JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2007**, *55*, 10081–10085 **10081**

ARTICLES

Influence of Drying Methods and Agronomic Variables on the Chemical Composition of Mate Tea Leaves (*Ilex paraguariensis* A. St.-Hil) Obtained from High-Pressure CO_2 Extraction

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The main objective of this work is to assess the influence of two drying methods (microwave and vacuum oven) and some agronomic variables (plant fertilization conditions and sunlight intensity) on the characteristics of mate tea (*Ilex paraguariensis*) leaves extracts obtained from high-pressure carbon dioxide extractions performed in the temperature range from 20 to 40 °C and from 100 to 250 bar. Samples of mate were collected in an experiment conducted under agronomic control at Ervateira Barão LTDA, Brazil. Chemical distribution of the extracts was evaluated by gas chromatography coupled with a mass spectrometer detector (GC/MS). In addition to extraction variables, results showed that both sample drying methods and agronomic conditions exert a pronounced influence on the extraction yield and on the chemical distribution of the extracts.

KEYWORDS: ***Ilex paraguariensis*; drying methods; GC/MS; carbon dioxide extraction; agronomic variables**

INTRODUCTION

Mate tea leaves (*Ilex paraguariensis* A. St.-Hil) are an important natural product in the economic and cultural context of South Brazil, with anti-inflammatory, therapeutic, antirheumatic, stimulating and diuretic properties (*1*–*3*). Jacques et al. (*4*, *5*) studied 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) and thermal behavior of tea leaves.

To take a glance at the mate market, we shall consider that only in this region of Brazil one can find more than 40 mate processing industries and about 180000 medium and small rural properties dedicated almost exclusively to cultivation of this raw material (*6*). Considering the fact that all of those industries direct their efforts to produce the same basic product (mate leaves for teas) processed in a very rudimentary way and the recent availability of this raw material from other countries (*7*), it is not surprising that the strong competition established has required company investments toward improved agronomic techniques and also in the development of new process to produce higher-value products.

Recently, the development of new separation techniques has gained increased importance in the chemical and food industries, due primarily to the imposed environmental and public health regulations and also the necessity of minimizing energy requirements (*8*, *9*). It is well-known that carbon dioxide is an appropriate solvent for the food industry, since it is nontoxic, nonflammable, nonexplosive, and readily available and has a low critical temperature and pressure that avoids degradation of thermolabile compounds (*9*–*11*). The advantages of using near critical carbon dioxide extraction prevail when small raw material amounts and high-quality products are processed. Despite the importance of mate tea leaves in the social and economic context of South Brazil, there is little work focusing

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10.1021/jf071544o CCC: $37.00
Published on Web 11/07/2007

on the oil extraction of this raw material with high-pressure carbon dioxide extraction (*3, 12*). Saldanã et al. (*12*) presented a study on the extraction of methylxanthines from mate tea leaves using CO_2 as solvent and ethanol as cosolvent in the temperature range of 40–80 °C and pressures up to 400 bar.

In the literature, some work can be found regarding the chemical characterization of volatile compounds of mate tea leaves (*13–16*) and also concerning the use of mate extracts obtained using organic solvent extraction, for antibacterial activity tests (*15*). Esmelindro et al. (*17*) investigated the extraction of mate tea leaves with carbon dioxide on the chemical distribution of selected compounds focusing mainly on the industrial steps. Esmelindro and co-workers (*18*) investigated the influence of light intensity and age of leaves on the composition of mate tea leaf extracts obtained from CO_2 extraction at 30 °C and 175 bar. However, investigations regarding the effects of high-pressure carbon dioxide extraction variables (temperature and solvent density) on the extraction yield and on the distribution of chemical components of the extracts of mate tea leaves produced under agronomic control are scarce in the literature. The literature is somewhat vast concerning the application of microwave ovens to investigate the mechanism of food cooking, to evaluate the effects of microwaves on nutrient value of foods, and as an energy source in organic synthesis (*19–22*). Nevertheless, there is a lack of information in the literature regarding the use of microwave ovens to dry natural herbaceous matrices and a consequent comparison with the conventional vacuum oven technique.

In this context, the aim of this work is to assess the influence of two drying methods and agronomic conditions on the characteristics of the extracts of mate tea leaves obtained from high-pressure CO_2 extraction. The extraction experiments were performed in a semibatch laboratory-scale unit in the temperature range 20–40 °C and from 100 to 250 bar. The instrumental analyses were conducted in a GC/MS. The extraction yield, extraction kinetics, and extract chemical composition are reported in this work.

MATERIALS AND METHODS

Sample Preparation. Mate tea leaf samples were collected in an experiment conducted under agronomic control at Ervateira Barão LTDA. 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 months. Half of the plants were covered with a covering device (a plastic sieve commercially sold as “sombrite”) that absorbed 75% of the natural light incidence.

The plants were grown under four distinct fertilization conditions (*4*): 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, plants were fertilized with 120 g/year of a potassium source (potassium chloride), and the last treatment comprised the use of potassium and nitrogen sources simultaneously (300 g/year of urea and 120 g/year of potassium chloride).

For each fertilization condition, seven plants with age of leaves of 18 months were used to represent the agronomic treatment. For each tree, fractions from the top, middle, and bottom were sampled and homogenized. The samples were dried by two methods: in a vacuum oven (24 h at 35 °C) and in a microwave oven (5 min). The final moisture of all samples was around 2%. After being dried, samples were stored under nitrogen atmosphere until the extraction to prevent oxidation. The CO_2 employed in the extractions (White-Martins, 99.9%) was used without further purification.

Apparatus and Extraction Procedure. The experiments were performed in a laboratory-scale unit, presented in detail elsewhere (*18*), which consists basically of a CO_2 reservoir, two thermostatic baths, a syringe pump (ISCO 500 D), a 0.1 dm^3 jacketed extraction vessel, an absolute pressure transducer (Smar, LD301) equipped with a portable programmer (Smar, HT 201) with a precision of ±0.3 bar, a collector vessel with a glass tube, and a cold trap. Typically, approximately 25 g of comminuted mate tea leaves were charged into the extraction vessel. The CO_2 was pumped at a constant flow rate of 2 g min^{-1} into the bed, which was supported by two 300 mesh wire disks at both ends, and was kept in contact with the herbaceous matrix for at least 1 h to allow system stabilization. Afterward, the extract was collected by opening the micrometering valve, and the pump recordings accounted for the CO_2 mass flow. After that, the mass of the extract was weighed, the glass tube was reconnected to the equipment, and this procedure was performed until no significant mass was extracted or, as in some cases, the extraction period exceeded a pre-established limit. The experiments took up to 400 min, isothermally at constant pressure. Duplicate runs were performed for all conditions leading to an overall standard deviation of the extraction yields of about 0.02.

Extracts Characterization. All extracts were analyzed by GC/MS on Shimadzu equipment, model QP5050-A with a split–splitless injector (split mode ratio 1:20) at 280 °C and interface at 300 °C. An OV-5 (Ohio Valley Company, cross-linked 5% phenyl-methyl-silicone, 30 m × 0.25 mm × 0.25 μm) capillary column was employed. The oven temperature was programmed to start at 100 °C (1 min), heated to 280 °C at 4 °C/min, and held for 15 min at this temperature. The extracts were identically treated and analyzed using equal chromatographic conditions. The analysis of the extracts was performed by injecting 1 μL of each extract directly into the GC/MS, after its dissolution in 1 mL of dichloromethane.

Quantification of compounds (caffeine, palmitic acid, phytol, squalene, stearic acid, and vitamin E) by GC/MS was made by comparing the areas of their chromatographic peaks, in the SIM mode, versus the biphenyl (internal standard) area with the authentic standards areas. The standards such as caffeine, palmitic acid, phytol, squalene, stearic acid, and vitamin E were purchased from Sigma Aldrich (St. Louis, MO, USA).

All chemical analyses were carried out in triplicate (duplicate extractions and triplicate injections of samples and standards).

Derivatization. The extracts were derivatized prior the chromatographic analysis due to the potential presence of some fatty acids, which can damage the column and inhibit the identification of the constituents. The derivatizing agent used was the BSTFA (*N*,*O*-bis(trimethylsilyl)trifluoracetamide) with 1% of TMCS (trimethylchlorosilane). This process transforms the carboxylic acids in their trimethyl-silyl-esters (*23–27*).

Statistical Analysis. The effects of agronomic conditions (sunlight intensity and fertilization sources) on the quantitative content of the selected compounds were statistically analyzed by means of analysis of variance. The analyses were conducted with Statistica 5.5 software, using the Analysis of Variance, ANOVA, package. The comparison between two levels of a variable was accomplished by the Tukey test at 95% of confidence level ($p < 0.05$); i.e., ANOVA with Tukey test, $p < 0.05$.

RESULTS AND DISCUSSION

Table 1 presents the liquid yield (mass %) of mate tea leaf extractions with carbon dioxide at high pressures for plants exposed to full sunlight incidence and without additional fertilization. Results present in this table are in fact mean values of two extractions. It can be observed that liquid yields varied from 0.39 to 2.46 and the extraction process variables exert a pronounced effect on the amount of semivolatile matter extracted CO_2 at 40 °C, 250 bar, and 0.880 g/cm^3 produced higher yields in both conditions of drying. It can also be observed from this table that slightly higher yields were obtained when the samples were dried in a vacuum oven, probably due to the less aggressive drying mechanism imposed on herbaceous samples.

Figure 1 presents the kinetics of the extractions of mate tea leaves for the samples dried under vacuum (**A**) and in a microwave oven (**B**). From Figure 1, it seems clear that the extraction variables exert similar effects on liquid yield independent of the drying methods. The pressure presented a positive

Table 1. Extraction Liquid Yield of Mate Tea Leaf Extraction with CO_2 at High Pressures for Plants Exposed to Direct Sunlight and without Fertilization

run	drying method	T (°C)	P (bar)	density[a] (g/cm^3)	extraction liquid yield (g of extract/100 g of raw material)
01	vacuum oven	30	175	0.868	1.93 ± 0.02
02	vacuum oven	20	250	0.963	1.73 ± 0.02
03	vacuum oven	40	250	0.880	2.46 ± 0.02
04	vacuum oven	20	100	0.854	1.19 ± 0.01
05	vacuum oven	40	100	0.612	0.53 ± 0.02
06	microwave oven	30	175	0.868	1.59 ± 0.02
07	microwave oven	20	250	0.963	1.70 ± 0.01
08	microwave oven	40	250	0.880	2.22 ± 0.02
09	microwave oven	20	100	0.854	1.11 ± 0.01
10	microwave oven	40	100	0.612	0.39 ± 0.015

[a] Taken from Angus et al. (*28*).

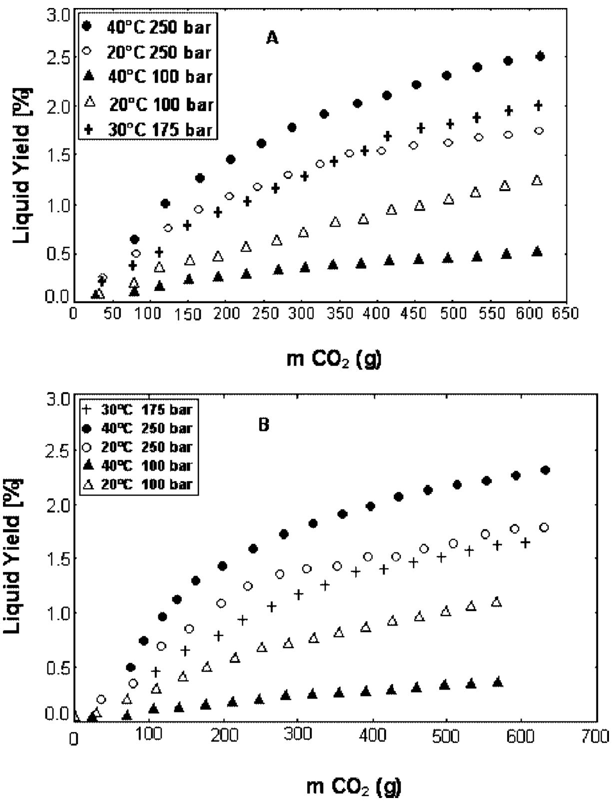

Figure 1. Effects of temperature and density on the kinetics of the extraction of mate tea leaves with compressed carbon dioxide: (**A**) samples dried in the vacuum oven, (**B**) samples dried in the microwave oven. In both cases, samples were collected from plants exposed to full sunlight and without additional fertilization.

effect on the liquid yield, as an increase in pressure causes an enhancement in the solvency power of the solvent. On the other hand, temperature presented a distinct effect depending on the operation pressure: at lower pressures (100 bar), the liquid yield decreased as the temperature increased from 20 to 40 °C. This fact can be explained in terms of the large reduction in CO_2 density (see **Table 1**). At higher pressures (250 bar) however, a rise in temperature leads to an increase in the liquid yield. At this pressure level, the reduction in density is less pronounced and is compensated by the enhancement in the vapor pressure of the compounds presented in the mate tea leaves extracts.

Effect of Fertilization Type on the Performance of CO_2 Extraction. To investigate the effects of the fertilization treatment, extractions were carried out at the conditions of the central point of the statistical design, i.e., temperature of 30 °C

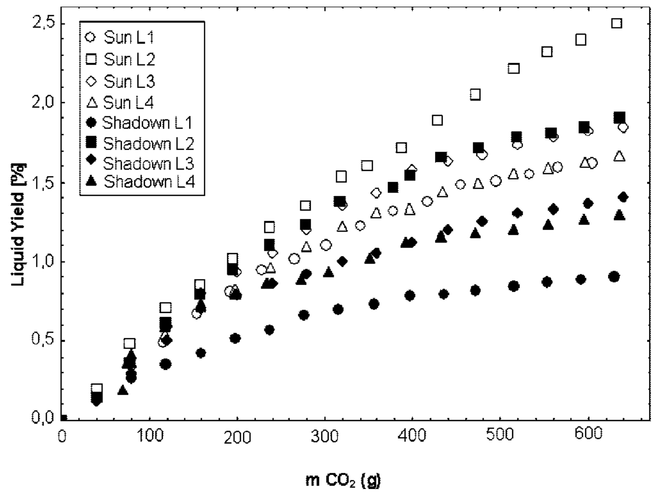

Figure 2. Effects of fertilization type and light intensity on the kinetics of the extraction of mate tea leaves. Samples were collected from plants with 18 months of age of leaves, dried in vacuum oven. Extractions were performed at 30 °C and 175 bar. (L1, plants that did not receive any type of fertilization; L2, plants that received urea as nitrogen source; L3, plants that were treated with potassium chloride; L4, plants that were treated simultaneously with the mentioned sources of potassium and nitrogen).

and pressure of 175 bar. In this case, samples of mate tea leaves dried in the vacuum oven were used.

To evaluate the effect of fertilization, plants exposed to full and partial sunlight incidence and with age of leaves of 18 months were employed in the extractions. According to the agronomic treatment, the following designation was ascribed: L1, plants that did not receive any type of fertilization; L2, plants that received urea as nitrogen source; L3, plants that were treated with potassium chloride; L4, plants that were treated simultaneously with the mentioned sources of potassium and nitrogen.

Figure 2 presents the effect of fertilization type and degree of sunlight incidence on the kinetics of the extraction of mate tea leaves. One can observe that when only nitrogen was used as fertilizer, there was an increase in the extraction liquid yield when compared to the plants without fertilization treatment. The same effect, though to a less extent, was verified to occur when a potassium source (potassium chloride) was used. Interestingly, when the plants were fertilized simultaneously with nitrogen and potassium sources, a negative synergism was observed; i.e., extraction yield did not present significant changes in relation to that without the addition of fertilizer.

We can also note from **Figure 2** that, independent of the fertilization condition, extraction yields were greater for plants exposed to full sunlight incidence than for shaded plants. However, when shaded plants were fertilized with a nitrogen source, a remarkable increase in the extraction yield was observed. In summation, the highest yield was obtained with N fertilization and high sunlight incidence.

Chemical Characterization of Mate Tea Leaf Extracts. As previously shown (*13, 14, 16–18*), the main compounds in mate leaf extracts were caffeine, palmitic acid, phytol, stearic acid, squalene, and vitamin E. Then these compounds were quantitatively determined in the extracts by using GC/MS and biphenyl as internal standard. For the quantification of compounds of mate tea extracts from, plants with age of leaves of 18 months and samples dried in a vacuum oven were employed. **Table 2** presents the concentrations of selected compounds of mate tea leaf extracts.

Table 2. Effect of fertilization type and light incidence on the concentration of selected compounds present in the high-pressure CO_2 extracts of mate tea leaves. Plants with age of 18 months dried in vacuum oven

	content (mg/kg ± std dev[a])							
	shadow				sun			
compound	without fertilization	N	K	N + K	without fertilization	N	K	N + K
caffeine	4290 ± 84.8	8688.9 ± 69.1	5487.0 ± 80.6	4689.3 ± 83.9	1705.0 ± 21.2	5418.1 ± 27.6	2889.8 ± 15.5	2058.2 ± 10.3
palmitic acid	242.2 ± 16.7	229 ± 9.3	358.4 ± 12.9	256.8 ± 11.5	129.2 ± 4.7	210.6 ± 9.6	288.8 ± 6.4	255.8 ± 10.5
phytol	336.3 ± 10.8	182.7 ± 8.2	229.9 ± 3.4	102.8 ± 5.9	332.8 ± 10.2	111.8 ± 5.6	132.7 ± 5.2	93.4 ± 2.2
stearic acid	29.75 ± 1.0	27.3 ± 0.9	28.6 ± 0.6	28.0 ± 0.7	28.4 ± 0.8	31.8 ± 0.4	31.4 ± 0.3	26.6 ± 0.7
squalene	10501.1 ± 158.7	9596.9 ± 118	8389.6 ± 40.7	9193.8 ± 52.6	12801.1 ± 71.8	9742.7 ± 104.2	8595.7 ± 87.7	9073.8 ± 24
vitamin E	1260.1 ± 39.1	1525.6 ± 50.1	1227.8 ± 46.5	1091.6 ± 29.6	1930.0 ± 40.1	2755.4 ± 56.1	1548.5 ± 26.7	999.2 ± 16.4

[a] Average values of three determinations.

Table 3. Statistical Analysis (ANOVA with Tukey Test, $p < 0.05$) of the Effect of Light Intensity and Fertilization Type on the Concentration (mg/kg of sample) of Selected Compounds in the Extracts of Mate Tea Leaves Obtained from High-Pressure CO_2 Extraction (Plants with Age of 18 Months Dried in a Vacuum Oven)

	light intensity		fertilization			
compound	shadow	sun	without fertilization	N	K	N + K
caffeine	5788.8[a]	3017.8[b]	4188.4[b]	7053.5[a]	2997.5[d]	3373.8[c]
palmitic acid	271.6[a]	221.1[b]	185.7[d]	219.8[c]	323.6[a]	256.3[b]
phytol	212.9[a]	167.7[b]	334.6[a]	147.2[c]	181.3[b]	98.2[d]
stearic acid	28.4[a]	29.6[a]	29.1[a]	29.6[a]	30.0[a]	27.3[a]
squalene	9420.3[b]	10053.4[a]	11651.2[a]	9669.8[b]	8492.7[d]	9133.6[c]
vitamin E	1808.3[a]	1276.3[b]	1595.1[b]	2140.5[a]	1388.2[c]	1045.4[d]

These compounds were also selected due to their characteristics and potential applications. Caffeine is an important alkaloid belonging to the group of methylxanthines (*29–31*), which comprises substances capable of stimulating the central nervous system, producing a short time alert state (*32*, *33*). Particularly, caffeine is one of the most consumed stimulant substances worldwide. Phytol, a diterpenic alcohol, is a raw material used for the synthetic production of vitamins K and E (*34*). Squalene is a natural bactericidal compound, widely used in the cosmetic industries to manufacture creams and emulsions (*35*, *36*). Vitamin E is crucial for the proper functioning of the organism and, currently, has attracted much attention as a powerful antioxidant agent (*37*).

Table 3 presents the results of statistical analysis of the influence of light intensity and fertilization type on the amount of selected compounds present in the extracts of mate tea leaves. The values presented in this table are in fact average values in each level of the variable. Equal letters between two levels of a factor means that there is no significant difference at 95% between these two levels, according to the Tukey test ($p < 0.05$), as in the case of the stearic acid content.

An inspection of **Tables 2** and **3** reveals that the intensity of sunlight has a negative effect on the concentrations of caffeine, phytol, palmitic acid, and vitamin E; i.e., greater amounts of caffeine, phytol, palmitic acid and vitamin E can be obtained from plants protected from the sun. In relation to squalene and stearic acid, concentrations of these compounds increase with increasing sunlight incidence. Similar results regarding caffeine were reported by Mazzafera (*38*) that found greater concentrations of caffeine in mate leaves cultivated in shadow areas.

The effect of fertilization type on the chemical composition revealed sufficient correlation with light intensity. When mate plants were fertilized with nitrogen sources a significant increase in the amount of caffeine and vitamin E was observed. Phytol and squalene presented greater concentrations in plants without fertilizer, while the amount of palmitic acid increased in plants fertilized with K. The effect of nitrogen fertilization on the alkaloids production has been already subject of research. In a general trend the literature points out an increase in the alkaloids content in leaves of mate with nitrogen fertilization. In some cases, however, results can vary depending on the nitrogen source used in the fertilization (nitrate, ammonia or urea) (*39*). According to Mazzafera (*40*), as alkaloids are nitrogen compounds, low amounts of caffeine are expected to occur in treatments with limited availability of nitrogen. The caffeine level varies as an integrated part of nitrogen compounds metabolism in the plant as a function of availability of this element.

It can also be noticed from **Table 3** that the caffeine concentration diminished with the simultaneous use of potassium and nitrogen sources. Also, lower levels of fertilization with potassium source, generally results in an increase of alkaloids percentage in plants (*38*). Mazzafera (*40*), when studying the effect of nutrients supplement on the content of caffeine in coffee leaves (Arabian Coffea L.), observed that the decrease of potassium fertilization induced an increase in the content of caffeine in leaves.

According to the literature, the concentration of caffeine in mate leaves can vary from 0.16% in old to 1.4% in young leaves (*29–31*). Saldaña et al. (*3*) identified and quantified the caffeine content in extracts of mate leaves obtained from supercritical fluid extraction (extractions performed at 70 °C and 255 atm) and found values around of 4300 mg/kg of this compound. In another work, Saldaña et al. (*12*) observed that the solvent (CO_2) modification with different amounts of ethanol resulted in an increase in the concentration of caffeine from 3320 mg/kg to 6110 mg/kg in supercritical fluid extracts of mate leaves.

Conclusions. In this work, the effects of two drying methods and different agronomic conditions on the extraction yield and on the chemical characteristics of extracts of mate tea leaves obtained from high-pressure carbon dioxide extractions were investigated. Results indicated that these variables exert a remarkable influence on the extraction liquid yield and on chemical content of selected compounds in the extracts. In this sense, it could be stressed that extracts with specific characteristics can be obtained advantageously by manipulating the investigated variables. When the plants are fertilized only with nitrogen or potassium sources, improved extraction yields were obtained. Nevertheless, when plants are fertilized simultaneously with nitrogen and potassium sources, no significant changes are verified in the extraction yield. It was experimentally verified that light intensity has a negative effect on the concentration of caffeine, phytol, palmitic acid, and vitamin E, whereas the concentration of squalene is increased with increasing light intensity.

ACKNOWLEDGMENT

The authors thank Ervateira Barão LTDA for the supply of the mate leaves.

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Received for review May 26, 2007. Revised manuscript received September 4, 2007. Accepted September 16, 2007. The authors thank CNPq, FAPERG, and SCT/RS for financial support and scholarships.

JF071544O