differences in the polyphenol content

514 of different commercial brands of yerba mate, in contrast to other plants (*Camellia*
515 *sinensis* and *Melissa officinalis*) that showed a significant variation (Marques & Farah,
516 2009).
517

518 *3.3. Identification and quantification of methylxanthines by HPLC in yerba mate extracts*

519 Methylxanthines (MX) were characterized by comparison with UV spectra and
520 retention times of standards. Overlapping chromatograms registered at 272 nm of yerba
521 mate extracts (Figure1a, in blue) and MX standards (caffeine, theophylline and
522 theobromine, Figure1a, in green) allowed to identify the presence of theobromine and
523 caffeine at 7.28 and 18.68 min, respectively. However no theophylline was detected in
524 any of the analyzed commercial brands of yerba mate in agreement with previous studies
525 (Blum-Silva, Chaves, Schenkel, Coelho, & Reginatto, 2015; Dugo et al., 2009). Only
526 Mazzafera (1994) detected traces of theophylline in yerba mate. In this sense, Heck et
527 al. (2008) indicated that it is difficult to detect theophylline in yerba mate since this is an
528 intermediate in the catabolism of caffeine in plants, being transformed into 3-
529 methylxanthine or theobromine.

530 Quantitative analysis of mate showed that caffeine was more abundant than
531 theobromine in all samples of yerba mate (Table 2), accounting to 88.5% of the total MX
532 content, which is in line with the previously reported values (Meinhart et al., 2010, among
533 others). Commercial brands B and C presented the richest and poorest MX content,
534 respectively, in contrast to their respective phenolic concentration. In this sense, null
535 correlation was found between both parameters (R^2 = 0.0002 MX *vs.* phenolic content
536 determined both colorimetrically and chromatographically).

537 Yerba mate, with contents of methylxanthines between 8.2-40.2 mg/g, can be
538 considered a modest source of these purine alkaloids compared with coffee (16.1-38.6
539 mg/g of caffeine, 0.5-2.3 mg/g of theobromine and 0.003 mg/g of theophylline,
540 Tzanavaras, Zacharis, & Themelis, 2010), tea (6.8-23.8 mg/g of caffeine and 0.01-1.8

541 mg/g of theobromine, Tzanavaras et al., 2010) or kola nuts (10-25 mg/g of caffeine and
542 1 mg/g of theobromine, Burdock, Carabin, & Crincoli, 2009).
543

544 *3.4. Determination of total phenolic compounds by Folin-Ciocalteu and antioxidant*
545 *capacity in yerba mate extracts*

546 The extracts of yerba mate had a similar content of polyphenols determined by Folin-
547 Ciocalteu method (10% in basis of dry matter) (Table 3), slightly higher than the results
548 reported by HPLC (7.6-8.4%, Table 2). This measurement was performed to compare
549 with published data on this and other beverages, since the Folin-Ciocalteu method has
550 been traditionally used to quantify the phenolic content in different samples in spite of
551 the poor specificity of the reagent, which reacts with any reducing substance present in
552 the sample leading to an overestimation of the real total phenolic content (Singleton,
553 Orthofer, & Lamuela-Raventos, 1999). Nevertheless, phenolic contents determined by
554 both spectrophotometric and chromatographic methods in the present work showed a
555 good correlation. These results are in agreement with the literature and confirm that
556 yerba mate is an important source of polyphenols, with higher phenolic content than
557 other polyphenol-rich beverages like black tea or orange juice (Blum-Silva et al., 2015;
558 Bravo et al., 2007).

559 The antioxidant capacity of the yerba mate extracts was characterized by the FRAP
560 assay, that measures the reducing power of antioxidants, and ABTS and ORAC assays,
561 to evaluate their radical scavenging activity. The results (summarized in Table 3)
562 highlighted the high antioxidant capacity of all the evaluated commercial brands (A-D),
563 showing a direct relationship with the total polyphenolic (TPP) content determined by
564 Folin-Ciocalteu (R^2 = 0.93 TPP *vs.* FRAP, R^2 = 0.95 TPP *vs.* ABTS and R^2 = 0.74 TPP
565 *vs.* ORAC) and chromatographically (R^2 = 0.74 TPP *vs.* FRAP, R^2 = 0.85 TPP *vs.* ABTS
566 and R^2 = 0.75 TPP *vs.* ORAC). In addition, null or low correlations between MX content
567 and antioxidant activity were observed (R^2 = 0.06 MX *vs.* FRAP, R^2 = 0.007 MX *vs.* ABTS

568 and R^2 = 0.18 MX *vs*. ORAC), contrary to the antioxidant capacity associated to MX by
569 other authors (Azam, Hadi, Khan, & Hadi, 2003). These results suggest that phenolic
570 compounds are the main contributors to the antioxidant activity of mate beverages. The
571 direct relationship between total phenolic compounds and antioxidant capacity was in
572 agreement with results previously reported in yerba mate (Valerga, Reta, & Lanari,
573 2012).
574 Compared with other foods widely consumed, yerba mate showed an important
575 antioxidant activity (FRAP = 711-820 µmol TE/g; ABTS = 410-438 µmol TE/g and ORAC
576 = 2173-2434 µmol TE/g, Table 3) being even higher than other foods such as cocoa (620
577 µmol TE/g for ORAC), orange juice (515 and 249 µmol TE/g for FRAP and ABTS,
578 respectively) and white (154 and 181 µmol TE/g for FRAP and ABTS, respectively) or
579 rose wine (286 and 261 µmol TE/g for FRAP and ABTS, respectively), among others
580 (Martin et al., 2008; Saura-Calixto & Goñi, 2006). In addition, the values of TPP and
581 antioxidant capacity are in the range of those reported by others authors in yerba mate
582 (TPP = 6.7 g/100 g; FRAP = 506.6 µmol TE; ABTS = 457.1 µmol/g) (Oh, Jo, Cho, Kim,
583 & Han, 2013; Zielinski et al., 2014).
584
585
586
587 *Contributions and limitations of the study*
588 The optimized chromatographic method used in the present study allowed a more
589 complete characterization of the polyphenols and methylxanthines in yerba mate
590 extracts from four commercial branches widely consumed in Argentina and exported to
591 other countries like Spain, where its consumption is steadily increasing. A total of 58
592 polyphenols were detected and identified using LC-DAD-MS-QToF and subsequent LC-
593 MS^n analysis including one hydroxycinnamic acid, 12 caffeoyl-glycosides, 38
594 hydroxycinnamate esters, 2 caffeoylquinic lactones and 6 flavonols. As far as the authors
595 know, 4-sinapoylquinic acid, di- and tri-methoxycinnamoylquinic acids,

596 trimethoxycinnamoylshikimic acid and four isomers of caffeoyl-2,7-anhydro-3-deoxy-2-
597 octulopyranosonic acid (CDOA), caffeic acid esterified with a monosaccharide of eight
598 carbon atoms, have been identified for the first time in yerba mate. Additionally, 46
599 phenolic compounds and two methylxanthines have been quantified in mate using
600 HPLC-DAD, which represents an important achievement. However, it is important to
601 highlight that this study was performed in commercial mate products; processing might
602 have altered the initial phenolic composition of mate leaves, since high temperatures
603 during drying and roasting, and the prolonged storage during the "estacionado" of the
604 dried leaves may have caused oxidation and chemical transformations of the phenolic
605 compounds. Therefore, it cannot be inferred whether the newly identified polyphenols
606 were initially present in the fresh mate leaves or formed during processing and storage.
607 Nevertheless, since the analyzed mates are the products actually used by consumers,
608 we consider of interest the exhaustive chemical characterization of the phenolic fraction
609 here provided.

610 In conclusion, the results revealed that *Ilex paraguariensis* is an important source of
611 polyphenols with a moderate content of methylxanthines and, therefore, with high
612 antioxidant potency mainly associated to their polyphenolic content.
613
614

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622
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624

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772 **Figure captions**

773 **Figure 1.**Typical HPLC-DAD chromatograms of yerba mate extracts at 272 nm (a, in
774 blue), 320 nm (b), 320 nm enlarged (c) and 360 nm (d). Chromatographic profile of
775 methylxanthine standards (theobromine –TB-, theophylline –TP- and caffeine –CF-)
776 registered at 272 nm (a, in green).

777 **Figure 2.** Chemical structures of methylxanthines (a) and hydroxycinnamic acids
778 derivatives (b) identified in yerba mate.

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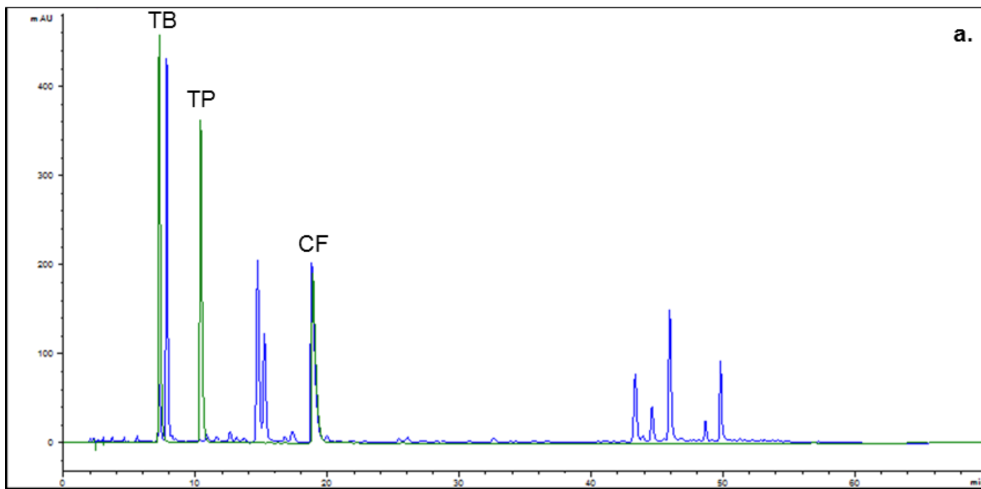
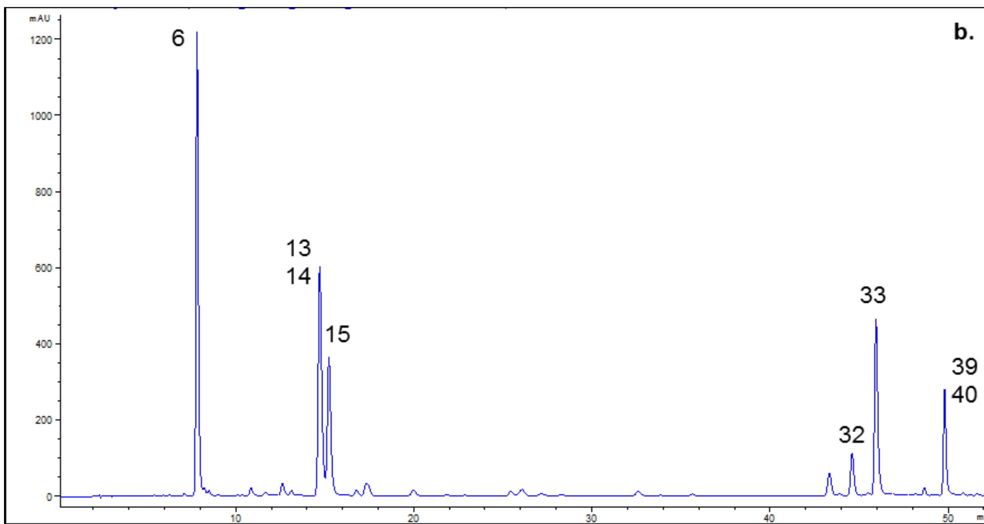
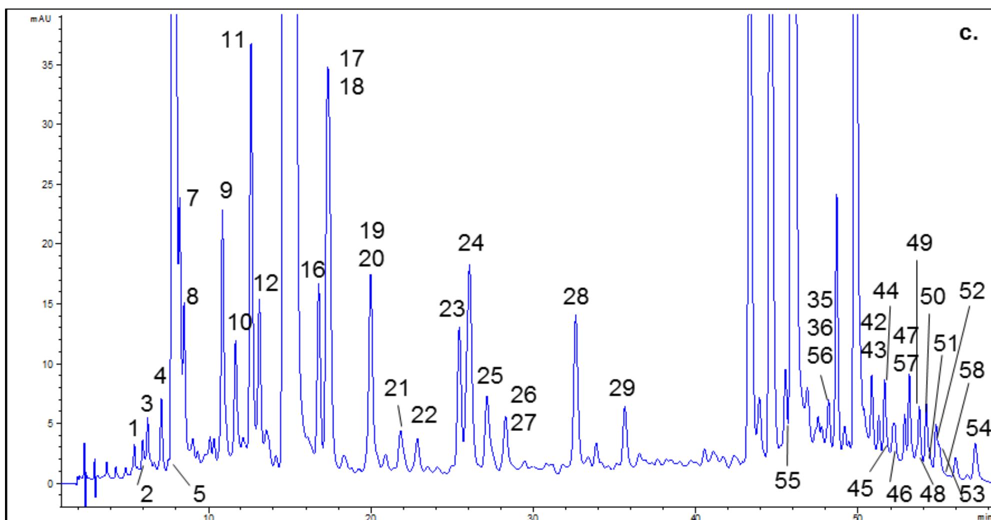
796 **Figure 1.**

797 

798 

799 

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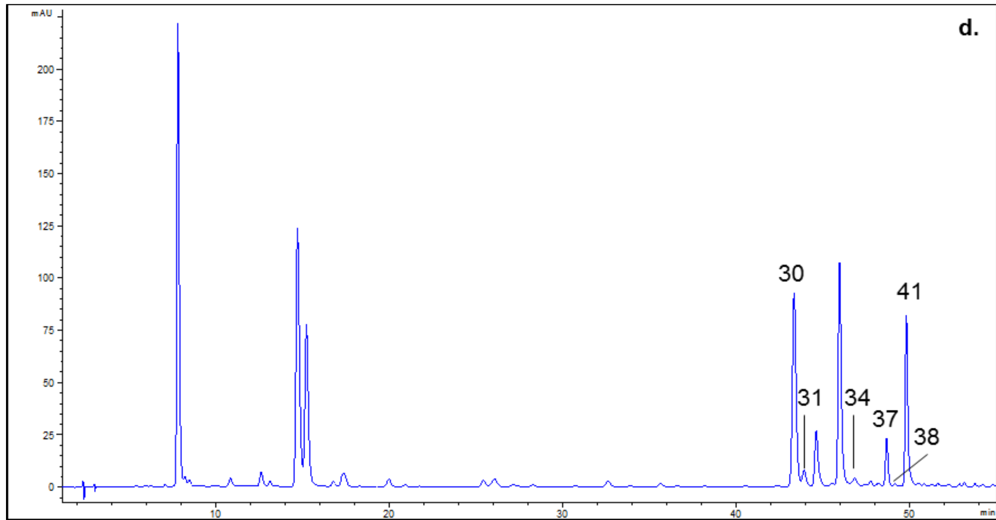

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820 **Figure 2.**

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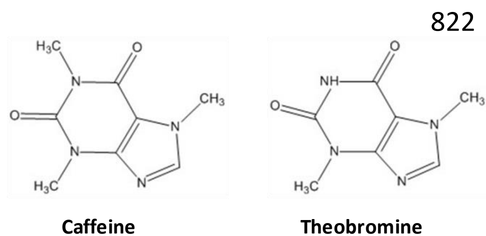

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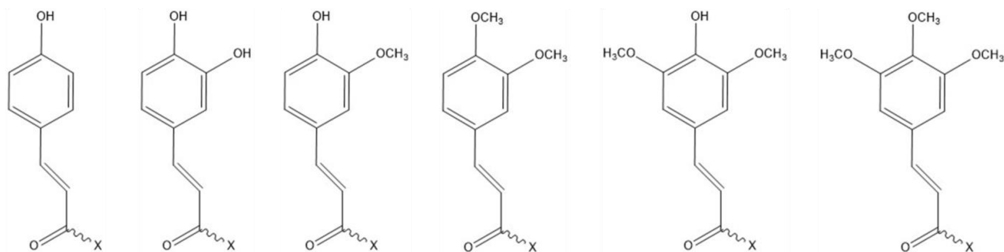

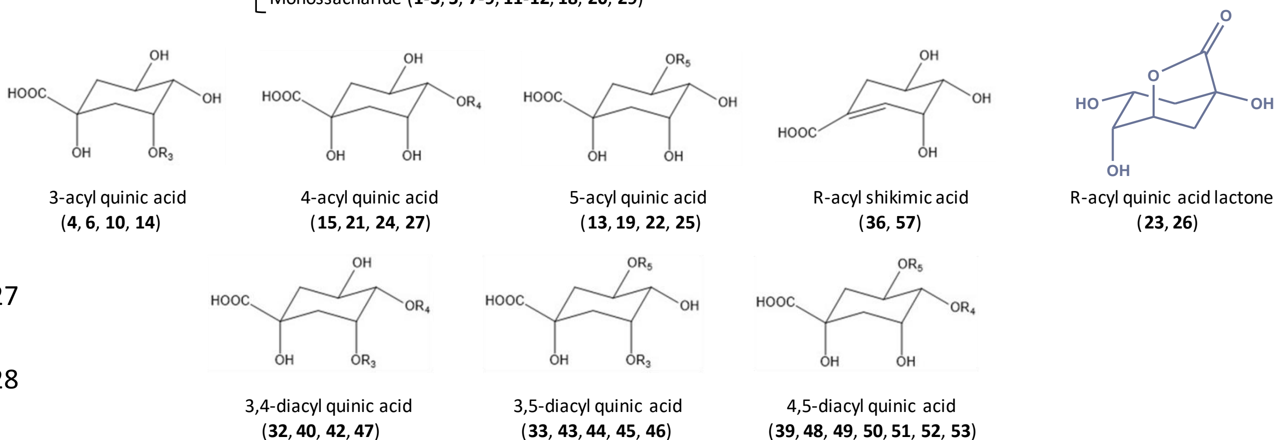

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837 **Table 1.** HPLC-DAD characterization and negative ion MS/MS fragmentation of phenolic compounds in yerba mate.

No.	Name	RT (min)	λ_{max}	MS^1 *m/z*	MS^2 Base peak *m/z*	MS^2 Secondary peak *m/z*	% RA	*m/z*	% RA	*m/z*	% RA	*m/z*	% RA
1	Caffeoylglucose	5.43	324	341.0880	161	323	5.0	221	11.6	203	33.0	179	33.0
2	Caffeoylglucose	5.93	325, 294sh	341.0800									
3	β-1-Caffeoylglucose	6.24	322, 294sh	341.0700	203	281	15.0	221	31.0	179	90.0	161	100.0
4	*cis*-3-Caffeoylquinic acid	7.09	321, 296sh	353.0877	191	179	90.0	135	21.4				
5	Caffeoylglucose	7.6	326	341.0880									
6	3-Caffeoylquinic acid	7.81	324, 296h	353.0891	191	179	76.9	135	19.2				
7	Caffeoylglucose	8.22	327, 298sh	341.0880	161	203	55.0	179	77.0	135	18		
8	Caffeoylglucose	8.46	326, 298sh	341.0880	161	203	55.0	179	77.0	135	18		
9	6-Caffeoylglucose	10.85	324, 294sh	341.0880	281	251	37.0	221	58.0	179	95	161	43
10	3-*p*-Coumaroylquinic acid	11.66	312	337.0925	163	191	12.0						
11	CDOA	12.81	326, 296sh	381.0820									
12	α-1-Caffeoylglucose	13.12	326, 296sh	341.0880	179	281	89.0	251	55.0	221	43.0	161	31.6
13	5-Caffeoylquinic acid	14.70	325, 296sh	353.0893	191								
14	3-Feruloylquinic acid	14.70	*	367.1028	193	173	23.4	134	14.7				
15	4-Caffeoylquinic acid	15.22	326, 296sh	353.0890	173	179	65.5	191	50.0	135	20.9		
16	Caffeic acid	16.77	322, 296sh	179.0351	135								
17	Feruloylquinic acid	17.34	325, 296sh	367.1030	161								
18	CDOA	17.34	*	381.0820									
19	*cis*-5-Caffeoylquinic acid	19.97	326, 296sh	353.0877	191								
20	CDOA	19.97	*	381.0820									
21	4-*p*-Coumaroylquinic acid	21.81	312	337.0917	173	191	10.0	145	5.4				
22	5-*p*-Coumaroylquinic acid	22.86	312	337.0917	191	173	9.0						
23	Caffeoylquinic lactone	25.43	326, 296sh	335.0779	161	335	39.0	135	14.2				
24	4-Feruloylquinic acid	26.06	326, 296sh	367.1044	173	161	100	193	18.0	135	30.0		
25	5-Feruloylquinic acid	27.13	325, 296sh	367.1030	191	173	14.3						
26	Caffeoylquinic lactone	28.25	325, 296sh	335.0750	161	335	33.8	191	12.5	135	5.7		
27	4-Sinapoylquinic acid	28.25	*	397.1137	173	191	35.4						
28	Feruloylquinic acid	32.50	326, 296sh	367.1040	179	135	31.8						
29	CDOA	35.62	326, 294sh	381.0820									

30	Rutin	43.32	255, 354	609.1464							
31	Quercetin-glycoside	43.90	256, 353	463.0870	301						
32	3,4-Dicaffeoylquinic acid	44.6	325, 296sh	515.1200	515	353	81.9	173	30.1		
33	3,5-Dicaffeoylquinic acid	45.94	327, 296sh	515.1212	353	191	21.2				
34	Isorhamnetin-glycoside	46.86	262, 372	477.1030							
35	Dicaffeoylquinic acid	48.16	324, 296sh	515.1180	353	515	6.8	179	5.7		
36	Trimethoxycynnamoylshikimic acid	48.16	*	393.1190							
37	Kaempferol-rhamnoglucoside	48.66	265, 349	593.1509	285						
38	Kaempferol-glycoside	49.15	262, 350	447.0930							
39	4,5-Dicaffeoylquinic acid	49.79	326, 296sh	515.1217	353	515	20.5	173	23.2		
40	3-*p*-Coumaroyl-4-caffeoylquinic acid	49.79	*	499.1260	353	173	19.0				
41	Isorhamnetin-glycoside	49.79	250, 337	477.1030							
42	3-Caffeoyl-4-feruloylqinic acid	50.82	322, 296sh	529.1354	367	529	100.0	173	13.3	161	22.3
43	3-Caffeoyl-5-*p*-coumaroylquinic acid	50.82	*	499.1246	353						
44	3-Feruloyl-5-caffeoylquinic acid	51.61	328, 294sh	529.1347	367	193	55.7				
45	3-Sinapoyl-5-caffeoylquinic acid	51.61	326, 296sh	559.1444	397	223	14.9				
46	3-Caffeoyl-5-feruloylquinic acid	52.20	326, 296sh	529.1300	353	367	73.1	191	47.4	135	18.6
47	*cis*-3-Caffeoyl-4-feruloylquinic acid	53.13	328, 296sh	529.1362	529	367	19.1	173	15.7	161	12.7
48	4-*p*-Coumaroyl-5-caffeoylquinic acid	53.74	312	499.1240	337	173	72.9				
49	4-Sinapoyl-5-caffeoylquinic acid	53.74	326	5591451	397	223	14.1	173	56.2		
50	4-Feruloyl-5-caffeoylquinic acid	54.17	326, 296sh	529.1350	367	529	5.4	173	50.2		
51	4-Caffeoyl-5-*p*-coumaroylquinic acid	54.17	312	499.1240	353	337	6.3	173	52.3		
52	*cis*-4,5-Dicaffeoylquinic acid	54.77	325, 296sh	515.1197	353	515	10.7	173	39.1		
53	4-Caffeoyl-5-feruloylquinic acid	54.77	326, 294sh	529.1350	353	529	28.0	191	12.7	173	40.4
54	Caffeoyl-feruloylquinic acid	57.19	328, 300sh	529.1360	529	367	17.0	179	28.5		
55[a]	Trimethoxycynnamoylquinic acid	45.51	324, 296sh	411.1299							
56[a]	Dimethoxycynnamoylquinic acid	48.16	325, 264sh	381.1191							
57[a]	Trimethoxycynnamoylshikimic acid	53.13	328, 296sh	393.118							
58[a]	Tentative identification as Diferuloylquinic acid	55.50	325,296sh	543.151							

Abbreviations: RT = retention time; % RA = percentage of relative abundance; CDOA = caffeoyl-2,7-anhydro-3-deoxy-2-octulopyranosonic acid.

*λmax not detected by overlapping with the previous chromatographic compound.

[a] Compounds detected only in mate

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843 **Table 2.** Content of hydroxycinnamic acid derivatives and flavonols in addition to methylxanthines in extracts from different commercial brands of yerba mate. Values are
844 means ± SD expressed on mg/g dry matter (n = 3 analyzed in duplicate)

Polyphenolic Group	Compound	Mate A	Mate B	Mate C	Mate D
Hydroxycinnamic acid	Caffeic acid	0.198 ± 0.008	0.173 ± 0.007	0.231 ± 0.008	0.197 ± 0.007
Hydroxycinnamic-glycosides	Caffeoyl-glycosides	2.613 ± 0.052	3.043 ± 0.077	2.900 ± 0.065	2.352 ± 0.056
Hydroxycinnamoylquinic acids	Caffeoylquinic acids	48.180 ± 1.406	53.564 ± 0.802	54.134 ± 1.784	50.646 ± 1.304
	Feruloylquinic acids	3.103 ± 0.258	1.764 ± 0.060	2.033 ± 0.184	1.592 ± 0.059
	p-Coumaroylquinic acids	0.413 ± 0.013	0.417 ± 0.008	0.358 ± 0.016	0.406 ± 0.011
	Dicaffeoylquinic acids	13.894 ± 0.879	15.722 ± 0.693	16.608 ± 0.748	13.022 ± 0.684
	Caffeoyl-feruloylquinic acids	0.514 ± 0.041	0.462 ± 0.015	0.548 ± 0.019	0.357 ± 0.019
	Caffeoyl-*p*-coumaroylquinic acids	0.055 ± 0.003	0.052 ± 0.003	0.053 ± 0.003	0.043 ± 0.002
	Caffeoyl-sinapoylquinic acids	0.079 ± 0.006	0.108 ± 0.008	0.091 ± 0.005	0.070 ± 0.005
Hydroxycinnamoylquinic lactones	Caffeoylquinic lactones	0.487 ± 0.014	0.358 ± 0.009	0.326 ± 0.016	0.459 ± 0.016
TOTAL HIDROXYCINNAMATES		**66.725 ± 1.693**	**72.447 ± 1.071**	**74.152 ± 1.959**	**66.596± 1.490**
Flavonols	Rutin	5.597 ± 0.159	6.738 ± 0.322	6.043 ± 0.313	6.030 ± 0.330
	Quercetin-glycoside	0.365 ± 0.026	0.504 ± 0.016	0.581 ± 0.032	0.458 ± 0.023
	Kaempferol-rhamnoglucoside	0.943 ± 0.040	1.019 ± 0.072	0.922 ± 0.025	0.880 ± 0.080
	Kaempferol-glycoside	0.037 ± 0.004	0.052 ± 0.003	0.059 ± 0.005	0.040 ± 0.004
TOTAL FLAVONOLS		6.942 ± 0.166	8.313 ± 0.330	7.604 ± 0.315	7.408 ± 0.340
TOTAL POLYPHENOLS		**76.478 ± 1.688**	**83.976 ± 1.115**	**84.887 ± 1.970**	**76.552 ± 1.514**
Theobromine		1.16 ± 0.04	1.30 ± 0.07	1.05 ± 0.05	0.95 ± 0.006
Caffeine		8.83 ± 0.27	8.95 ± 0.51	7.16 ± 0.33	7.34 ± 0.49
TOTAL METHYLXANTHINES		9.98 ± 0.27	10.25 ± 0.51	8.21 ± 0.33	8.28 ± 0.49

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848 **Table 3.** Total polyphenolic content and antioxidant capacity of extracts from different commercial brands of yerba mate. Values are means ± SD expressed on a dry matter
849 basis (d. m.) (n = 3 analyzed in triplicate). Different letters within a column indicate statistically differences ($p < 0.05$). TE: Trolox equivalents.

	Total phenolic content	Antioxidant capacity		
	Folin-Ciocalteu (g/100 g d.m.)	FRAP (μmolTE/g d.m.)	ABTS (μmolTE/g d.m.)	ORAC (μmol TE/g d.m.)
Mate A	10.32 ± 0.17 ^{b}	733.64 ± 32.62 ^{c}	417.15 ± 17.76 ^{bc}	2172.96 ± 169.15 ^{a}
Mate B	10.45 ± 0.11 ^{ab}	759.04 ± 25.19 ^{b}	426.99 ± 20.57 ^{ab}	2270.10 ± 162.07 ^{a}
Mate C	10.79 ± 0.39 ^{a}	820.59 ± 25.50 ^{a}	438.15 ± 18.39 ^{a}	2433.98 ± 241.71 ^{a}
Mate D	9.95 ± 0.27 ^{c}	711.41 ± 25.26 ^{c}	410.50 ± 15.97 ^{c}	2192.89± 237.64^{a}

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