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Study of the chlorogenic acid content in yerba mate (*Ilex paraguariensis* St. Hil.): Effect of plant fraction, processing step and harvesting season

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ABSTRACT

Chlorogenic acid (CGA) is a fine chemical used in different food and pharmaceutical industries. CGA is currently extracted from various plant materials, particularly green coffee beans. Yerba mate (*Ilex paraguariensis* St. Hil.) contains significant amounts of CGA with very low levels of extraction interfering substances (fatty materials). Both of these reasons prompted us to evaluate yerba mate as a novel source for extraction of this compound.

CGA content was quantified in various yerba mate fractions during different processing steps, and in samples taken from two companies at early and late harvesting seasons. Samples were exhaustively extracted with hot water and total CGA content (including its three isomeric compounds) was determined by HPLC. Total CGA content (on a dry weight basis) ranged from 45.8 ± 0.4 to 80.8 ± 1.0 g CGA kg^{-1} of leaves and from 31.6 ± 0.6 to 78.9 ± 5.3 g CGA kg^{-1} of stems. A substantial reduction in CGA content was found along the processing steps. The highest CGA content was found in samples from freshly harvested (green) yerba mate, for both leaves and stems; with no significant differences in their CGA content ($P < 0.05$). CGA content at the early harvesting season was substantially higher to that obtained at the end of the harvesting season, for both green leaves and stems.

Green stems, a residue from yerba mate processing obtained at the early harvesting season, could be considered as a promising and valuable raw material for the production of CGA-enriched extracts.

1. Introduction

Structurally, chlorogenic acid (CGA, CAS number: 327-97-9) is an ester formed between caffeic and L-quinic acids (5-*O*-caffeoyl-quinic acid) (Fig. 1). Isomers of CGA include 3-*O*-caffeoyl-quinic acid (neochlorogenic acid, neo-CGA) and 4-*O*-caffeoyl-quinic (cryptochlorogenic acid, crypto-CGA). CGA is a kind of polyphenol derivative widely present in higher plants (Delage et al., 1991) that is extensively used in medicine and industries such as in consumer chemicals and food industries (Kweon et al., 2001). It is employed as additive in beverages, cosmetics, tea products and foods as well as medical substances (Jiang et al., 2000; Jin et al., 2005). Potentially beneficial properties to humans such as antioxidant, hypoglycaemic, antiviral and hepatoprotective activities have been also attributed to CGA in *in vitro*, *in vivo* and epidemiological studies (Farah and Donangelo, 2006).

CGA is currently available in the international market both as analytical grade reagent, as well as food grade and bulk product, being considered within the category of Fine Chemicals. Current FOB price of CGA (food grade, bulk product, 95% w/w purity) is estimated in around US$ 3500 per kilogram (personal communication from a trader, 2014). Its present commercial sources are from plant extracts of *Lonicera japonica* Thunb and *Eucommia ulmoides* Oliver (Chun and Kim, 2004; Clifford et al., 2006; Li et al., 2005; Rønsted et al., 2002). These sources are generally limited and therefore expensive. CGA is also present in relative high concentrations in other plant resources, such as apples, pears and potato tubers, and mainly in coffee berries, particularly in green (or raw) coffee beans, material which is considered to date as the most important CGA natural source (Clifford, 1999, 2000). Green coffee beans are

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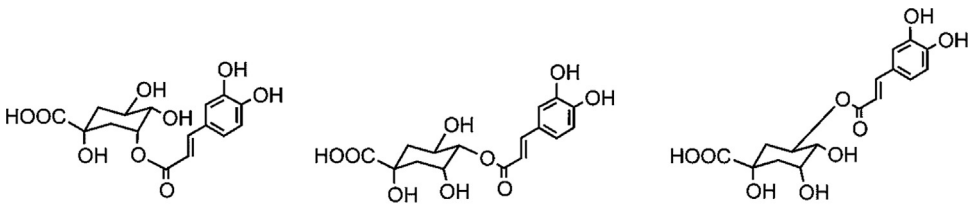

Fig. 1. Chemical structure of chlorogenic acid and its isomeric compounds.

basically just unroasted coffee beans. Total CGA content of green coffee beans may vary according to genetics—species and cultivar, degree of maturation and, less importantly, agricultural practices, climate and soil (Farah and Donangelo, 2006). The diversity of methodology employed in the analysis of CGA is another important factor when comparing levels. Depending on the species, green coffee beans contain some 6–10% CGA on a dry weight basis. Due to thermal instability, CGA is degraded into low molecular weight phenol derivatives during coffee roasting, resulting in a progressive destruction and transformation of CGA with some 8–10% being lost for every 1% loss of dry weight (Clifford, 1999, 2000).

Ilex paraguariensis St. Hil. (Aquifoliaceae) (yerba mate) is an arboreal species that naturally grows and is cultivated in the temperate and subtropical climatic regions of Argentina, Brazil, and Paraguay. Argentina is the main producer of yerba mate (≈60% of the worldwide production accounting for 750,000 ton/year in average). Yerba mate is traditionally employed as a decoction or infusion due to its nutritional and medicinal properties (tonic, choleretic, diuretic, anti-rheumatic, etc.). Values as high as 8–10% (w/w) CGA (including its various isomeric compounds) on a dry weight basis have been reported for different yerba mate derived materials (Isolabella et al., 2010; Marques and Farah, 2009; Pagliosa et al., 2010). Therefore, yerba mate could be considered as a completely new, still untapped and highly competitive source for CGA extraction.

The production process of yerba mate involves harvesting of the leaves and stems, which are roasted, dried over fire, minced, aged, milled, and packed for their commercialization. Processing may vary among industries although the procedure is basically the same. Fig. 2 shows a typical process flow chart for yerba mate (Schmalko and Alzamora, 2001).

During the industrial processing steps of yerba mate (harvesting, roasting, drying, and aging) some changes in the profile and concentration of bioactive compounds may be produced (López et al., 2006). Only a few recent studies have been carried out to assess the changes that occur in yerba mate during its processing. Bastos et al. (2006) and Isolabella et al. (2010) have studied CGA variation, among other compounds, of yerba mate leaves along the different processing steps. Nevertheless, to date there is no detailed information of the CGA content in the different fractions of yerba mate (particularly leaves and stems) during its industrial processing and at different harvesting seasons.

Scientific information available on yerba mate includes studies on its composition, physiological effects, and potential health implications as well as technological considerations for its processing (Heck and Gonzalez de Mejia, 2007). Nevertheless, no examples about yerba mate utilization as raw material for bio-transformations have been described. A novel strategy for industrial production of various useful materials of pharmaceutical significance from our traditional yerba mate is under development. This strategy is based on its high CGA content, comparable to that in coffee products, but with a lower level of extraction interfering substances, particularly fatty materials. Therefore, yerba mate seems to be a quite special and unique source of CGA, which can be bio technologically converted into value added compounds of pharmaceutical significance. For instance, CGA can be readily converted to shikimate via quinate, 3-dehydroquinate and 3-dehydroshikimate (Adachi et al., 2006a,b,c, 2008). Shikimate is important as the direct precursor for Oseltamivir synthesis (Roche's brand name Tamiflu®), the potent and selective competitive inhibitor of influenza A and B neuraminidase (Enserink, 2006) preventing people from pandemic flu infection, as well as for the synthesis of antibiotics, amino acids and agrochemicals.

According to the previously described, we investigated the CGA content, in both leaves and stems, along the different processing steps in two yerba mate companies at the beginning and at the end of the harvesting season. This information is mandatory for selecting the most adequate yerba mate fraction as raw material for the preparation of CGA-enriched aqueous extracts for subsequent CGA-biotransformation into valuable products.

2. Materials and methods

2.1. Yerba mate materials

Samples of fresh (just harvested) yerba mate branches and processed yerba mate were supplied by two yerba mate processing plants (Plant A and Plant B) located in Apóstoles, Misiones, Argentina. Collection and storage of the plant material were carried out under strict controlled conditions. Plant material was mechanically harvested during the beginning (April and May, 2014) and at the end of the harvesting season (September 2014). Analysis was carried out on samples taken from the same lot (≅10 ton) during the following processing steps:

i) Harvesting (samples labeled H): samples of fresh yerba mate branches (≅20 kg, randomly chosen) just harvested were conveniently cut into pieces and treated in our laboratory in a microwave oven at maximum power (700 W) for 5 min to inhibit enzymatic activity.
ii) Roasting (R, locally called zapecado): in this stage, green yerba mate branches are exposed to direct fire at temperatures between 250 and 550 °C during 2–4 min depending on processing plant to inactivate oxidizing enzymes. Samples of yerba mate branches (≅20 kg, randomly chosen) were analyzed.
iii) Drying (D): in both processing plants it was performed employing a continuous belt system. In the case of Plant A drying consisted of the following sub-steps: pre-drying (PD) (250 °C for 7 min), first drying belt (1st D) and second drying belt (2nd D) (105–120 °C for 1.5 h). In Plant B drying was performed at 90 °C during 5 h. Samples (≅20 kg, randomly chosen from each drying process) of yerba mate branches were analyzed. In addition, in the case of Plant B, samples taken after coarse milling were also analyzed. In these cases (processing steps i, ii and iii),

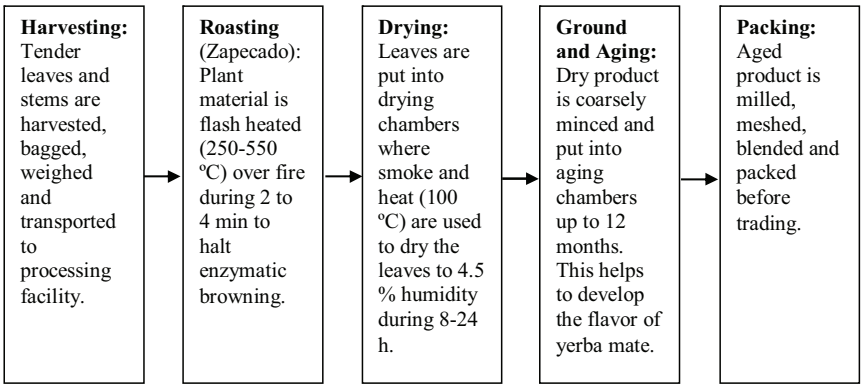

Fig. 2. Flow chart for the industrial processing of yerba mate (adapted from Schmalko and Alzamora, 2001).

leaves and small stems were manually separated from yerba mate branches before analysis.

iv) Processed yerba mate (PYM, material subjected to the previous stages of aging, milling and sieving). These samples correspond to the three fractions (leaves, stems and powder, ≅5 kg in each case) that are mixed in varying proportions (blends) depending on the nature and brand of the yerba mate being produced to obtain the final product before packing and commercialization.

In order to facilitate extraction, all samples above mentioned were ground separately to fine powder (except for the case of the powder from PYM) with a cutter mill with 500 μm pore mesh (Ø particle size <500 μm). Powdered samples thus obtained were packed into airtight plastic bags and stored at 4 °C until required.

2.2. Chemicals and reagents

CGA was a product of Sigma–Aldrich (St. Louis, USA). HPLC grade water, formic acid and acetonitrile were from Panreac (Barcelona, Spain), Tedia (Fairfield, USA) and J.T. Baker (Phillipsburg, USA), respectively. Distilled water was used for CGA extractions while for determination of CGA content the reagents used were of analytical or chromatographic grade.

2.3. Moisture content

In order to express the amount of CGA on a dry weight basis, moisture content of the samples was determined according to the AOAC method (AOAC, 2000).

2.4. Preparation of yerba mate extracts

Water extracts were prepared by boiling 500 mg of each sample (duplicate) in 50 mL of distilled water (98 °C) during 5 min with periodic agitation, according to Heck et al. (2008) and Isolabella et al. (2010), with modifications. Then, samples were left to cool down at room temperature, centrifuged (15 min 6000 rpm) and the volume made up to 50 mL to recover water lost during boiling. The extraction step was carried out again on the solid remaining in the same conditions described above. Reported values for CGA correspond to the sum of those obtained from the first and second extractions. All extracts were kept at 4 °C after preparation or opening of the container until analysis, which was performed within no more than 4 days.

2.5. Identification and quantification of chlorogenic acid in yerba mate extracts

Identification of CGA and its isomeric compounds was conducted with a high performance liquid chromatography (HPLC) method adapted from Bravo et al. (2006). Analysis was carried out using a Shimadzu Prominence gradient liquid chromatograph, equipped with a Shimadzu SIL-20 A HT autosampler and a Shimadzu SPD-M20A diode array detector (DAD) UV/VIS. Samples (20 μL) were injected into the HPLC system, and separation was performed on a C18 RP Phenomenex Prodigy ODS3 column (5 μm, 250 mm × 4.6 mm I.D.) protected with an ODS RP18 guard column. Runs were done at room temperature and at a flow rate ranged from 1 to 1.2 mL min^{-1}. A binary gradient of 1% formic acid in deionized water (solvent A) and acetonitrile (solvent B) was as follows: from 10% to 17.5% solvent B over 15 min, 17.5% to 30% solvent B over 2 min, and then isocratically for 8 min, and, finally, it increased linearly to 10% solvent B over 1 min. The DAD was set at 326 nm. The identification of CGA and its isomeric compounds was carried out by comparing the retention times and absorption spectra (between 250 and 400 nm) of the peaks with those of pure standard compounds, absorbances at 326 nm (maximum for CGA), and elution patterns with those reported in literature (Bravo et al., 2006; Carini et al., 1998; Fang et al., 2002). Concentration of CGA and its isomers in yerba mate samples was calculated using a calibration curve of standard compound commercially available ($y = 6 \times 10^8\, x - 18511$, $R^2 = 1$) due to equality in the chemical structure. In all cases, values were expressed on a dry weight basis (grams per kilogram of dried material, g CGA kg^{-1} dw).

2.6. Statistical analysis

Data were expressed as means ± standard error of the mean of two independent determinations carried out by triplicate. A one way ANOVA with the *a posteriori* Tukey test was used to evaluate the significance of results. A probability (P) value <0.05 was considered significant. Multiple sample comparison was performed using the Statgraphics Centurion program version XVII.

3. Results and discussion

The moisture content for all samples analyzed varied according to the processing step, as it was expected (Table 1). The moisture average values found for the different samples were similar to those reported by other authors (Bastos et al., 2006).

The identification and quantification of CGA and its isomers (neo-CGA and crypto-CGA) was monitored by diode-array. All extracts obtained (from leaves, stems and powder) showed a sim-

Table 1
Moisture content of yerba mate materials along different processing steps in two processing plants.

Processing plant	Processing step	Moisture content (%)[A]		
		Leaves	Stems	Powder
A	Harvesting	60.78 ± 0.13^{a}	61.81 ± 0.00^{b}	–
	Roasting	20.69 ± 0.13^{c}	47.38 ± 0.01^{d}	–
	Pre-drying	13.22 ± 0.09^{e}	34.71 ± 0.10^{f}	–
	1st Drying	5.27 ± 0.17^{g}	7.19 ± 0.02^{h}	–
	2nd Drying	2.00 ± 0.04^{i}	2.26 ± 0.05^{i}	–
	Processed yerba mate	2.49 ± 0.08^{i}	2.80 ± 0.01^{i}	2.63 ± 0.11^{i}
B	Harvesting	62.30 ± 0.05^{b}	65.22 ± 0.08^{j}	–
	Roasting	24.21 ± 0.14^{k}	47.96 ± 0.10^{d}	–
	Drying	5.95 ± 0.16^{g}	5.61 ± 0.12^{g}	–
	Milling	5.63 ± 0.00^{g}	5.95 ± 0.03^{g}	–
	Processed yerba mate	6.42 ± 0.13^{h}	7.37 ± 0.13^{h}	7.31 ± 0.18^{h}

[A] Results are expressed as g water per 100 g of wet material (mean ± SD, $n = 3$).
Different superscripts indicate significant difference ($p < 0.05$).

Table 2
UV characteristics of the chromatographic peaks.

Peak	RT (min)	λ_{max} (nm)	Compound
1	9.0	326, 296 sh	Neochlorogenic acid
2	13.5	326, 296 sh	Chlorogenic acid
3	14.6	326, 296 sh	Cryptochlorogenic acid

RT: retention time; sh: shoulder.

ilar phenolic profile. A typical elution chromatogram is shown in Fig. 3.

Table 2 gives the UV characteristics of the chromatographic peaks. The three compounds (peaks 1, 2 and 3) had the same UV spectra, with a maximum at 326 nm and a shoulder at 296 nm, typical of caffeoylquinic acid derivatives. The retention time and UV spectrum of the compound corresponding to peak 2 were identical to those of standard CGA. The other compounds would correspond to isomers of CGA. Considering the elution profile of CGA isomers from plant foods reported in the literature on C18 HPLC columns (Fang et al., 2002) and their UV spectra, compounds corresponding to peaks 1 and 3 were identified as 3-*O*-caffeoylquinic acid (neo-CGA) and 4-*O*-caffeoylquinic acid (crypto-CGA), respectively.

Water extracts were prepared from various yerba mate materials. A high solvent volume/sample weight ratio was chosen in order to assure a complete and exhaustive extraction of water soluble compounds. Extraction procedure was repeated twice resulting that total amount of CGA plus its isomers in the second extract was around 5 to 10% of the values obtained in the first extraction. A subsequent third extraction did not result in significant CGA values (lower than 1% compared with those of the first extraction). Therefore, our extraction procedure proved successful for the determination of total CGA content in yerba mate derived materials.

Some authors reported the use of different solvents such as alcohols (methanol, ethanol, isopropanol) as well as other organic compounds (acetone, dimethylformamide) for CGA extraction from different plant materials (Li et al., 2005; Naczk and Shahidi, 2004). Nevertheless, it should be mentioned that the use of these solvents could lead to distortion of the HPLC chromatographic peaks. While these solvents have been used in order to obtain the maximum extraction of active constituents, the use of organic solvents makes subsequent applications of these extracts in food and pharmaceutical industries inconvenient and also produces toxic wastes, thus being a severe pollution issue (Sonaglio et al., 2007). In addition, enzymatic hydrolysis of CGA to obtain quinate (the first step in the bioconversion pathway above mentioned to produce shikimate, ultimate objective of our investigation) is carried out under aqueous conditions, thus confirming the convenience of preparing CGA-water extracts.

Table 3 lists the concentration of the different CGA isomers individually determined and the total CGA concentration (calculated as the sum of all three isomers), for the three fractions of yerba mate evaluated (leaves, stems and powder when corresponds). Samples were obtained during the different processing steps at the early harvesting season (April and May, 2014) from processing plants A and B.

All the three CGA isomers quantified in the extracts prepared from samples (leaves and stems) obtained from processing plant A showed a quantitative variation during the processing steps. The highest levels of each CGA isomer were found in the extracts pre-

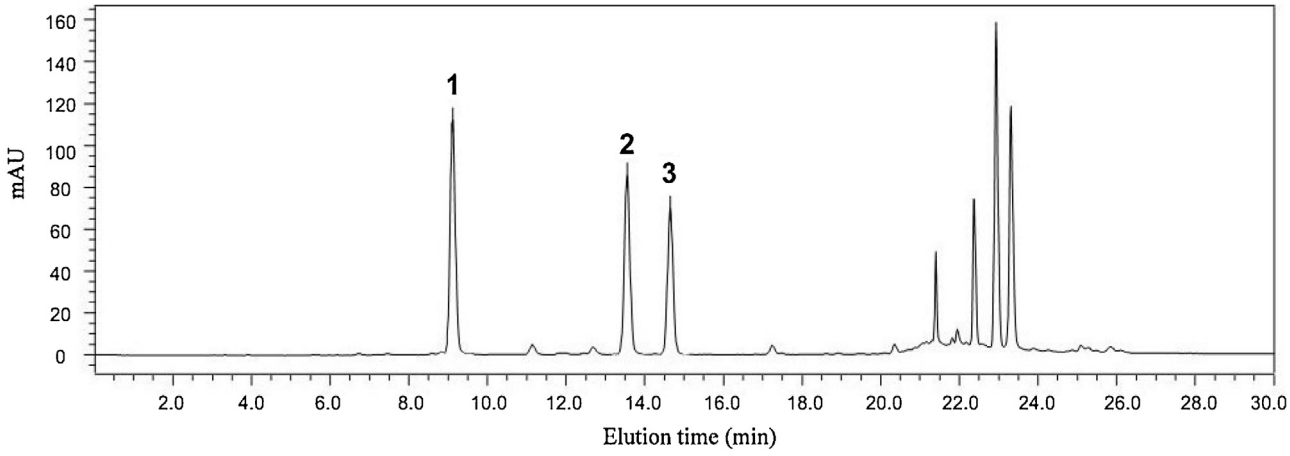

Fig. 3. Representative HPLC chromatogram of a yerba mate extract. Signal at 326 nm. (1): neochlorogenic acid, (2): chlorogenic acid and (3): cryptochlorogenic acid.

Table 3
Concentration of CGA–isomers and total CGA (neo-CGA + CGA + crypto-CGA) in three fractions of yerba mate evaluated (leaves, stems and powder when corresponds) during different processing steps (mean ± SD; $n = 3$). Samples were obtained from two processing plants (A and B) during the early harvesting season (April and May, 2014).

P	P.S.	neo-CGA (g CGA kg^{-1} dw)			CGA (g CGA kg^{-1} dw)			crypto-CGA (g CGA kg^{-1} dw)			Total CGA (g CGA kg^{-1} dw)		
		Leaves	Stems	Powder	Leaves	Stems	Powder	Leaves	Stems	Powder	Leaves	Stems	Powder
A	H	35.9 ± 0.7^{a}	26.5 ± 1.9^{a}	–	23.9 ± 0.2^{a}	31.8 ± 2.1^{a}	–	21.1 ± 0.0^{a}	20.6 ± 1.3^{a}	–	80.8 ± 1.0^{a}	78.9 ± 5.3^{a}	–
	R	31.4 ± 0.2^{b}	nd	–	20.4 ± 0.1^{b}	nd	–	19.0 ± 0.3^{a}	nd	–	70.8 ± 0.2^{b}	nd	–
	PD	28.4 ± 0.6^{b}	nd	–	21.1 ± 0.6^{b}	nd	–	18.6 ± 0.3^{ab}	nd	–	68.1 ± 1.4^{b}	nd	–
	1st D	28.9 ± 0.5^{b}	19.2 ± 0.2^{b}	–	20.0 ± 0.7^{b}	21.6 ± 0.2^{b}	–	17.6 ± 0.9^{b}	17.6 ± 0.1^{b}	–	66.5 ± 2.1^{b}	58.4 ± 0.5^{b}	–
	2nd D	30.2 ± 0.2^{b}	17.1 ± 1.7^{b}	–	20.7 ± 0.0^{b}	17.8 ± 1.7^{c}	–	17.4 ± 0.2^{b}	15.4 ± 1.4^{b}	–	68.3 ± 0.4^{b}	50.3 ± 4.8^{b}	–
	PYM	27.3 ± 0.4^{b}	1.0 ± 0.4^{c}	21.9 ± 0.9	20.8 ± 0.3^{b}	16.2 ± 0.2^{c}	24.6 ± 0.9	15.4 ± 0.3^{b}	9.3 ± 0.2^{c}	16.5 ± 0.4	63.5 ± 1.0^{b}	35.6 ± 0.8^{c}	62.9 ± 2.1
B	H	20.0 ± 1.1^{a}	17.5 ± 0.4^{a}	–	17.0 ± 1.2^{a}	19.6 ± 0.2^{a}	–	13.1 ± 1.0^{a}	13.4 ± 0.3^{a}	–	50.0 ± 2.3^{a}	50.5 ± 1.8^{a}	
	R	18.4 ± 0.1^{a}	14.8 ± 0.1^{ab}	–	14.8 ± 0.3^{a}	19.3 ± 0.3^{a}	–	12.5 ± 0.3^{a}	13.2 ± 0.2^{a}	–	45.8 ± 0.4^{a}	47.3 ± 0.5^{ab}	–
	D	21.4 ± 0.9^{a}	15.2 ± 0.1^{ab}	–	15.0 ± 0.6^{a}	15.5 ± 0.1^{b}	–	12.4 ± 0.5^{a}	11.8 ± 0.0^{ab}	–	48.8 ± 2.1^{a}	42.6 ± 0.3^{bc}	–
	M	19.5 ± 0.4^{a}	12.0 ± 0.1^{bc}	–	15.4 ± 0.1^{a}	13.4 ± 0.0^{bd}	–	12.3 ± 0.1^{a}	9.7 ± 0.0^{b}	–	47.2 ± 0.6^{a}	35.2 ± 0.0^{cd}	–
	PYM	21.8 ± 2.1^{a}	8.9 ± 0.1^{c}	17.5 ± 0.3	16.9 ± 1.7^{a}	12.8 ± 0.1^{cd}	15.6 ± 0.2	12.6 ± 1.4^{a}	7.4 ± 0.1^{c}	11.5 ± 0.1	51.3 ± 5.2^{a}	31.6 ± 0.6^{d}	44.6 ± 0.6

In each column, different superscripts indicate significant difference ($P < 0.05$) for the data corresponding to each yerba mate processing plant.
P: yerba mate processing plant.
P.S.: processing step. H: harvesting (green yerba mate), R: roasting, PD: pre-drying, 1st D: 1st drying belt, 2nd D: 2nd drying belt, D: drying, M: milling, PYM: processed yerba mate (final product).
nd: not determined.

Fig. 4. Total CGA content (on a dry weight basis) in just harvested yerba mate samples (green leaves and stems) obtained from two processing plants at the beginning of the harvesting season (April and May, 2014). Bars represent the media ± standard deviation of two independent experiments carried out by triplicate. Different superscripts indicate significant difference ($P < 0.05$).

pared from green leaves and stems (samples H). During roasting, a significant loss in neo-CGA and CGA occurred but not for crypto-CGA. No significant differences were observed in the levels of the three CGA isomers in leaves throughout roasting, pre-drying and drying steps. In the case of stems, a significant reduction in CGA isomers was observed after the drying step. In plant B, the content of each CGA isomer in leaves was almost constant throughout the different processing steps, from the green yerba mate up to the final product. There was a small decrease in the content of neo-CGA isomer in stems since the roasting step until the end of the processing whereas the variations observed in CGA and crypto-CGA contents could well be due to uncertainty (Table 3).

Significant differences in the concentration of the different CGA isomers as well as total CGA were observed when compared the values obtained for plant A and B at equivalent processing steps (Table 3). No significant differences for total-CGA were found between green leaves and stems ($P < 0.05$) for samples taken from the same processing plant (Fig. 4). On the contrary, substantial differences were found when compared the results obtained in both processing plants evidencing that the raw material processed in Plant A had higher amounts of CGA than that of Plant B. This fact was tentatively ascribed to variations in environmental conditions and genetic variability of the plant material processed. It is known that age of the tissue and exposition to light or shadow influence the amount of bioactive substances in yerba mate tissues (Ashihara and Crozier, 2001; Fernandez et al., 2002; Gulati and Ravindranath, 1996; Mazzafera, 1994).

While there have been several studies on the identification of CGA and its isomeric compounds in yerba mate, there are not comprehensive studies on the total quantification of these substances and their variation in different yerba mate derived materials. Total CGA (all three isomers combined, on a dry weight basis) concentration ranged from 45.8 ± 0.4 to 80.8 ± 1.0 g CGA kg^{-1} of leaves, and from 31.6 ± 0.6 to 78.9 ± 5.3 g CGA kg^{-1} of stems irrespectively from the processing plant (Table 3). Whichever the case, the highest CGA values were found in samples from green yerba mate. The main loss in the concentration of all CGA isomers took place during roasting, particularly in plant A.

Table 4
Concentration of total CGA (neo-CGA + CGA + crypto-CGA) in green yerba mate samples (leaves and stems) obtained from two processing plants at early (April and May, 2014) and late (September 2014) harvesting seasons (mean ± SD; $n = 3$).

Processing plant	Harvesting season	Total CGA (g kg^{-1} dw)	
		Green leaves	Green stems
A	Early	80.8 ± 1.0^{a}	78.9 ± 5.3^{a}
	Late	22.7 ± 1.7^{b}	20.4 ± 1.2^{b}
B	Early	50.0 ± 2.3^{c}	50.5 ± 1.8^{c}
	Late	21.5 ± 2.9^{b}	26.1 ± 0.7^{b}

Different superscripts indicate significant difference ($P < 0.05$).

The individual isomer distribution was different in green leaves compared with green stems. In green leaves, neo-CGA was the predominant isomer (≅40–44%) in samples from plants A and B whereas crypto-CGA was the less abundant (≅26%). Although, difficult to compare due to differences in the yerba mate material analyzed, the predominance of neo-CGA is in accordance with reports from Marques and Farah (2009). Regarding green stems, the predominant isomer was CGA (≅39–40%) being crypto-CGA the less abundant again (≅26%) in samples from plants A and B.

Bastos et al. (2006) reported that CGA (as well as caffeine) content in infusions prepared with yerba mate leaves increased with the processing steps of yerba mate, indicating that the use of dried leaves for the production of yerba mate beverages would be more advantageous than the use of fresh ones. They postulated that cell disruption and mechanical impact during the processing steps could increment the extraction yield from dried processed leaves. Similarly, Isolabella et al. (2010), who have studied the variation of bioactive compounds during yerba mate processing, reported an increase in the total CGA content in yerba mate leaves after roasting when compared with the green leaves, followed by a decrease during the drying process. These authors postulated that CGA is trapped by caffeine and that the temperature and humidity conditions employed during roasting would release these compounds resulting in an increase in their concentrations. On the contrary, our results indicate that green leaves and stems contain higher concentrations of CGA than industrialized materials. It should be noted that green yerba mate materials are quite unstable to oxidation (browning takes place soon after harvesting). In contrast, processed yerba mate materials are already stabilized by heating (roasting) and CGA analysis can be carried out days after sampling without a significant variation in the results. In our case, green yerba mate materials displaying even minor changes in their color (incipient browning) were discarded in order to ensure the freshness of the material under analysis. Moreover, samples were thermally treated to inhibit enzymatic activity within 12 h of harvesting (transportation time was substantially reduced because our laboratory is located in a yerba mate producing region). Therefore, the original CGA content in green yerba mate samples was soon stabilized, resulting in a convenient and advantageous raw material for CGA extraction.

According to the previous results, it was decided to study the influence of the developmental stage of yerba mate plant on total CGA content, particularly in green yerba mate materials. For this purpose, a new set of samples taken during September 2014 (late harvesting season) was analyzed. Total CGA content in both leaves and stems is strongly influenced by the harvesting season (Table 4). Samples taken at the late harvesting season showed a substantial reduction in CGA content as compared with those taken at the early harvesting season.

The growth of yerba mate tree is monopodial and rhythmic (Hallé et al., 1978), comprising two annual growth flushes, one in spring (September to December) and another in autumn (March to June) (Rakocevic et al., 2006). Among other factors, leaf maturity (or age) could influence CGA content because foliar growth is between intermediate to full (i.e.: with leaves that completed their foliar growth and others that not) from March to April, while in September the harvest would have a greater proportion of new outbreaks (Escalada et al., 2011). These authors also reported higher total polyphenol content in yerba mate leaves obtained at the beginning of the harvesting season in good agreement with our results. Similarly, Da Croce (2002) reported a significant influence of the harvesting season on the caffeine content in yerba mate cultivated in Brazil. Chemical composition of *Ilex* species, as any agricultural product, may vary significantly with the type of soil, climate, season, age of plant and leaves, sexual dimorphism and genetic characteristics (Oliva et al., 2006). In conclusion, changes in CGA content could be attributed to the plant developmental stage (Silva and Rakocevic, 2010) as well as different climatic conditions (temperature, humidity, solar intensity, etc.) and others unidentified factors. Whichever the case, it was demonstrated that green yerba mate materials (leaves and stems) harvested at the early season are the most convenient sources for CGA extraction due to their higher CGA content in comparison with those harvested at the late season.

4. Conclusions

Yerba mate samples obtained at different processing steps from two companies were analyzed for CGA content. The highest CGA content was found in green leaves and stems (just obtained after harvesting) with no significant differences in their CGA content ($P < 0.05$). A substantial loss in CGA was found during roasting. The harvesting season had a significant influence on CGA content for both yerba mate leaves and stems. CGA content at the early harvesting season was substantially higher to that obtained at the end of the harvesting season, for both green leaves and stems. It is noteworthy that a substantial amount of green stems is discarded in the processing of yerba mate. These results could provide a guideline for the preparation of yerba mate extracts enriched in CGA from one of the major and undervalued residues derived from the yerba mate industry: the green stems obtained at early harvesting season. Studies on the optimization of water extraction of CGA from green stems of yerba mate and its subsequent bioconversion into quinate (and caffeate) are under development and results will be published elsewhere.

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References

Adachi O, Ano Y, Toyama H, Matsushita K, 2006a. High shikimate production from quinate with two enzymatic systems of acetic acid bacteria. Bioscience, Biotechnology and Biochemistry 70, 2579–2582.

Adachi O, Ano Y, Toyama H, Matsushita K, 2006b. Purification and characterization of shikimate dehydrogenase from *Gluconobacter oxydans* IFO 3244 and its application to enzymatic shikimate production. Bioscience, Biotechnology and Biochemistry 70, 2786–2789.

Adachi O, Ano Y, Toyama H, Matsushita K, 2006c. Enzymatic preparation of metabolic intermediates, 3-dehydroquinate and 3-dehydroshikimate, in the shikimate pathway. Biotechnology and Biochemistry 70, 3081–3083.

Adachi O, Ano Y, Akakabe Y, Shinagawa E, Matsushita K, 2008. Coffee pulp koji of *Aspergillus sojae* as stable immobilized catalyst of chlorogenate hydrolase. Applied Microbiology and Biotechnology 81, 143–151.
AOAC, 2000. Official Methods of Analysis of the Association of Official Analytical Chemists, 17th ed. Association of Official Analytical Chemists, Gaithersburg, MD, USA.
Ashihara H, Crozier A, 2001. Caffeine: a well known but little mentioned compound in plant science. Trends in Plant Science 6, 407–413.
Bastos DHM, Fornari AC, Queiroz YS, Torres EAFS, 2006. Bioactive compounds content of chimarrão infusions related to the moisture of yerba maté (*Ilex paraguariensis*) leaves. Brazilian Archives of Biology and Technology 49, 399–404.
Bravo L, Goya L, Lecumberri E, 2006. LC/MS characterization of phenolic constituents of mate (*Ilex paraguariensis* A. St. Hil) and its antioxidant activity compared to commonly consumed beverages. Food Research International 40, 393–405.
Carini M, Facino RM, Aldini G, Calloni M, Colombo L, 1998. Characterization of phenolics antioxidants from mate (*Ilex paraguariensis*) by liquid chromatography mass spectrometry and liquid chromatography tandem mass spectrometry. Rapid Communications in Mass Spectrometry 12, 1813–1819.
Chun OK, Kim DO, 2004. Consideration on equivalent chemicals in total phenolic assay of chlorogenic acid-rich plums. Food Research International 37, 337–342.
Clifford MN, 1999. Chlorogenic acids and other cinnamates: nature, occurrence and dietary burden. Journal of the Science of Food and Agriculture 79, 362–372.
Clifford MN, 2000. Chlorogenic acids and other cinnamates: nature, occurrence, dietary burden, absorption and metabolism. Journal of the Science of Food and Agriculture 80, 1033–1043.
Clifford MN, Wu W, Kuhnert N, 2006. The chlorogenic acids of Hemerocallis. Food Chemistry 95, 574–578.
Da Croce DM, 2002. Características fisicoquímicas de extractos de erva-mate (*Ilex paraguariensis* St. Hil) no estado de Santa Catarina. Ciencia Forestal 12 (2), 107–113 (in Portuguese).
Delage E, Bohuon G, Baron A, Drilleau JF, 1991. High-performance liquid chromatography of the phenolic compounds in the juice of some French cider apple varieties. Journal of Chromatography A 555, 125–136.
Enserink M, 2006. Oseltamivir becomes plentiful-but still not cheap. Science 312, 382–383.
Escalada G, Brumovsky LA, Hartwig VG, 2011. Influencia de la zona de cultivo y procesamiento de la yerba mate sobre su contenido de polifenoles totales y capacidad antioxidante. Revista de Ciencia y Tecnología 13 (15), 66–74 (in Spanish).
Fang N, Yu S, Prior RL, 2002. LC/MS/MS characterization of phenolic constituents in dried plums. Journal of Agricultural and Food Chemistry 50, 3579–3585.
Farah A, Donangelo CM, 2006. Phenolic compounds in coffee. Brazilian Journal of Plant Phytopatology 18, 23–36.
Fernandez PL, Pablos F, Martin MJ, Gonzalez AG, 2002. Study of catechin and xanthine tea profiles as geographical tracers. Journal of Agricultural and Food Chemistry 50, 1833–1839.
Gulati A, Ravindranath SD, 1996. Seasonal variations in quality of kangra tea (*Camellia sinensis* (L) O Kuntze) in Himachal Pradesh. Journal of the Science of Food and Agriculture 71, 231–236.
Hallé F, Oldeman RAA, Tomlinson PB, 1978. Tropical Trees and Forests: An Architectural Analysis. Springer-Verlag, Berlin.
Heck CI, Gonzalez de Mejia E, 2007. Yerba mate tea (*Ilex paraguariensis*): a comprehensive review on chemistry, health implications, and technological considerations. Journal of Food Science 72 (9), 138–150.
Heck CI, Schmalko M, Gonzalez de Mejia E, 2008. Effect of growing and drying conditions on the phenolic composition of mate teas (*Ilex paraguariensis*). Journal of Agricultural and Food Chemistry 56, 8394–8403.
Isolabella S, Cogoi L, López P, Anesini C, Ferraro G, Filip R, 2010. Study of the bioactive compounds variation during yerba mate (*Ilex paraguariensis*) processing. Food Chemistry 122, 695–699.
Jiang Y, Satoh K, Kusama K, 2000. Interaction between chlorogenic acid and antioxidants. Anticancer Research 20, 2473–2476.
Jin UH, Lee JY, Kang SK, Kim JK, Park WH, Kim JG, Moon SK, Kim CH, 2005. A phenolic compound, 5-caffeoylquinic acid (chlorogenic acid), is a new type and strong matrix metalloproteinase-9 inhibitor: isolation and identification from methanol extract of *Euonymus alatus*. Life Science 77, 2760–2769.
Kweon MH, Hwang HJ, Sung HC, 2001. Identification and antioxidant activity of novel chlorogenic acid derivatives from bamboo (*Phyllo stachys edulis*). Journal of Agricultural and Food Chemistry 49, 4646–4655.
Li H, Chen B, Yao S, 2005. Application of ultrasonic technique for extracting chlorogenic acid from *Eucommia ulmodies* Oliv. (*E. ulmodies*). Ultrasonics Sonochemistry 12, 295–300.
López O, Isolabella S, Anesini C, Ferraro G, Filip R, 2006. Estudio cuali-cuantitativo por HPLC de los principios activos presentes en los extractos de *Ilex paraguarienses* (yerba mate) en las diferentes etapas del procesamiento industrial. In: Annals of IV South-American Congress on Yerba Mate, Posadas, Argentina, November 2011, pp. 5–8 (in Spanish).
Marques V, Farah A, 2009. Chlorogenic acids and related compounds in medicinal plants and infusions. Food Chemistry 113, 1370–1376.
Mazzafera P, 1994. Caffeine: theobromine and theophylline distribution in *Ilex paraguariensis*. Revista Brasilera de Fisiología Vegetal 6, 149–151.
Naczk M, Shahidi F, 2004. Extraction and analysis of phenolics in food. Journal of Chromatography A 1054, 95–111.
Oliva EV, Reissmann CB, Gaiad S, Sturion JA, De Olivera EB, Wisniewski C, Miaqui DP, 2006. Composicao química foliar de macronutrientes em precedencias de erva-mate *Ilex paraguariensis* St. Hil. In: Annals of IV South-American Congress on Yerba Mate, Posadas, Argentina, November, pp. 5–8, 2011 (in Portuguese).
Pagliosa CM, Vieira MA, Podestá R, Maraschin M, Bertello Zeni AL, Amante ER, Dias de Mello Castanho Amboni R, 2010. Methylxanthines, phenolic composition, and antioxidant activity of bark from residues from mate tree harvesting (*Ilex paraguariensis* A. St. Hil). Food Chemistry 122, 173–178.
Rakocevic M, Medrado MJS, Lucambio F, Valduga TA, 2006. Caracterização de crescimento do caule da erva-mate (*Ilex paraguariensis* St. Hil.) cultivada em em dois ambientes luminosos contrastantes. In: Annals of IV South-American Congress on Yerba Mate, Posadas, Argentina, November, pp. 5–8, 2011 (in Portuguese).
Rønsted N, Strandgaard H, Jensen SR, Mølgaard P, 2002. Chlorogenic acid from three species of hydrostachys. Biochemical Systematics and Ecology 30, 1105–1108.
Schmalko ME, Alzamora SM, 2001. Color, chlorophyll, caffeine, and water content variation during yerba mate processing. Drying Technology 19 (3-4), 599–610.
Silva MHM, Rakocevic M, 2010. Software for interpolation of vegetative growth of yerba mate plants in 3D. Pesquisa Agropecuária Brasileira 45, 244–251.
Sonaglio D, Ortega GG, Petrovicck PR, Bassani VL, 2007. Desenvolvimento tecnológico e produção de fitoterápicos. In: Simões CM, Schenkel EP, Gosmann G, Mello JCP, Mentz LA, Petrovick PR (Eds.), Farmacognosia—da Planta ao Medicamento. Editora da UFRG, Porto Alegre, Brazil, pp. 289–326 (in Portuguese).