7510 *J. Agric. Food Chem.* **2007,** *55,* 7510–7516

JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

Influence of Agronomic Variables on the Macronutrient and Micronutrient Contents and Thermal Behavior of Mate Tea Leaves (*Ilex paraguariensis*)

Rosângela A. Jacques,[*,†] Eduardo J. Arruda,[‡,#]
Lincoln C. S. de Oliveira,[†] Ana P. de Oliveira,[§] Cláudio Dariva,[⊥,⊗]
J. Vladimir de Oliveira,[§,∇] and Elina B. Caramão[||,◆]

Universidade Federal do Pampa (UFPel/UNIPAMPA), rua carlos Barbosa, s/n°, 96412-420 Bagé, RS, Brazil, Farmácia/Biotecnologia Programa MSMT - UCDB, Av. Tamandaré, 6000, CEP 79117-900, C.P 100 Campo Grande, MS, Brazil, Instituto de Pesquisa e Tecnologia-ITP, Programa de Mestrado em Engenharia de Processos PEP/UNIT, Aracaju, SE, Brazil, Universidade Regional Integrada do Alto Uruguai e Missões/Campus de Erechim, Av. Sete de Setembro, 1621 Erechim, RS, Brazil, 3520-9000, and Instituto de Química, UFRGS, Av. Bento Gonçalves, 9500, 91501-970, Porto Alegre, RS, Brazil

The influence of agronomic variables (light intensity, age of leaves, and fertilization type) on the content of macronutrients and micronutrients (potassium, calcium, sodium, magnesium, manganese, iron, zinc, and copper) of tea leaves was assessed by acid digestion, followed by flame atomic absorption spectrometry (FAAS). The thermal behavior of mate tea leaves (*Ilex paraguariensis*) was also studied in this work. Samples of mate (*Ilex paraguariensis*) were collected in an experiment conducted under agronomic control at Erva-Mate Barão Commerce and Industry LTD (Brazil). The results showed that the mineral content in mate is affected by the agronomic variables investigated. In general, the content of mineral compounds analyzed is higher for younger leaves and for plants cultivated in shadow. Thermal analysis of samples indicated a similar behavior, with three typical steps of decomposition: loss of water, degradation of low-molecular weight compounds, and degradation of residual materials.

KEYWORDS: FAAS; mate tea leaves; *Ilex paraguariensis*; agronomic variables; mineral content; thermal analysis; TG; DTG; DSC

INTRODUCTION

Ilex paraguariensis St. Hil. is a South American native perennial tree belonging to Aquifoliaceae family (*1*). It has been used historically as a source of a mild stimulant beverage called mate ("erva mate" or "yerba mate"), prepared by infusion of its dried leaves and ramifications (*2*). Yerba mate is an important natural product in South America with many attributed relevant properties like anti-inflammatory, therapeutic, antirheumatic, stimulating, and diuretic activities (*3*−*5*). Mate (*Ilex paraguariensis*) is an important natural product of South Brazil (*6*−*8*). To take a glance of the mate market, we shall consider that only at the south region of Brazil one can find more than 40 mate processing industries and about 180 000 medium and small properties crowded together in a narrow area dedicated almost exclusively to cultivate this raw material (*2*, *9*).

Considering the fact that all of those industries direct their efforts to produce the same base product (comminuted mate leaves for teas), considering that the processing of mate leaves within an industrial environment is nowadays conducted in a very rudimentary way (*10*), and considering the recent availability of this raw material from other countries (*11*), it is not surprising that the strong competition established has required company investments toward producing higher-value products and also to ensure a quality standard of the products.

The literature points out that some agronomic variables, like age of leaves and light intensity, exert a remarkable influence on the semivolatile chemical distribution in mate tea leaf extracts (*12*). Regarding the mineral composition, the extent to which plants take up metals depends on the binding of trace analytes to soils constituents (*13*), and in this sense, intrapopulation agronomic conditions may be of primary importance in order to ensure a standard quality of mate products. In this sense, the macro- and microenvironmental conditions (*14*) in which the

---

[*] To whom correspondence should be addressed. Phone: 55 53 3247 2367. E-mail: rjacques.unipampa@ufpel.edu.br.

[†] Universidade Federal do Pampa (UFPel/UNIPAMPA).

[‡] Farmácia/Biotecnologia Programa MSMT - UCDB.

[#] phone: 55 51 3308 7213, e-mail: elina@ufrgs.br.

[§] Instituto de Pesquisa e Tecnologia-ITP.

[⊥] Universidade Regional Integrada do Alto Uruguai e Missões/Campus de Erechim.

[⊗] Phone: 55 54, 3520-900. E-mail: claudio.dariva@yahoo.com.br.

[∇] E-mail: vladimir@uri.edu.br.

[||] Instituto de Química, UFRGS.

[◆] Phone 55 67 3312 3467. Fax 55 67 3312 3301/ 3302. E-mail: ejarruda@acad.ucdb.br.

10.1021/jf071545g CCC: $37.00

plants grow, along with genetic factors, should be responsible for the absorption of minerals by the plants.

The high content of minerals and several oligoelements, especially Mn, in mate tea is considered to be of nutritional interest (*15*). The total aluminum, magnesium, zinc, cadmium, iron, copper, calcium, potassium, and phosphorus contents of tea leaves collected in different countries were determined, as were the contents of these elements that could be extracted with hot distilled water. Atomic spectroscopic methods were used. Little difference in elemental composition was found for tea leaves from different sources. The [Mg]/[Al] ratio in the hot aqueous extracts was twice as high as that in tea leaves. It is presumed that the aluminum in tea is present in a complex form, which has a less unhealthy effect than its free ionic form (*16*).

Yerba mate exhibits an unusually high manganese content that is readily available for hot water extraction. Despite the high elemental manganese levels in *Ilex paraguariensis* extract, no manganese-related toxicity of yerba mate has been observed even among heavy yerba mate drinkers, indicating that the manganese in the extract has only a very low bioavailability. Besides, chemical content of raw medicinal plant materials may differ significantly for species of different origins but also for plants belonging to the same species (*17*−*21*).

The thermal behavior and the physical−chemical characterization of mate tea leaves can furnish important information about the quality and stability of the plants to the industrial processing steps. The effect of these industrial steps was important, and the results show that the industrial processing steps have direct influence on the content of such compounds. This fact proves the relevance of analyzing these results when the use of the raw material for the development of new food products and biotechnological processes is intended (*22*).

Plants representing a wide range of mineral content (silica-free) were used to test the possibility that mineral elements in plants act in a similar way to flame retardants (*23*). Thermal analyses showed that the maximum rate of weight loss, the amount of volatilization between 175 and 350 °C, and the temperature at which these plant materials undergo thermal decomposition are related to the silica-free mineral content. However, tested plant material having more than 12% silica-free mineral content did not fit all of these relationships; generally the effect leveled off between 5% and 7%. There is evidence that only some parts of the mineral elements present are probably active in the pyrolytic pathways of plant carbohydrates (*23*) and also play a role in the thermal decomposition process or thermal stability which could modify sensorial properties.

To our knowledge there is no available work reported in literature about the influence of some agronomic variables (light intensity, age of leaves, and fertilization of the plants) on the content of macro- and micronutrients in mate tea leaves and thermal properties of *Ilex paraguariensis*. In this work, the mineral content in mate tea leaves was determined by acid digestion followed by flame atomic absorption spectrometry (FAAS) and thermal analysis, because the literature points out that this technique provides high reproducibility and recovery for metal in plants (*24*−*28*). The quantitative analysis of K, Ca, Na, Mg, Mn, Zn, Cu, and Fe and thermal properties in mate tea leaves conducted under two distinct light intensity levels, for four types of fertilization, and at four ages of leaves are reported in this work.

MATERIALS AND METHODS

Sample Collection. Mate tea leaf samples were collected in an experiment conducted under agronomic control at Erva-Mate Barão Commerce and Industry LTD (Brazil). At the beginning of the experiment, all plants were about 7 years old and all leaves and ramifications were totally cut off. At this time, the age of leaves was set to zero month. Half of the experiment was covered with a covering device that absorbed 75% of the natural light incidence. The plants were handled with four distinct fertilization conditions. In the first treatment, no additional fertilization was added to the plants; in the second treatment, each plant was fertilized with 300 g/year of a nitrogen source (urea); in the third treatment, each plant was fertilized with 120 g/year of a potassium source (potassium chloride), and in the fourth treatment, each plant was fertilized with potassium and nitrogen sources simultaneously (300 g/year of urea and 120 g/year of potassium chloride). Seven plants of each treatment were selected, from which fractions from the top, middle, and bottom of each tree were sampled and homogenized to form the sample of each treatment. The material of each individual treatment was homogenized and dried in a vacuum oven (24 h at 35 °C). The mate samples were stored under nitrogen atmosphere until the analysis.

Table 1. Instrumental Parameters Employed in the FAAS for the Determination of K, Ca, Na, Mg, Mn, Zn, Cu, and Fe in Mate Tea Leaves

element	wavelength (nm)	lamp intensity (mA)	slit width (nm)	working range (mg L^{-1})
Ca	239.9	10	0.2	2–800
Na	303.2	5	0.2	2–400
Mg	202.6	4	1.0	0.15–20
Mn	403.1	5	0.2	0.5–60
Zn	213.9	5	1.0	0.01–2.0
Cu	324.7	4	0.5	0.03–10
Fe	248.3	5	0.2	0.06–15

Chemicals. Concentrated nitric acid and hydrogen peroxide 30% (v/v) were of analytical grade (Merck). Bidistilled water was used throughout all the work. All glassware and plastic were washed with 5% w/v nitric acid and rinsed with bidistilled water. Stock standard solutions of K, Ca, Na, Mg, Mn, Fe, Zn, and Cu (Spectrosol, E. Merck, Germany) containing 1000 mg L^{-1} of each element were used. Calibration standard solutions of each element were obtained by appropriately diluting the stock solutions. All standards were acidified to obtain proper acid concentrations.

Digestion Procedure. From each treatment sample, triplicate samples were selected (3 g) and submitted to carbonization in an electric plate at 350 °C during 2 h. Afterward, tea leaves were incinerated at 700 °C for approximately 4 h. The ashes were dissolved in 4 mL of HNO_3 (1 mol L^{-1}) and 4 mL of H_2O_2 (30% v/v) and heated until a clear extract was obtained. The volume was then increased to 50 mL with HNO_3 (1 mol L^{-1}) (*15*).

Mineral Determination Procedure. A Varian atomic absorption spectrometer model AA55 equipped with 10 cm burner head was used for metal determination. The hollow-cathode lamps of Ca, Mg, Na, Mn, Zn, Fe, and Cu were used as radiation sources. The elements were measured under optimized operating conditions by FAAS in air/acetylene or nitrous oxide/acetylene flame. The instrumental parameters are shown in **Table 1**.

The determination of Ca, Na, Mg, Cu, Mn, Zn, and Fe was performed in FAAS in the absorption mode while the determination of K was accomplished in the emission mode (*15*−*18*). All measurements were performed in triplicate for the sample and standard solutions. Calculations of metal contents in the samples were based on a calibration curve obtained from aqueous standards. In order to avoid possible interferences for Ca and Mg determination, lantane chloride was added to both acid solutions (ash samples and standard solutions) in a final proportion of 1% (w/v).

Statistical Analysis. The effects of investigated agronomic variables on the quantitative content of the selected metals were statistically analyzed by analysis of variance coupled with the Tukey test at 5% of significance level ($p < 0.05$). All statistical analyses were performed using the Statistica 5.5 software (*29*).

Thermal Analysis. Thermal analysis is defined by the International Confederation of Thermal Analysis as any technique that measures a

Table 2. Variation in the Amount of Ashes with the Age of the Mate Leaves Samples

	ash amount (g/100 g) ± SD							
	6 months		12 months		18 months		24 months	
fertilization	sun	shadow	sun	shadow	sun	shadow	sun	shadow
without[a]	5.39 ± 0.04	6.43 ± 0.03	5.30 ± 0.10	7.47 ± 0.15	5.88 ± 0.14	5.94 ± 0.21	6.23 ± 0.04	6.71 ± 0.06
with N[a]	5.25 ± 0.05	6.40 ± 0.04	5.30 ± 0.14	7.63 ± 0.05	5.11 ± 0.06	5.60 ± 0.14	5.85 ± 0.01	6.84 ± 0.01
with K[a]	4.97 ± 0.05	5.69 ± 0.06	5.40 ± 0.10	7.10 ± 0.20	6.07 ± 0.10	6.23 ± 0.01	5.47 ± 0.01	6.26 ± 0.06
with N + K[a]	5.34 ± 0.05	5.77 ± 0.11	5.32 ± 0.05	7.00 ± 0.10	6.40 ± 0.36	6.44 ± 0.13	6.35 ± 0.06	7.21 ± 0.10
without[b]	5.71	7.46	6.65	7.87	6.57	6.70	6.66	7.31

[a] Determined by simple gravimetric assay. [b] Determined by TG assay.

Table 3. Macronutrients of Mate Tea Leaves as a Function of Age of Leaves, Fertilization Type, and Incidence of Natural Light

		amount of metals (mg/100 g dry basis) ± SD							
		6 months		12 months		18 months		24 months	
mineral	fertilization	sun	shadow	sun	shadow	sun	shadow	sun	shadow
K	without	1476.3± 7.9	1746.9 ± 55.5	1072.9 ± 15.5	1136.2 ± 11.8	612.6 ± 5.2	649.6 ± 5.3	545.0 ± 4.0	590.4 + 7.7
	N	1034.9 ± 7.4	1430.3 ± 31.5	810.8 ± 4.6	857.1 ± 13.9	513.9 ± 13.7	597.3 ± 0.7	513.9 ± 13.7	543.2 ± 2.5
	K	1637.2 ± 18.6	2084.6 ± 12.5	1220.3 ± 17.9	1535.1 ± 12.0	653.2 ± 8.5	696.0 ± 14.1	653.2 ± 8.5	673.4 ± 8.8
	N + K	1159.1 ± 29.5	1933.5 ± 3.2	1082.0 ± 10.1	1260.5 ± 18.4	612.6 ± 2.5	667.2 ± 8.9	612.6 ± 2.5	554.3 ± 6.0
Ca	without	464.6 ± 16.1	755.1 ± 6.1	345.3 ± 6.4	480.9 ± 16.8	415.0 ± 2.2	648.1 ± 10.4	414.5 ± 1.6	514.1 ± 13.4
	N	814.9 ± 2.9	852.0 ± 16.2	630.7 ± 8.8	811.9 ± 17.6	639.0 ± 1.4	815.0 ± 2.7	550.7 ± 7.9	720.1 ± 8.2
	K	631.2 ± 12.4	826.0 ± 9.7	598.1 ± 0.4	691.4 ± 7.4	521.1 ± 6.9	721.0 ± 4.4	521.1 ± 6.9	703.1 ± 3.1
	N + K	598.1 ± 0.4	793.4 ± 12.2	517.0 ± 9.5	628.7 ± 28.1	429.4 ± 5.4	661.9 ± 3.0	424.3 ± 5.3	569.6 ± 6.6
Mg	without	761.8 ± 1.8	743.0 ± 7.1	788.8 ± 4.7	777.5 ± 2.1	748.5 ± 4.0	757.6 ± 4.8	726.8 ± 3.7	753.8 ± 6.0
	N	768.5 ± 3.0	780.5 ± 7.2	797.7 ± 5.0	797.0 ± 0.5	768.0 ± 8.2	773.7 ± 13.4	797.5 ± 3.8	786.9 ± 3.3
	K	744.7 ± 1.6	729.7 ± 1.1	765.3 ± 7.5	765.2 ± 2.8	727.7 ± 10.7	749.2 ± 10.4	709.5 ± 1.3	726.8 ± 3.8
	N + K	762.0 ± 1.0	755.0 ± 2.0	794.0 ± 1.4	784.0 ± 5.7	754.5 ± 4.7	773.4 ± 6.5	775.6 ± 2.5	775.9 ± 2.8
Mn	without	182.7 ± 3.3	186.5 ± 7.7	128.5 ± 2.3	172.6 ± 2.7	116.8 ± 3.8	149.8 ± 2.1	122.1 ± 0.8	148.1 ± 0.8
	N	219.3 ± 6.1	216.2 ± 4.1	170.0 ± 1.1	182.4 ± 5.7	149.1 ± 0.9	158.7 ± 1.2	152.4 ± 0.8	152.7 ± 2.1
	K	271.2 ± 7.4	318.4 ± 9.5	180.2 ± 2.9	210.0 ± 2.8	267.1 ± 2.6	201.8 ± 1.6	240.6 + 1.6	182.6 + 1.6
	N + K	275.8 + 8.8	354.2 + 10.5	258.8 + 2.5	221.7 + 4.7	282.6 + 2.2	217.5 + 2.1	262.1 + 2.4	239.7 + 3.2

change in a physical property of a substance as a function of temperature while this substance is subjected to a controlled temperature program. Recently the use of thermal analytical methods (thermogravimetry (TG) and derivative thermogravimetry (DTG)) has increased the interest of food industries. These methods provide stability data that are important for purposes of fast application.

The TG-DTG and DSC curves of raw material were obtained in a simultaneous thermoanalyzer system SDTQ600 of the TA Instruments, with continuous air flow at 100 mL min^{-1}, using a sample mass in the range of 5−12 mg in alumina crucibles without cover, at heating rate of 20 °C min^{-1}, with scanning from ambient temperature to 900 °C. To obtain this measure, the equipment SDTQ600 was previously calibrated for TG with standard weight, for temperature with indium, zinc, and aluminum metal, for DSC heat flow with sapphire, and for DSC cell constant with zinc metal (*30*). For this assay, the samples obtained without artificial fertilization were used.

RESULTS AND DISCUSSION

Table 2 shows the total ash amount in mate tea leaves, along with the agronomic conditions of the samples investigated. The common amount of ashes cited in the literature for mate leaves is in the range of 5.20−6.20 (*31*). It should be noted that the ash content was in the interval of 4.97−7.50. The values obtained here were slightly higher, mainly for samples of plants protected from direct sun exposure, because the plant metabolism is diminished due to the decrease of photosynthesis of leaves cultivated at low light intensity. The results agree with those of Kaspary (*25*) who found that the growing of mate trees in shadow conditions induced an increase in the amount of mineral material.

The ash content did not show a significant variation with the kind of fertilizer used, while the age of the sample showed little influence on this property. Old plants (24 months) showed values of ash content slightly greater than those for younger ones.

Tables 3 and **4** present the results in terms of amount (mg) of macronutrients and micronutrients in 100 g of mate tea leaves, respectively. In these tables, the uncertainty (standard deviation) of each determination of metal content is also presented. It can be observed that potassium is the major macronutrient (K, Ca, Mg, and Mn), with concentrations up to 2100 mg/100 g of mate tea leaves. On the other hand, among the micronutrients (Na, Zn, Fe, and Cu), sodium is the major element, with composition up to 50 mg/100 g of mate tea leaves.

An increase in the amount of macro- and micronutrients was observed for plants cultivated in shadow, with the exception of magnesium, which was not influenced by the solar intensity. The fertilization showed a similar behavior for macro- as for micronutrients, that is, the use of nitrogen increased the amount of calcium and magnesium, while the use of nitrogen and potassium, increased the amount of manganese, and the major amount of potassium was found with potassium fertilization, as was expected. For micronutrients, the use of nitrogen and the mixture of nitrogen and potassium produced an increase in zinc and ferrous amounts, respectively, while copper and sodium did not show any influence from the fertilization.

The amounts of the macro- and micronutrients varied randomly with the age of the plants, not allowing any conclusion about the influence of age on mineral content.

Table 5 presents the statistical analysis of the influence of light intensity, age of leaves, and fertilization on the quantitative content of macro- and micronutrients in mate leaves. The values presented in this table are, in fact, mean values of the metal content for each condition. Also in this table, the same letter

Table 4. Micronutrients of Mate Tea Leaves as a Function of Age of Leaves and Incidence of Natural Light

		amount of metals (mg/100 g dry basis) ± SD							
		6 months		12 months		18 months		24 months	
mineral	fertilization	sun	shadow	sun	shadow	sun	shadow	sun	shadow
Zn	without	4.7 ± 0.1	6.5 ± 0.2	3.6 ± 0.1	6.8 ± 0.2	4.2 ± 0.1	5.7 ± 0.1	3.3 ± 0.1	4.9 ± 0.1
	N	6.5 ± 0.1	8.1 ± 0.1	5.1 ± 0.1	9.8 ± 0.1	5.9 ± 0.1	6.4 ± 0.1	5.4 ± 0.1	9.1 ± 0.1
	K	5.3 ± 0.1	6.9 ± 0.2	4.1 ± 0.1	8.7 ± 0.1	5.4 ± 0.1	6.4 ± 0.1	3.9 ± 0.1	8.7 ± 0.1
	N + K	5.4 ± 0.1	7.7 ± 0.1	4.4 ± 0.1	9.2 ± 0.1	4.5 ± 0.1	6.4 ± 0.1	4.3 ± 0.1	8.2 ± 0.2
Fe	without	5.4 ± 0.1	8.8 ± 0.1	6.4 ± 0.1	11.6 ± 0.2	7.9 ± 0.1	8.2 ± 0.1	7.6 ± 0.1	7.8 ± 0.1
	N	5.8 ± 0.1	9.0 ± 0.1	6.6 ± 0.1	11.6 ± 0.1	8.1 ± 0.1	8.5 ± 0.1	7.7 ± 0.1	8.1 ± 0.1
	K	6.0 ± 0.1	9.3 ± 0.1	6.7 ± 0.1	11.8 ± 0.1	8.3 ± 0.1	8.7 ± 0.1	8.0 ± 0.1	8.3 ± 0.1
	N + K	6.2 ± 0.1	9.2 ± 0.1	7.0 ± 0.1	12.0 ± 0.1	8.3 ± 0.1	8.9 ± 0.1	8.2 ± 0.1	8.4 ± 0.1
Cu	without	1.00 ± 0.1	1.50 ± 0.1	0.88 ± 0.1	1.47 ± 0.1	0.73 ± 0.2	1.10 ± 0.1	0.80 ± 0.1	0.91 ± 0.1
	N	0.93 ± 0.1	1.33 ± 0.1	0.77 ± 0.1	1.27 ± 0.1	0.68 ± 0.1	0.89 ± 0.1	0.79 ± 0.1	0.89 ± 0.1
	K	0.88 ± 0.1	1.17 ± 0.1	0.74 ± 0.1	1.17 ± 0.1	0.64 ± 0.1	0.82 ± 0.1	0.75 ± 0.1	0.76 ± 0.1
	N + K	0.84 ± 0.1	1.00 ± 0.1	0.70 ± 0.1	0.98 ± 0.1	0.62 ± 0.1	0.78 ± 0.1	0.73 ± 0.1	0.74 ± 0.1
Na	without	31.9 ± 1.2	48.7 ± 1.1	21.1 ± 0.6	50.1 ± 1.9	37.2 ± 0.6	56.5 ± 0.8	30.6 ± 2.1	22.0 ± 0.1
	N	27.9 ± 1.3	46.3 ± 0.5	22.5 ± 0.6	41.1 ± 1.3	26.1 ± 0.7	34.4 ± 0.6	29.9 ± 2.5	26.2 ± 0.2
	K	30.9 ± 1.8	38.8 ± 2.3	29.4 ± 0.3	34.6 ± 0.7	27.0 ± 0.7	32.8 ± 2.3	29.5 ± 1.1	32.8 ± 0.1
	N + K	20.8 ± 1.3	40.2 ± 1.5	24.3 ± 0.8	29.6 ± 0.4	34.3 ± 0.3	27.6 ± 0.8	31.7 ± 1.3	30.4 ± 3.5

Table 5. Statistical Analysis (ANOVA Coupled with a Tukey Test at 5%) of the Metal Contents in Mate Tea Leaves

	age of leaves				light intensity		fertilization			
mineral	6 months	12 months	18 months	24 months	sun	shadow	without	N	K	N + K
K	1562.9 a	1121.9 b	625.3 c	585.8 d	926.7 b	1030.8 a	978.7 b	787.7 c	1144.1 a	985.2 b
Ca	716.9 a	588.0 b	606.3 c	522.2 d	532.2 b	699.5 a	504.7 d	729.3 a	651.6 b	577.8 c
Mg	755.6 b	783.7 a	756.6 b	756.7 b	761.9 a	764.3 a	757.2 c	783.7 a	739.7 d	771.8 b
Mn	253.0 a	190.5 b	192.9 b	187.5 b	205.0 a	207.0 a	150.9 d	175.1 c	234.0 b	264.0 a
Na	35.67 a	31.56 b	34.58 a	29.12 b	28.4 b	37.0 a	37.30 a	31.79 b	31.97 b	29.88 c
Fe	7.45 d	9.22 a	8.37 b	8.01 c	7.14 b	9.39 a	7.95 c	8.18 bc	8.40 ab	8.52 a
Zn	6.37 a	6.45 a	5.59 c	5.97 b	4.73 b	7.45 a	4.95 c	7.01 a	6.19 b	6.25 b
Cu	1.08 a	1.00 b	0.78 c	0.80 c	0.80 b	1.05 a	1.05 a	0.94 b	0.87 c	0.80 d

Table 6. Thermal Analysis Data of TG-DTG and DSC for the Samples Cultivated under Sun and Shadow Exposure without Fertilization

	TG-DTG data				DSC data	
plants	temp range (°C)	mass loss (%)	attribution	residue (%)	temp max (°C)	heat (kJ/g)
sun 6 months	20–130	6.9	water, volatile			
	130–352	45.4	thermal decomposition		340	1.5
	352–534	42.0	thermal decomposition	5.71	493	7.1
shadow 6 months	20 – 110	6.2	water, volatile			
	110 – 350	42.9	thermal decomposition		343	1.2
	350–548	43.4	thermal decomposition	7.46	493	8.2
sun 12 months	20 – 120	5.7	water, volatile			
	120 – 365	46.5	thermal decomposition		343	1.3
	365–548	41.1	thermal decomposition	6.65	493	7.5
shadow 12 months	20 – 109	6.9	water, volatile			
	105 – 352	43.1	thermal decomposition		341	1.4
	352–545	41.2	thermal decomposition	7.87	480	7.4
sun 18 months	20 – 125	6.7	water, volatile			
	113 – 358	44.2	thermal decomposition		343	1.5
	358 – 539	42.5	thermal decomposition	6.57	480	7.7
shadow 18 months	20 – 99	6.2	water, volatile			
	99 – 348	41.9	thermal decomposition		340	1.6
	348 – 533	45.2	thermal decomposition	6.70	488	6.9
sun 24 months	20 – 123	8.4	water, volatile			
	123 – 363	41.9	thermal decomposition		343	1.4
	363–534	43.0	thermal decomposition	6.66	482	7.8
shadow 24 months	20 – 108	6.4	water, volatile			
	108 – 346	40.8	thermal decomposition		346	1.6
	346–527	45.5	thermal decomposition	7.31	481	7.0

between two levels of a factor means that there is no significant difference at 5% (Tukey test).

It should be noted from this table that the age of leaves exert a remarkable and variable influence on the mineral content in mate tea leaves. The behavior of macro- and micronutrient contents is quite distinct in relation to age of leaves, light intensity, and fertilization. In young leaves (6 months), the macronutrients and micronutrients are generally at their highest levels. The concentration of macronutrients, except for magnesium, decreases progressively with the age of leaves. On the other hand, some micronutrient contents are maximized in leaves with intermediate age, around 12 months, and some of them do not present a significant decrease in content with time.

Regarding the light intensity, plants cultivated in shadow conditions presented significantly (except for magnesium and manganese) higher levels of the investigated metals than those plants cultivated under direct sun exposure. The mate is an arboreal species that naturally occurs in southern Brazilian, Argentinian, Paraguayan, and Uruguayan forests, where the plants are subject to natural shadow due to the higher trees. When mate trees are cultivated under direct exposure to sun, there is a trend of reducing the surface of leaves to diminish the photoinhibition, the excessive warmth, and the loss of water (*26*). According to Coelho and Mariath (*27*), mate trees cultivated in shadow conditions had enhanced metabolism of chemical substances that inhibit the presence of insects, increasing the lifetime of the leaves in the plants. In this sense, the plant could also assimilate higher amounts of nutrients.

The plants absorb magnesium in the Mg^{2+} ion form, which is the central site of chlorophyll molecule, essential for the plant photosynthesis. The concentration of this compound was not improved under the shadow conditions, suggesting that other factors rather than photosynthesis could be responsible for the accumulation of other nutrients (K, Ca, Cu, Zn, and Fe). It could be possible that in shadow the lower evaporation and the lower temperature of the leaves promote a more constant metabolic activity, with a more efficient photosynthesis, as lower losses of water induce the stomata to be open for a longer period of time.

In **Table 5**, an expressed increase in the manganese content is also observed when the plants are fertilized with nitrogen and potassium sources at the same time. Because no evidence of synergic effects between potassium and manganese is presented in the literature, it could be possible that a displacement of Mn^{2+} ions due to the cation exchange capacity (CEC) of the soil by the potassium fertilization is taking place, increasing the Mn^{2+} availability for the plants. This hypothesis is supported by the fact that in acid soils ($pH < 5.0$) the manganese can occupy 20−30% of the soil CEC as cation electrostatically adsorbed. In this sense, the concentration of Mn^{2+} in the soil can be enhanced due to its substitution by the K^+ ion furnished by the potassium fertilization.

The content of potassium in the mate leaves in both conditions of light intensity (shadow and sun) presented lower values when the plants were fertilized with a nitrogen source. Although the literature does not present evidence of antagonist effects between nitrogen and potassium, the results obtained in this work suggest that the nitrogen in the soil could inhibit the absorption of potassium in the mate leaves. On the other hand, the fertilization of soil with a potassium source promotes an increase in the content of this mineral in the leaves. These observations are in agreement with the work of Zampier (*28*) who concluded that the content of this mineral in mate leaves is proportional to its quantity in the soil.

The results obtained by TG-DTG and DSC showed that thermal decomposition process occurs in three steps **(Table 6)**. In the first step, a small loss of mass is observed connected with a wide but little pronounced endothermic effect on the DSC curve. This endothermic effect is due to desorption of water of samples together with evaporation of volatile components at 121.83 ± 9.15 °C for samples at high sun and 106.40 ± 6.92 °C for those at low sun with initial decomposition. The second step of thermal decomposition is accompanied by an exothermic effect on the DSC curve and high mass loss, as reflected by the TG and DTG curves. These effects are due to degradation and combustion of low-molecular-weight compounds contained in the samples. In the third step occurs the degradation of residues of second stage and thermal decomposi-

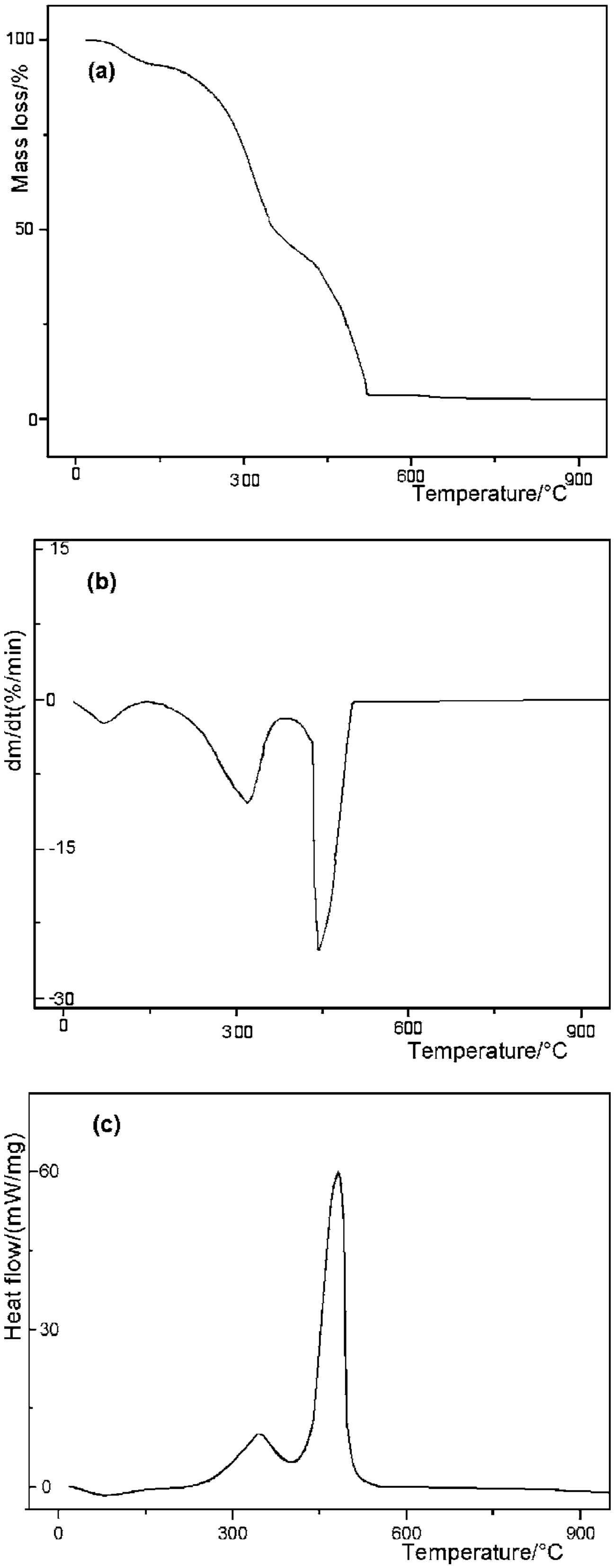

Figure 1. TG-DTG and DSC curves of *Ilex paraguariensis* cultivated without fertilization, under sun exposition, and until 6 months old: (a) TG curve; (b) DTG curve; (c) DSC curve.

tion of inorganic compounds, probably carbonates, and it is shifted to the higher temperatures. Mineral residue, probably metallic oxides, is the final decomposition product of all samples. The last line in **Table 2** shows the ash content obtained from the TG and DTG data. It is possible to note that the values are slightly greater than those obtained by simple gravimetric measurements. These data show correlation between ash contents and first stage temperatures (stability temperature); samples with higher ashes contents have lower stability temperatures. The agronomical variables did not show influence in the thermal behavior of the samples. The TG, DTG, and DSC graphs are similar for all the samples. The thermal analysis data of TG-DTG and DSC for the sample of 6 month cultivates at sun exposition and without fertilization are shown in **Figure 1**.

The thermal stability of mate leaves was evaluated by thermogravimetric curves (**Figure 1**). The results showed that the thermal decomposition process is very similar for all samples studied. The DSC curves showed endo- and exothermic peaks in correspondence with the mass loss verified in TG-DTG curves. However, the mass loss and residues obtained are characteristic for each sample (see **Table 5**) with variations in disagreement with ash content obtained by classical methods of analysis.

CONCLUSIONS

Based on analysis of the influence of the age of leaves on the mineral content, it is noted that in general, the content is higher for younger leaves. It is also possible to conclude that plants that are cultivated in shadow areas present higher metal amounts than those cultivated without protection from the sun. The mate plant metabolism is strongly influenced by its agronomic cultivation conditions, which reflects directly in the chemical mineral composition of the mate leaves. The variables that demonstrated higher influences were light intensity and age of leaves.

These results showed the influence of mineral content and agronomics variables in the stability temperature of tea leaves. Samples of mate cultivated in shadow areas, denominated shadow 6, 12, 18, and 24 months, have higher ash contents and lower thermal stability, than samples cultivated in sun areas. Minerals play a role in the decomposition and stability thermal of the leaves in the dry processing of *Ilex paraguariensis*. These results contribute to the establishment of standard quality and thermal treatment for mate leaves.

LITERATURE CITED

(1) Cansian, R. Variabilidade Genética e de Compostos Voláteis e Semi-Voláteis em Populações Nativas de *Ilex paraguariensis* (St. Hil.) do Brasil, Visando a Conservação da Espécie, Ph.D. Thesis, São Carlos, SP, Brazil, 2003 (in Portuguese).

(2) Mosele, S. H. A Governança na Cadeia Agro-Industrial da Erva-Mate na Região Alto Uruguai Rio-Grandense sob a Ótica da Cadeia de Suprimentos, M.Sc. Dissertation, Porto Alegre, Ed. UFRGS, 2002, 231 (in Portuguese).

(3) Saldanã, M. D. A.; Mazzafera, P.; Mohamed, R. S. Extraction of purine alkaloids from maté (*Ilex paraguariensis*) using supercritical CO_2. *J. Agric. Food Chem.* **1999**, *47*, 3804−3808.

(4) Saldanã, M. D. A.; Zetzl, C.; Mohamed, R.; Brunner, G. Extraction of Methylxantines from Guaraná Seeds, Maté Leaves and Cocoa Beans Using Supercritical Carbon Dioxide and Ethanol. *J. Agric. Food Chem.* **2002**, *50*, 4820−4826.

(5) Alikaridis, F. Natural Constituents of *Ilex* species, *J. Ethnopharmacol.* **1987**, *20*, 121−144.

(6) Jacques, R. A.; Oliveira de, A. P.; Freitas, L. S.; Peres, V. F.; Dariva, C.; Oliveira, de J. V.; Caramão, E. B. The use of ultrasound in the extraction of *Ilex paraguariensis* leaves: A comparison with maceration. *Ultrason. Sonochem.* **2007**, *14*, 6−12.

(7) Jacques, R. A.; Freitas, L. S.; Peres, V. F., Dariva, C., Oliveira de, J. V.; Caramão, E. B. Chemical composition of mate tea leaves (*Ilex paraguariensis*): A study of extraction methods. *J. Sep. Sci.* **2006**, *29*, 2780−2784.

(8) Jacques R. A.; Santos, J. G.; Dariva C.; Oliveira de, J. V.; Caramão, E. B. GC/MS characterization of mate tea leaves extracts obtained from high-pressure CO_2 extraction. *J. Supercrit. Fluids* **2007**, *40*, 354−359.

(9) Mosele, S. H; Peluso R. M. B. A influência do Plano Real e as Perspectivas para o Produtor de Erva-Mate do Alto Uruguai Rio-Grandense. *Perspectiva* **2000**, *24*, 17−20 (in Portuguese).

(10) Esmelindro, M. C.; Toniazzo, G.; Lopes, D.; Oliveira, D.; Dariva, C. Effects of processing conditions on the chemical distribution of mate tea leaves extracts obtained from CO_2 extraction at high pressures. *J. Food Eng.* **2005**, *70*, 588−592.

(11) Maccari, A. J.; Santos, A. P. R. Produtos alternativos e desenvolvimento da tecnologia industrial na cadeia produtiva da erva-mate. *MCT/CNPq/PADCT*; Curitiba, PR, 2000 (in Portuguese).

(12) Esmelindro, A. A.; Girardi, J. S.; Mossi, A.; Jacques, R. A.; Dariva, C. Influence of Agronomic Variables on the Composition of Mate Tea Leaves (*Ilex paraguariensis*) Extracts Obtained from CO_2 Extraction at 30 ° C and 175 bar. *J. Agric. Food Chem.* **2004**, *52*, 4820−4826.

(13) Kabata-Pendias, A., Pendias, H., Eds. *Trace elements in soils and plants*; CRC: Boca-Raton, FL, 1984.

(14) Azcón, R.; Ambrosano, E.; Charest, C. Nutrient acquisition in mycorrhizal lettuce plants under different phosphorous and nitrogen concentration. *Plant Sci.* **2003**, *165*, 1137−1145.

(15) Wrobel, K.; Urbina, E. M. C. Determination of total aluminum, Chromium, copper, iron, manganese, and nickel and their fractions leached to the infusions of black tea, green tea, Hibiscus sabdariffa, and Ilex paraguariensis(mate) by ETA-AAS. *Biol. Trace Elem. Res.* **2000**, *78*, 1−3.

(16) Ajasa, A. M. O.; Bello, M. O.; Ibrahim, A. O.; Ogunwande, I. A.; Olawore, N. O. Heavy trace metals and macronutrients status in herbal plants of Nigeria. *Food Chem.* **2004**, *85*, 67−71.

(17) Ishida, H.; Suzuno, H.; Sugiyama, N.; Innami, S.; Tadokoro, T.; Maekawa, A. Nutritive evaluation on chemical components of leaves, stalks and stems of sweet potatoes (*Ipomoea batatas poir*) *Food Chem.* **2000**, *68*, 359−367.

(18) Anderson, K. A.; Smith, B. W. Chemical Profiling to Differentiate Geographic Growing Origins of Coffee *J. Agric. Food Chem.* **2002**, *50*, 2068−2075.

(19) Wesolowski, M.; Konieczynski, P. Thermal decomposition and elemental composition of medicinal plant materials − leaves and flowers. Principal component analysis of the results. *Thermochim. Acta.* **2003**, *397*, 171−180.

(20) Ferrara, L.; Montesano, D.; Senatore, A. The distribution of minerals and flavanoids in the tea plant (*Camellia sinensis). Il Farmaco* **2001**, *56*, 397−401.

(21) Wesolowski, M.; Konieczynski, P. Thermoanalytical, chemical and principal component analysis of plant drugs. *Int. J. Pharm.* **2003**, *262*, 29−37.

(22) Esmelindro M. C.; Toniazzo G.; Waczuk, A.; Dariva, C.; Oliveira, D.; Caracterização físico-química da erva-mate: influência das etapas do processamento industrial. *Cienc. Tecnol. Aliment., Campinas* **2002**, *22* (2), 193−204 (in Portuguese).

(23) Fernández-Cácerez, P. L.; Martín, M. J.; Pablos, F.; González, A. G. *J. Agric. Food Chem.* **2001**, *49*, 4775−4779.

(24) Garcia, R. V.; Basualdo, I.; Peralta I.; Herebia de, M.; Caballero, S. Mineral contents of Paraguay Yerba mate (*Ilex paraguariensis*). *Arch. Lat. Nutr.* **1997**, *47*, 77−80.

(25) Kaspary, R. Efeitos de diferentes graus de sombreamento sobre o desenvolvimento e trocas gasosas de plantas jovens de erva-mate, M.Sc. Thesis, Porto Alegre, Ed. UFRGS, 2002, 231 (in Portuguese).

(26) Camacho, M.; Bellefleur, P. Aclimatación morfológica a la luz en seis espécies arbóreas de los bosques montanos de Costa Rica. *Rev. Biol. Trop.* **1996**, *44*, 71−79 (in Spanish).

(27) Coelho, G. C.; Mariath, J. E. A. Inflorescences morphology of *Ilex* L. (Aquifoliaceae) species from Rio Grande do Sul, Brazil. *Feddes Repertorium (Berlin)* **1996**, *107*, 19−30.

(28) Zampier, A. C. Avaliação dos níveis de nutrientes, cafeína e taninos em erva-mate após adubação e sua relação com a produtividade, M.Sc. Dissertation, Curitiba, Ed. UFPR, 2001 (in Portuguese).

(29) Statistica for Windows, 5.5, Statsoft, 2000.

(30) Wendlandt, W. W. *Thermal Methods of Analysis, Chemical Analysis*; John Wiley & Sons Inc.: New York, 1974.

(31) Sanz, M. D. T.; Isasa, M. E. T., Elementos minerales en la yerba-mate (*Ilex paraguariensis St. H.). Arch. Lat. Nutr*. **1991**, *41*, 441−454 (in Spanish).

Received for review May 26, 2007. Accepted June 29, 2007. The authors thank CNPq, SCT-RS, UCDB, FUNDECT-MS, and Erva-Mate Barão Commerce and Industry LTD (Brazil) for the financial support and scholarships.

JF071545G