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Bioactive Compounds Content of Chimarrão Infusions Related to the Moisture of Yerba Maté *(Ilex Paraguariensis)* Leaves

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ABSTRACT

The aim of this study was to evaluate the effects of the processing stages of yerba maté (Ilex paraguariensis) on the moisture content of the leaves and the efficiency of the aqueous extraction of some bioactive substances. Samples of yerba maté were analyzed for caffeine, phenolic acids (caffeic acid, 5-caffeoilquinic acid) and flavonoids (quercetin, kaempferol and myricetin) by HPLC equipped with a diode array detector. Processing widely influenced the caffeine and 5-caffeoilquinic acid content of the aqueous extract ($p < 0.05$), which was related to the moisture content of the leaves. Caffeic acid was present in 45% of the in infusions from dried mate leaves. Quercetin, myricetin and kaempferol were not detected.

Key words: Yerba maté (*Ilex paraguariensis*), processing, bioactive substances

INTRODUCTION

Yerba maté was being consumed by native South American Indians when the new world was discovered by the European people. Native South Americans were aware of yerba maté's stimulating properties due to caffeine. Nowadays, yerba maté beverages are also recognized as a rich source of antioxidant substances, the phenolic acids (Carini et al., 1998; Clifford and Ramirez-Martinez, 1990; Filip et al., 2000; Mazzafera, 1997), which are readily absorbed by the body (Bravo, 1998; Olthof et al., 2001; Olthof et al., 2003) and are responsible for the *in vitro* and *in vivo* antioxidant effect of these beverages (Baisch et al., 1998; Bracesco et al., 2003). Other physiological effects of yerba maté have also been reported and explain its popular use as a choleretic beverage, among others (Gugliucci, 1996; Gorzalczany et al., 2001; Gugliucci and Menini, 2002).

The processing of yerba maté consists of three different stages: a) a rapid drying process called "sapeco", aiming to inhibit enzymatic activity and lower the moisture level; b) a partial drying stage, which usually takes place in rotating drums heated by the burning of wood or gas in places called "barbaqua", and c) a further drying and subsequent grinding stage, after which the yerba maté is called "cancheada" (Schmalko and Alzamora, 2001; Esmelindro et al., 2002). Process parameters (time/temperature of the drying stages) differ among diverse producers (Esmelindro et al., 2002; Nunez and Kanzig, 1995), depending greatly on the driers' design and operation. The processed herb is usually a blend of leaves harvested by different producers who form a cooperative to

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build a common “barbaqua”. The “cancheada” herb is used in two beverages, the “chimarrão” (made with hot water) and the “tererê” (made with cold water). Chimarrão is largely consumed by South Americans. Daily consumption ranges from 1.5 to 6 L.

Some authors suggested that fresh maté leaves should be used to produce maté beverages instead of dried leaves because the drying process greatly diminished the caffeine content (Schmalko and Alzamora, 2001; Esmelindro et al., 2002). It would be advantageous for the maté industry that herbal beverages should contain high amounts of phenolic compounds, responsible for antioxidant properties already observed, and caffeine, a stimulating compound. Although drying steps destroy caffeine, it should be verified whether the drying process enhances aqueous extraction. This study aimed at evaluating the effects of the processing stages of yerba maté (*Ilex paraguariensis*) on the moisture content of the leaves and on the efficiency of the aqueous extraction of some bioactive substances.

MATERIAL AND METHODS

Plant material employed in this study was composed of leaves (fresh leaves, just harvested from the bushes), partially dried leaves (leaves after the “sapeco” stage) and dried leaves (after the drying in rotating drums and grinding) obtained directly from producers in Paraná, Brazil, between May and June 2003. The samples were kept in a freezer at -18^{o}C until analysis (no longer than 90 days). Fresh leaves were blanched in our laboratory before freezing to inhibit enzymatic activity within 36 h of harvesting.

Standards (caffeine, 5-caffeoylquinic acid, caffeic acid, kaempferol, quercetin and myricetin) and HPLC grade methanol were obtained from Sigma Chemical Co. (St. Louis, USA), and acetic acid was purchased from Merck (Darmstadt, Germany).

Blanching of yerba maté fresh leaves at the laboratory before freezing

The fresh leaves were washed with tap water, immersed in boiling water (~97^{o}C) for one minute and immediately cooled in ice bath. After removing the moisture excess with absorbent paper, the leaves were kept in sealed plastic bags at -18^{o}C until analyses were performed.

Determination of the moisture content of the samples

The samples were dried to constant weight at 105°C in an oven.

Preparation of the infusions (extracts)

Different maté samples of 3.6 g of fresh leaves (60% of moisture content), 1.8 g of blanched leaves (21% of moisture content) and 1.5 g of dried ground leaves (6% of moisture content) were mixed with 30 mL of ultrapure water at 85^{o}C and left for 3 minutes. The infusion was then filtered trough n^{o} 1 Whatman filter paper. This procedure was carried out one more time and the extracts were combined to form the analytical sample. Total extraction time was 6 minutes.

Determination of soluble solids

A measured volume of aqueous extract (10 mL) was transferred into a tared beaker and evaporated to dryness. The residue was dried to constant weight at 105^{o}C in an oven.

Determination of 5-caffeoylquinic acid, caffeic acid and caffeine

Infusions were analyzed with no other modification than the appropriate dilution to fit the standard curves, as described by several authors (Ewald et al., 1999; Astill et al., 2001; Bispo et al., 2002). A Shimadzu HPLC chromatograph, equipped with an LC-10ATvp quaternary pump, a Rheodyne manual injection valve with a 20 μL sample loop and a diode array detector SPD M10AVP, was used for the determinations. All the modules were controlled by a personal computer equipped with the HPLC System Manager software CLASS-VP. A 4.6 x 250 mm, 5μm C18 Microsorb column was used for the separation. The analytical determination of caffeine, caffeic acid and 5-cafeoylquinic acid was carried out by means of high-performance liquid chromatography using a two-solvent isocratic elution. The composition of the solvents was: (A) water/acetic acid (99.5:0.5 v/v) and (B) methanol. The mobile phase composition was 75% of solvent A and 25% of solvent B. The flow rate was 1 mL/min. Data were obtained at 272 nm for caffeine and 323 nm for phenolic acids. Identification was based on the comparison of the spectra obtained between 250-350 nm and the retention time of the unknown substances in relation to that of pure standards. Quantification was achieved by external

calibration, using a five-point curve of different dilutions of a standard solution. Pearson's correlation coefficient (r) was always > 0.99. Peak purity, which was determined using the average of similarity of the apex *vs*. the upslope spectrum and the apex *vs*. the download spectrum performed by the photodiode array detector, was higher than 0.99 for both compounds.

Determination of flavonols (quercetin, myricetin and kaempferol)

Aqueous extracts were submitted to acid hydrolysis as described by Matsubara (2001).The flavonoid aglycons were analyzed using the same apparatus and conditions previously described, except that high-performance liquid chromatography was performed using a two-solvent gradient elution. The composition of the solvents was: (A) water/acetic acid (99.5:0.5 v/v) and (B) methanol. The mobile phase composition started at 75% of solvent A and 25% of solvent B. It was then linearly increased to 30% of solvent B in 10 min., and the final condition was held for an additional 4 minutes. The flow rate was 1 mL/min. Data were obtained at 370 nm.

Data analysis

Statistical analysis was performed using SPSS (version 11.0/2001- SPSS Inc Chicago). Results are presented as means ± SD. Data were analyzed for statistical significance by ANOVA and Scheffé's test. Significance level was set at $p < 0.05$.

RESULTS AND DISCUSSION

The moisture content of the leaves varied according to the processing stage. And, as the heat treatment becomes more intense, the amount of soluble solids in the aqueous extract increased. Therefore, the amount of soluble solids depended on the moisture content of the yerba maté sample (Table 1). This could be due to cell disruption, which occured as the heat and the mechanical impact became more intense throughout the processing stages of yerba maté.

The caffeine, 5-caffeoylquinic acid (5-cqa) and caffeic acid content of infusions prepared with fresh leaves are shown in Table 2. Caffeic acid was detected in only 45% of the dried yerba maté samples, and its content ranged from 2.6 to 5.8 µg/mL. The wide range of variation observed for the bioactive compounds in Table 2 was due to environmental conditions, genetic variability, the possible presence of other *Ilex* species that could have been harvested together with yerba maté and also due to the fact that the age of the tissue and the exposition to light or shadow seemed to influence the amount of bioactive substances in the maté leaves (Mazzafera, 1994; Ashihara and Crozier, 2001; Fernandez et al., 2002; Gulati and Ravindranath, 1996). Despite this variation, there was a significant difference ($p < 0.05$) in the mean caffeine and 5-cqa content of the infusions, which was related to the processing stage of yerba maté (Table 3).

Quercetin, myricetin or kaempferol were not detected in this study. Matsubara (2001) detected quercetin in amounts comparables to those present in black and green tea and smaller amounts of kaempferol in commercial yerba maté samples from Campinas, Brazil but did not detect myricetin. The presence of flavonols in mate leaves was described by Filip et al (2001).

A linear relationship between the 5-cqa and caffeine content of chimarrão and the soluble solids content was observed ($r = 0.95$ for 5-cqa and $r=0.89$ for caffeine) (Fig. 1a,b). The determination coefficients (r^2) were 0.77 and 0.90 for caffeine and 5-cqa respectively ($p < 0.05$), indicating that most of the observed behavior (efficiency of the aqueous extraction) could be explained by the studied variable (soluble solids content). Caffeine and 5-cqa content of the infusions increased with the processing stages of yerba maté, indicating that the use of dried leaves for the production of maté beverages was more advantageous than the use of fresh ones.

The caffeine content of infusions prepared with dried yerba maté found in this study was lower than that determined by Mazzafera (1997), who obtained values ranging from 290 to 790 µg/mL, and was similar to that found by Clifford and Ramirez-Martinez *(*1990). It is well known that the parameters involved in the extraction procedure (such as size of leaves and brewing conditions) greatly interfere in the solubility of bioactive compounds, as well as that growing conditions and genetic characteristics highly influence the content of such substances in plants (Astill et al., 2001).

Table 1 - Processing stage, moisture content and soluble solids content of yerba maté plant material

Processing stage	Moisture content (%)	Soluble solids (mg/mL)
Fresh leaves (n = 24)	$58.28^{a} \pm 7.16$	$1.60^{a} \pm 0.63$
After sapeco (partially dried leaves) (n = 15)	$20.99^{b} \pm 9.17$	$3.35^{b} \pm 1.34$
Dried/ground leaves (n = 33)	$6.02^{c} \pm 1.54$	$5.99^{c} \pm 1.16$

n = number of analyzed samples. Results are expressed as mean ± standard deviation
In each column, different superscripts indicate significant difference ($p < 0.05$)

Table 2 - Caffeine 5-cqa and caffeic acid content (μg/mL) of chimarrão infusion according to the locality (city), kind of production area (planted or native) and processing stage of yerba maté leaves (fresh leaves, after sapeco and dried leaves) collected in Paraná state, Brazil in 2003

City	Production area	Processing stage	5- CQA (μg/mL)	Caffeine (μg/mL)	Caffeic acid (μg/mL)
Paulo Frontin	planted	Fresh leaves (n=3)	139.2±13.1	114.1±12.8	nd
		After Sapeco (n=3)	217.9±24.6	150.5±20.8	nd
		Dried/ leaves (n=3)	376.5±24.2	144.6±15.3	5.7 ±0.2
	native	Fresh leaves (n=3)	92.0±37.2	86.4±26.6	nd
		After Sapeco (n=3)	231.21±40.5	207.1±58.2	nd
		Dried leaves (n=3)	320.3±51.5	263.0±42.3	2.6± 0.2
Cascavel	planted	Fresh leaves (n=3)	72.1±2.2	126.3±3.5	nd
		Dried leaves (n=6)	357.1±73.7	194.8±11.3	nd
	native	Fresh leaves (n=3)	88.6±13.0	114.0±5.9	nd
		After Sapeco (n=3)	108.1±20.9	87.3±15.6	nd
		Dried leaves (n=3)	245.4±8.7	159.2±14.3	nd
Santa Maria	planted	Fresh leaves (n=3)	71.1±10.0	106.7±10.7	nd
		Dried leaves (n=9)	447.1 ±31.0	294.0 ± 20.3	4.5± 0.6
	native	Fresh leaves (n=3)	74.0±30.9	108.8±20.6	nd
		After Sapeco (n=3)	125.3±14.3	88.4±18.7	nd
		Dried/ leaves (n=6)	258.6 ±31.2	157.8 ± 16.9	3.8± 0.2
Pinheiro Fernandes	native	Fresh leaves (n=3)	59.4±10.4	31.4±0.4	nd
		After Sapeco (n=3)	69.1±28.1	49.2±13.7	nd
		Dried leaves (n=3)	243.7±38.7	172.6±31.6	nd

n = number of analyzed samples. Results are expressed as mean ± standard deviation.
nd = not detected

Table 3 - Mean caffeine and 5-cqa content (μg/mL) of chimarrão infusion according to the processing stage

Processing stage	5-cqa μg/mL extract	Caffeine μg/mL extract
Fresh leaves (n = 24)	$83.8^{a} \pm 35.4$	$99.6^{a} \pm 33.4$
After sapeco - partially dried leaves) (n = 15)	$150.3^{b} \pm 69.6$	$116.5^{a} \pm 63.1$
Dried/ground leaves (n = 33)	$341.7^{c} \pm 90.4$	$211.5^{b} \pm 64.0$

n = number of samples. Results are expressed as mean ± standard deviation. In each column, different superscripts indicate significant difference ($p < 0.05$).

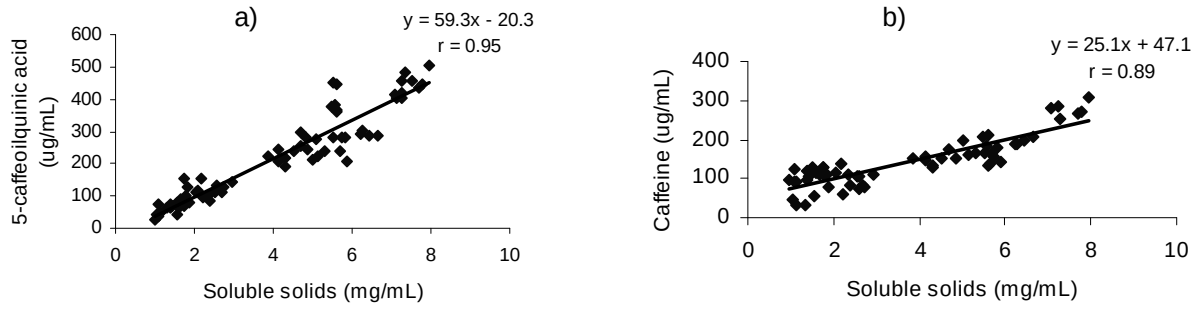

Figure 1 - Relationship between a) the 5-cqa and b) the caffeine content and soluble solids content of chimarrão infusions

The soluble solids content of chimarrão infusions highly depends on the moisture content of maté leaves. Dried yerba maté produced an aqueous extract with higher soluble solids content than the aqueous extract from fresh leaves or partially dried leaves ("sapecada").

CONCLUSION

There was a linear correlation between the soluble solids content and the bioactive substances of chimarrão, indicating that more efficient extraction resulted in a beverage with greater amounts of 5-caffeoilquinic acid and caffeine. Caffeic acid was detected in 45% of infusions obtained from the dried yerba maté samples analyzed. Yerba maté is an important source of phenolic acids which are absorbed by human and may protect against biological oxidative process, as demonstred by *in vivo* antioxidant experiments. Therefore, yerba maté consumption should be encouraged.

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RESUMO

A erva mate (*Ilex paraguariensis*) é a matéria prima para três tipos de bebidas largamente consumidas na América do Sul. Substâncias bioativas presentes neste produto como a cafeína e os ácidos clorogênicos têm recebido especial atenção da comunidade científica. O objetivo deste trabalho é avaliar o efeito do processamento da erva mate no teor de umidade das folhas e a eficiência da extração aquosa de algumas substâncias bioativas. Amostras de erva mate coletadas no Paraná, Brasil foram objeto deste estudo. Cafeína, ácidos fenólicos (ácido cafeico e ácido 5-cafeoilquinico) e flavonóides (quercitina, miricetina e caempferol) foram analisados por HPLC equipado com detector de arranjo de diodos. Os teores de ácido 5-cafeoilquinico e cafeína do extrato aquoso variam em função da etapa do processamento ($p<0,05$), que está relacionada com o teor de umidade das folhas ($r >0,9$). O ácido cafeico foi determinado em 45% das infusões obtidas das folhas secas e quercitina, miricetina e caempferol não foram detectados nesses extratos.

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