DOI:10.1021/jf903781w

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Methylxanthines and Phenolics Content Extracted during the Consumption of Mate (*Ilex paraguariensis* St. Hil) Beverages

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"*Chimarrao*" and "*terere*" are popular beverages consumed in South America prepared using mate (*Ilex paraguariensis* St. Hil.). "*Chimarrao*" consists of a partial infusion where hot water is added, while "*terere*" is a total infusion, with addition of cold water. This study was designed to simulate preparation of these beverages for consumption, in order to estimate the total amount of xanthines and phenolic compounds in aqueous extracts that would be ingested by the consumer. Different commercial types of mate were employed for "*chimarrao*" preparation (native, smooth, traditional, and course-ground), and these were compared to "*terere*". In "*chimarrao*", beverages from coarse-ground mate showed the highest levels of xanthines. However, "*terere*" presented quantities 2.5 times higher than the beverage of the coarse-ground mate. Considering the total phenolics in "*chimarrao*", there was no difference between the types of herbs, but in "*terere*", the extraction of almost all of the phenolics was observed.

KEYWORDS: *Ilex paraguariensis* St. Hil; mate herb; *"chimarrao"*; *"terere"*; caffeine; theobromine; total phenolic compounds; capillary electrophoresis.

INTRODUCTION

Mate, *Ilex paraguariensis* St. Hilaire (Aquifoliaceae), is a South America native species and grows naturally in Brazil, Paraguay, and Argentina (*1*). About 80% of the occurrence area belongs to Brazil, especially in the states of São Paulo, Mato Grosso do Sul, Rio Grande do Sul, Santa Catarina, and Paraná (*2*). According to the Brazilian Institute of Geography and Statistics, Brazilian production of mate in 2007 was 438,474 tons (*3*), concentrated in the South region, due to the tradition of "*chimarrao*" consumption (*2*). This crop has high social importance, since much of the production creates jobs and income for small farmers. Brazilian law defines mate as being composed exclusively of branches and leaves of *Ilex paraguariensis* St. Hil., obtained by a fragmentation and drying process and used for the preparation of "*chimarrao*" or "*terere*", to which sugar may be added (*4*).

Mate is traditionally consumed as two different hot infusions, one with the simple addition of boiling water to the dry material of the plant (tea) and another by repeated additions of hot water ("*chimarrao*") (*5*). "*Chimarrao*" is prepared in a container made from a Porongo fruit and is called "gourd", or "*cuia*" in Portuguese, where mate fills two thirds of its volume. The free volume is completed with hot water, forming a partial infusion (part of the mate stays dry). The resulting aqueous extract is sipped by the consumer through a metal straw known as a "*bomba*" (in Portuguese) or "*bombilla*" (in Spanish). The addition of water is repeated several times. In South America, approximately 30% of the population ingests more than 1 L per day of these beverages (*5*). Mate can also be consumed as "*terere*", a cold infusion, made with successive additions of cold water (*6*). Mate for "*terere*" can be pure (containing only leaves and branches of the plant) or composed (added with other plant species), and may contain flavoring and/or sugar (*4*).

The processing from mate to "*chimarrao*" basically consists of three steps: scorching, drying, and leaf grinding. The scorching is done with fire and comprises the rapid passage of the branches with leaves over the fire flames. The drying step may be performed in two types of mechanical dryers: rotary or belt. The main difference between the two types of dryers is related to the contact of the raw material with smoke during the drying process. After grinding, the herb is sieved and the final product is called ground herb. This can be used directly as raw material for tea production or, after going through a punch process, as herb for "*chimarrao*" (*7*).

The chemical constituents found in mate include tannins, polyphenols, amino acids, saponins, alkaloids, and others (*8*, *9*). Among alkaloids, methylxanthines (caffeine and theobromine) are highlighted. Caffeine in humans stimulates the central nervous system and the heart, besides enhancing brain activity and having diuretic action. However, the ingestion of high doses (10−15 mg/kg of body weight) can cause undesirable symptoms such as tremors, tachycardia, insomnia, irritability, anxiety, nausea, and

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 Published on Web 01/08/2010

Table 1. Composition of Mate Herbs Used in the Experiments

composition	smooth	traditional	native	course-ground	*"Terere"*
leaves of native plants	25%	39%	60%	41%	
leaves of cultivated plants	50%	53.5%	22.5%	46%	70%
branches	15%	7.5%	17.5%	13%	30%
sugar	10%				

gastrointestinal discomfort (*10*−*14*). Theobromine has diuretic and vasodilator effects and is regarded as a cardiac stimulant (*15*). It has been described that the content of methylxanthines in mate is extremely variable and indicates levels of caffeine between 0.16 and 1.4% and between 0.02 and 0.27% theobromine (*16*−*20*).

Phenolic compounds commonly found in mate are related to derivatives of chlorogenic and caffeic acids, as well small amounts of flavonoids. Phenolic compounds may possess antimutagenic and antioxidant properties, and they are therefore highlighted as important in preventing cancer, stroke, and chronic degenerative diseases such as cardiovascular disease (*21*, *22*). Levels of 9.608% of the phenolic derivatives were found (*23*) in the dry extract of *Ilex paraguariensis*, especially chlorogenic and caffeic acids and some derivatives of them, and 0.064% of flavonoids rutin, quercetin, and kaempferol.

Since "*chimarrao*" is a partial infusion, the fact that the matrix displays high levels of total phenolic and methylxanthines compounds does not necessarily mean that the aqueous extract normally ingested by the consumer has the same levels. This study aimed to estimate the amount of caffeine, theobromine, and phenolic compounds in aqueous extracts of "*chimarrao*" and "*terere*", obtained in the same way as they are traditionally consumed. Furthermore, the comparison was made among different commercial types of mate usually employed for "*chimarrao*" preparation (native, smooth, traditional, and course-ground), and these were compared to "*terere*".

MATERIALS AND METHODS

Samples. Samples of mate herbs were acquired in a local mate industry in the Northwest region of Rio Grande do Sul state (Brazil). The herbs analyzed were those commercially known as smooth, traditional, native, course-ground, and pure "*terere*". **Table 1** describes the composition of each type of mate, as provided by the manufacturer. One package of each herb was used to prepare the beverages.

Apparatus. Determination of xanthines levels was conducted in a G1600AX (Agilent Technology, Germany) capillary electrophoresis system equipped with a diode array detector. The separation occurred in a 37 cm × 50 μm i.d. fused silica capillary (Agilent Technology, Germany). To determine the level of total phenolic compounds, a UV-160 spectrophotometer was used (Pro-Análise, Brazil).

Total Xanthines in Mate. To assess the total xanthines content in mate used for the preparation of "*chimarrao*", the extraction was carried out based on the study from ref *24*. The sample was ground in a 100 mesh Marconi TE 600 (São Paulo, Brazil) mill. After milling, 1 g of the sample was added to 150 mL of water alkalinized with NaOH (pH 11.0). The mixture was kept boiling for 1 h. After cooling, the volume was set to 200 mL, and the sample was filtered. The extract was analyzed by capillary electrophoresis. The extraction method was evaluated by repeatability (n = 10) and recovery (with the addition of 350 and 700 mg of caffeine and 150 and 300 mg of theobromine per 100 g of sample).

Total Phenolic in Mate. The extraction of phenolic compounds was performed based on the method described in ref *25*. After milling, 20 g of the sample was placed in 100 mL of methanol and kept under constant stirring for 3 h. The extract was filtered, and the solid residue was taken back to shaking with 100 mL of methanol for another 1 h. The methanolic extracts were evaporated to dryness in a rotary evaporator at 38 ± 1 °C and stored under a temperature of −18 °C. Before analysis, the extract was resuspended in methanol (0.3 mg of extract per mL of methanol).

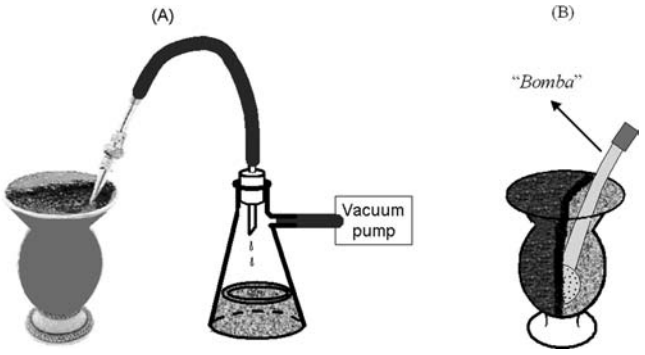

Figure 1. "*Chimarrao*" aqueous extracts obtention (**A**), and internal view of a "*chimarrao*" gourd packed with mate herb (**B**).

"*Chimarrao*" Preparation. 85 g of each mate (smooth, traditional, native, and course-ground) was weighed in a medium size gourd. This amount is approximately two-thirds the size of the gourd, following the method of preparation indicated on the product label. Mate was packed as shown in **Figure 1B**. Then, 150 mL of water at 75 ± 2 °C was added and, after 30 s, the liquid was extracted with the aid of a vacuum pump coupled with the *"chimarrao" "bomba"* (**Figure 1A**).

After a 2-min interval, the gourd was filled up again with 110 ± 10 mL of hot water and the liquid was extracted again. The gourd was supplemented with water 28 more times, always within a 2-min interval, thus simulating 30 aqueous extracts of "*chimarrao*" drunk successively by consumers. In general, after 30 water additions, mate beverages lose their flavor, and then, a new beverage must be prepared. During the experiments, care was taken to not move the mate, keeping it packed inside the gourd in the same way until the 30th extraction.

For "*terere*" extraction, 50 g was weighed into a glass. In this case, 180 mL of cold water was added at a temperature of 11 ± 2 °C, until complete herb infusion has occurred (30 s). Like "*chimarrao*", "*terere*" was extracted 30 times, always within a 2-min interval, adding 100 ± 5 mL of cold water in each subsequent extraction.

In all tests, a metal straw ("*bomba*", **Figure 1B**) was used with a sieve of 3.5 cm in diameter, 20 cm distant from the nozzle. The sieve contained 340 holes on each side with a 1 mm diameter each. For each type of mate studied, preparation of either "*chimarrao*" or "*terere*" was conducted in triplicate, and in each replicate, 30 additions of water were followed by vacuum extraction. Each extract had its volume recorded and reserved for phenolic and xanthines analysis (also in triplicate). All the extracts were analyzed separately from the first to the sixth, and then, each one was analyzed within a 4-extracts interval, until the 30th extract.

Analysis of Caffeine and Theobromine Content by Capillary Electrophoresis. The extracts for total xanthines content of mate leaves, as well as the aqueous extracts of each "*chimarrao*" and "*terere*", were filtered and analyzed by capillary electrophoresis. The method was based on ref 26. The electrolyte composition was 50 mmol/L sodium dodecyl sulfate and 10 mmol/L sodium carbonate (buffered in pH 11.0). The separation was performed at 25 °C with a voltage of 30 kV, injection of 50 mbar for 7 s, and detection at 206 nm. The method was validated for linearity, repeatability (n = 10), intermediate precision (n = 3), limits of detection, and quantification.

Analysis of Total Phenolic Content. Phenolic compounds were evaluated according to the method described in ref 27 with modifications, using the Folin-Ciocalteu reagent. Briefly, 0.5 mL of each methanolic extract (to determine the total phenolic content present in mate leaves) and of the aqueous extracts of "*chimarrao*" was taken in test tubes and then added with 2.5 mL of the Folin-Ciocalteu reagent 10% in distilled water. After 5 min, 2 mL of sodium carbonate 7.5% solution was added. The tubes were kept for 2 h protected from light. The absorbance was measured in a spectrophotometer at 740 nm. A blank sample was analyzed under the same conditions, replacing the extract by the same amount of solvent. An external calibration curve with gallic acid solutions between 0.003 and 0.100 mg/mL (R^2 = 0.998) was used. The result was expressed in grams of gallic acid equivalents (g GAE).

Statistical Analysis. The results obtained for caffeine, theobromine, and total phenolic compounds content present in the matrices were

Table 2. Total Content of Caffeine, Theobromine, and Phenolic Compounds Present in Mate Leaves, Obtained after Thorough Extraction of the Compounds[a]

mate herb	caffeine (g/100 g)[b]	theobromine (g/100 g)	phenolic compounds (g GAE/100 g)[c]
native	0.83 ± 0.01 b	0.203 ± 0.003 a	5.18 ± 0.30 a
traditional	0.66 ± 0.02 c	0.177 ± 0.003 c	5.22 ± 0.31 a
smooth	0.61 ± 0.02 c	0.166 ± 0.004 d	4.41 ± 0.19 ab
course-ground	0.79 ± 0.03 b	0.190 ± 0.003 b	4.92 ± 0.59 ab
"*Terere*"	1.02 ± 0.02 a	0.212 ± 0.006 a	4.18 ± 0.09 b

[a] The same letters in a row indicate there is no statistically significant difference (95% confidence level), considering the Tukey test. [b] Mean ± standard deviation (n = 3). [c] GAE = gallic acid equivalents.

subjected to analysis of variance (ANOVA) and Tukey test, both with 95% confidence, using the software Statistica 7.0 (Statsoft, USA).

RESULTS AND DISCUSSION

Method Validation. The method for extraction of total xanthines present in the matrix showed good repeatability, obtaining a relative standard deviation (RSD) of 2.40% for caffeine and 2.57% for theobromine. Recovery in the two levels studied was 103.7 and 101.0% for caffeine and 101.4% and 99.8% for theobromine. Separation and quantification of caffeine and theobromine were made by capillary electrophoresis. The method was validated for repeatability, where RSD values of 1.29% and 1.88% were observed for caffeine and theobromine, respectively, in ten measurements made on the same day. In the determination of intermediate precision (3 days), the RSD values were 0.93 and 2.95% for caffeine and theobromine, respectively. The method proved to be linear in the ranges from 5 to 200 mg of caffeine/L and from 2 to 100 mg of theobromine/L ($R^2 > 0.998$). The method allowed the separation of compounds in 2.7 min. The detection limits (3 times S/N (signal/noise)) were 0.103 mg/100 g of sample for caffeine and 0.037 mg/100 g for theobromine. The limits of quantification (10 S/N) were 0.342 mg/100 g of sample for caffeine and 0.123 mg/100 g for theobromine.

Methylxanthines and Phenolic Content in Mate Leaves. Table 2 shows the total content of caffeine, theobromine, and phenolics present in mate leaves used to prepare "*chimarrao*" and "*terere*", obtained after thorough extraction of the compounds. Caffeine content was higher in "*terere*" mate, followed by native and coarse-ground, whose values showed no significant difference. The herbs that contained the lowest amounts were the traditional and smooth. Both "*terere*" and native herb showed the highest contents of theobromine, followed by coarse-ground, traditional, and smooth mate. It was observed that native and traditional mate herbs showed the highest levels of total phenolic compounds, with statistically similar values of about 5.2 g GAE/100 g of sample. For coarse-ground, smooth, and "*terere*" mate herbs, levels were similar, being 4.9, 4.4, and 4.2 g GAE/100 g of sample, respectively.

Methylxanthines and Phenolic Content in Mate Beverages. Figure 2 shows the amounts of caffeine, theobromine, and phenolic compounds in aqueous extracts of "*chimarrao*" and "*terere*" that would normally be ingested by the consumer of these beverages. The extracts from native mate "*chimarrao*" had, on average, volumes of 95 ± 2.6 mL, those of the traditional 92 ± 2.3 mL, smooth 115 ± 5.2 mL, coarse-ground 67 ± 6.5 mL and 100 ± 10 mL of the "*terere*". Among different types of mate herbs used to prepare "*chimarrao*", it was observed that extracts from native, traditional, and smooth mate showed similar levels of caffeine and theobromine (**Figure 2A** and **B**). Extracts of

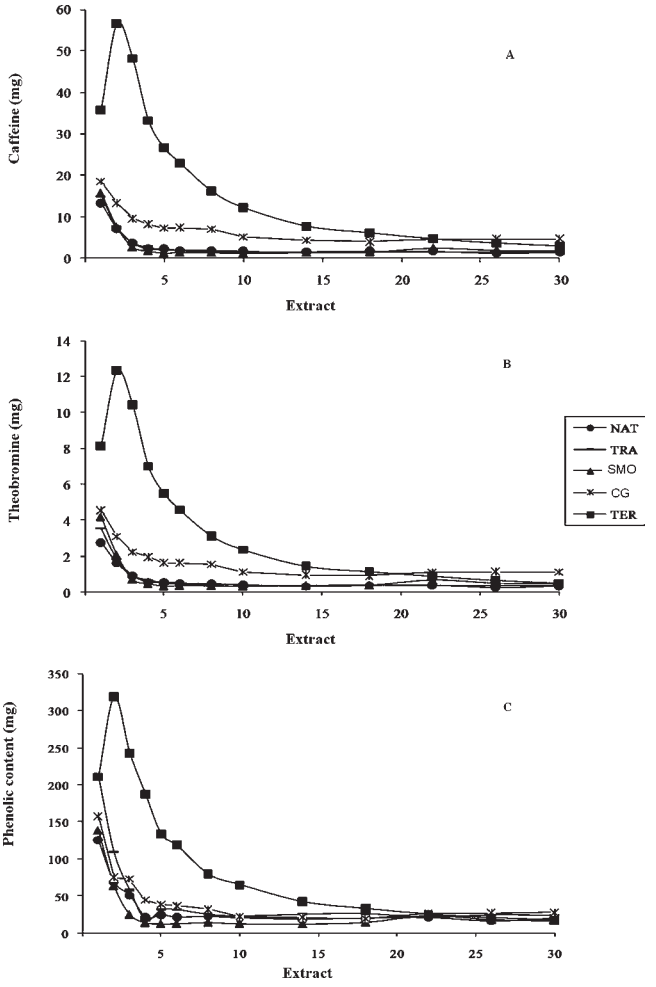

Figure 2. Caffeine (**A**), theobromine (**B**), and total phenolic compounds (**C**) content present in aqueous extracts from "*chimarrao*" and "*terere*". NAT: native; TRA: traditional; SMO: smooth; CG: course-ground; TER: "*terere*".

coarse-ground mate had the greatest levels of xanthines, in such a way that the 30th mate extract still contained about 4.0 mg of caffeine and 1.0 mg of theobromine.

During the withdrawal of the "*chimarrao*" extracts, it was noted that the volumes of water added to the gourd were similar for all mate. However, the coarse-ground extracted volumes were lower, indicating that there was a retention of greater amounts of water. "*Terere*" beverage showed levels much higher of methylxanthines and phenolic compounds when compared to the extracts from "*chimarrao*". It was observed that, in the second and third extracts, there was an increase in the levels of all the studied compounds in relation to the first extract, probably due to the fact that leaves and branches of "*terere*" mate were not completely immersed during the first extract, while in the second, and after, the mate was completely submerged in water. From the 4th to 10th extract, the levels still remained high, and after the 20th extract a reduction in the content was observed, close to those found in "*chimarrao*" extract of the coarse-ground mate.

Considering the total amount of caffeine in the 30 aqueous extracts from "*terere*" beverage, it showed average levels that were 2.5 times higher when compared to the amount found in the "*chimarrao*" extraction of coarse-ground mate. Compared to extractions made with native, traditional, and smooth mate herbs, "*terere*" showed levels 6 times higher. The extraction of higher levels in the beverage with cold water could be correlated with the complete infusion of mate, as in hot beverages

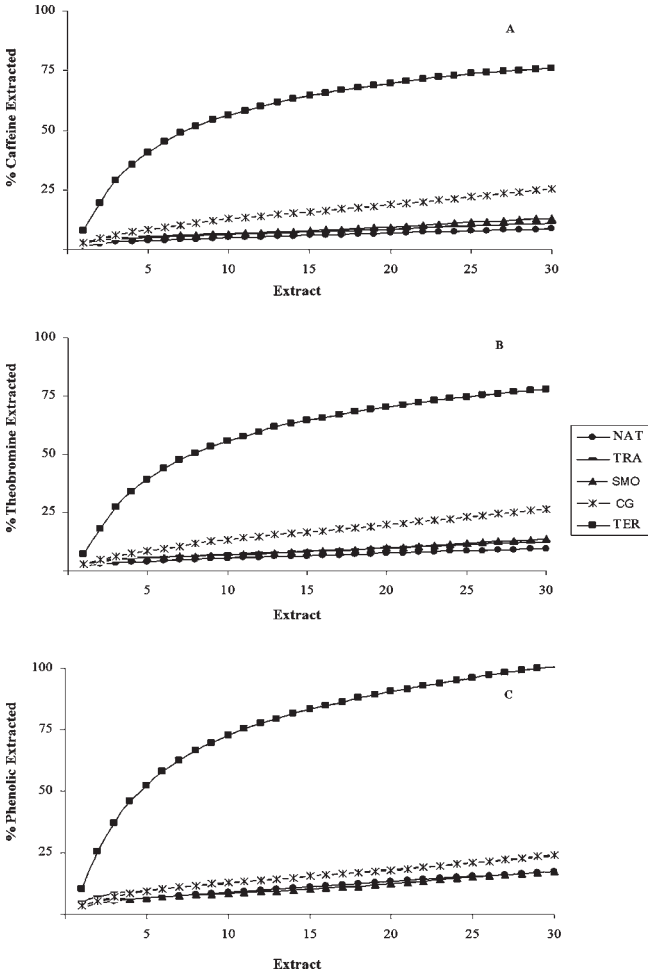

Figure 3. Accumulated percentage of caffeine (**A**), theobromine (**B**), and total phenolic compounds (**C**) extracted during successive water additions. NAT: native; TRA: traditional; SMO: smooth; CG: course-ground; TER: "*terere*".

("*chimarrao*") the water is partially in contact with the herb. For total phenolic compounds (**Figure 2C**), it was found that, among beverages with hot water, extracts from the traditional mate showed higher content, followed by extracts of coarse-ground, smooth, and native mate herbs.

Considering "*terere*" beverage, the first extract showed 210 mg of GAE, a value slightly higher than that obtained in the first extraction of traditional mate herb. But in the second extract, 319 mg of GAE was extracted; in the third, 243 mg; in the fourth, 187 mg; in the fifth and sixth, 133 and 118 mg of GAE, respectively. After the eighth extract, values declined from 79 to 17 mg of GAE in the 30th. The "*terere*" extracts showed greater content of total phenolics, being higher than all the hot beverages extracts, so that the 8th aqueous extract of "*terere*" (79 mg GAE) showed a higher amount of phenolics than the 3rd extract of any "*chimarrao*". Similarly to what occurred with xanthines, this greater efficiency of extraction may be related to the fact that in "*terere*" water is thoroughly in contact with the herb.

Percentage Extracted from the Matrices. Considering the total amount of xanthines and phenolic compounds present in the whole mate herbs put into the gourd, **Figure 3** shows the percentage extracted at the successive additions of water. It is possible to see that the percentages of theobromine and caffeine extracted are similar, indicating that both xanthines are equally solubilized during beverage consumption. It was observed that, despite the fact that native mate presented a superior amount of total xanthines when compared to the traditional and smooth mate herbs, it was not reflected in the percentage of caffeine and theobromine extracted. The graphs (**Figure 3A** and **B**) showed close percentages of extraction for the extracts from these mate herbs. Along the 30 aqueous extracts, 9.0% of the total xanthine content was extracted from native mate herb in the gourd, 11.7% from the traditional, and 13.3% from the smooth. It is worth mentioning that the first four "*chimarrao*" extracts removed 3.5% of the total caffeine and theobromine content present in the native mate herb, 4.9% in the traditional, and 5.3% in the smooth. In extracts of coarse-ground mate "*chimarrao*", the percentage extracted from the beverage was greater (up to three times) than in other matrices used for hot beverages. Until the fourth mate extract, 7.3% of total caffeine and theobromine was extracted, and until the 30th, 26.0% was removed. This fact could be correlated with better permeability of water in this type of mate herb.

Comparing "*terere*" to "*chimarrao*", a much higher percentage of extraction was observed for the first beverage. It could be noted that, in the 4th "*terere*" extract, 34.8% of total caffeine and theobromine had been extracted and, until the 30th, 77.1% had been extracted. It can be observed in **Figure 3** that the phenolic compounds are more efficiently extracted than caffeine and theobromine. For the "*terere*" mate herb, the content of phenolic compounds present in the matrix was the lowest when compared to other mate herbs. However, as observed for caffeine and theobromine, the percentage extracted was higher in this beverage.

Among the native, traditional, smooth, and coarse-ground mate herbs, it was observed that extracts of traditional and coarse-ground showed the highest percentages extracted. Over the 30 aqueous extracts, 17.5% of total phenolics present in the matrix from native and smooth mate herbs were extracted, 23.6% from the traditional mate, and 24.23% from the coarse-ground. In "*terere*" it was possible to extract almost all the total phenolic compounds, considering that, until the 4th extract, 45% had already been extracted. Over the 30 aqueous extracts, approximately 98% of total phenolic compounds was extracted from the herb.

Comparison with Other Foods and Beverages. Since many consumers ingest "*chimarrao*" extracts, a comparison was made between the content of these compounds present in extracts and in other foods or drinks. In the case of mate herbs that showed similar levels in the analyzed extracts, a mean was calculated for comparison (**Table 3**). It was found that the ingestion of the extract from the first "*chimarrao*" of native, traditional, or smooth mate herbs corresponds to the caffeine content found in approximately 16 mL of espresso coffee (*28*). No comparisons were made for the subsequent extracts, because the content extracted was very low when compared to that of the four first extracts.

By comparison with published studies, it was possible to observe that the fourth "*chimarrao*" extract prepared with coarse-ground mate herb had large amounts of caffeine, similar to those in 10 mL of espresso coffee (*28*), 118 mL of cola type soft drink (*29*), 34 mL of energy drink (*29*), or 0.5 g of guarana powder (*30*). The amount of theobromine in this extract is equivalent to 6.2 g of milk chocolate (*31*). Besides, the total phenolic compounds present correspond to 45.6 mL of red grape juice (*32*), 11.7 mL of red wine (*33*), 21 mL of green tea infusion (*34*), or 37 mL of orange juice (*34*).

Likewise, the fourth "*terere*" aqueous extract is similar in amounts of caffeine as those shown in 24 mL of espresso coffee (*28*), 277 mL of cola type soft drink (*29*), 80 mL of energy drinks (*29*), or 1.1 g of guarana powder (*30*). Theobromine content

Table 3. Xanthines and Phenolic Compounds Content Present in Mate Beverages

	"Chimarrao" (mg/100 mL)				
aqueous extract	native	traditional	smooth	course-ground	"Terere" (mg/100 mL)
			Caffeine		
1st	14.0	16.6	13.7	26.4	35.8
2nd	7.5	8.3	6.5	19.0	56.5
3rd	3.9	4.2	2.3	13.6	48.2
4th	2.3	3.0	1.6	11.6	33.2
10th	1.8	1.7	1.0	7.3	12.2
18th	1.8	1.8	1.3	5.7	6.1
30th	1.5	1.8	1.6	6.6	2.9
			Theobromine		
1st	2.8	3.9	3.7	6.4	8.2
2nd	1.7	2.0	1.8	4.4	12.3
3rd	0.9	1.0	0.6	3.1	10.4
4th	0.5	0.8	0.4	2.9	7.0
10th	0.4	0.3	0.3	1.6	2.4
18th	0.4	0.4	0.3	1.3	1.1
30th	0.3	0.4	0.3	1.6	0.5
			Phenolic Compounds		
1st	131.9	235.6	120.5	225.7	235.3
2nd	72.2	121.1	55.5	107.1	350.0
3rd	53.3	64.4	21.9	104.3	268.4
4th	22.3	20.0	12.3	62.9	208.5
10th	21.1	25.6	11.0	31.4	72.3
18th	20.7	29.2	12.8	27.0	36.3
30th	20.4	26.7	14.5	38.6	19.7

corresponds to 14.5 g of milk chocolate (*31*). The phenolic compounds present in the fourth extract of "*terere*" can also be found in 252 mL of red grape juice (*32*), 65 mL of red wine (*33*), 115 mL of green tea infusion (*34*), or 205 mL orange juice (*34*).

To summarize, the capillary electrophoresis method used to determine caffeine and theobromine showed validation parameters adequate to obtain a reliable quantification of these compounds. Considering the total content of xanthines and phenolic compounds present in different matrices, the mate herb used to prepare "*terere*" showed the highest levels of caffeine and theobromine, while the traditional mate herb (used for "*chimarrao*") exhibited the greatest amount of total phenolic compounds.

In the aqueous extracts obtained by simulating the consumption of "*chimarrao*", the highest levels of xanthines were found in coarse-ground mate herb. For "*terere*", the levels of xanthines extracted were higher than those for all the "*chimarrao*" extracts. Considering the sum of caffeine amount in the 30 aqueous extracts, "*terere*" beverage showed about 2.5 times more caffeine than the amount found for the coarse-ground mate herb.

The aqueous extracts of "*chimarrao*" made with traditional mate herb showed the highest amount of total phenolic compounds, but the extracted levels were much higher for "*terere*" beverage. For extraction percentage, "*terere*" again exhibited the highest values, since after 30 extractions almost all the phenolic compounds have been removed. Moreover, it could be noted that the phenolic compounds were extracted more efficiently than xanthines, for both beverages.

Chimarao had moderate levels of caffeine and theobromine, and relatively high amounts of total phenolic compounds, which makes it an interesting source for the intake of phenolic compounds in the human diet. Extracts from "*terere*" showed even greater levels of phenolics, with significant amounts of caffeine and theobromine. "*Terere*" consumption, besides providing significantly higher amounts of methylxanthines and phenolic compounds than "*chimarrao*", could prevent the negative effects associated with repetitive ingestion of this very hot drink on the development of oresophagic cancer.

ACKNOWLEDGMENT

This research was supported by FAPESP, CAPES, and CNPq.

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Received for review October 28, 2009. Revised manuscript received December 18, 2009. Accepted December 19, 2009.