Populational Diversity on Methylxanthines Content of Maté
(*Ilex paraguariensis* A. St.-Hil., Aquifoliaceae)

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**SUMMARY.** This work aimed to analyze the methylxanthines quantitative variability from different *Ilex paraguariensis* populations sampled in five regions, situated in four Brazilian states: Mato Grosso do Sul, Paraná, Santa Catarina and Rio Grande do Sul. Differences among populations concerning the caffeine, theobromine and total methylxanthines content were observed. The caffeine values varied from 0.04 to 9.60 mg/g and the theobromine values from 0.001 to 2.60 mg/g. Differences were observed also in the caffeine/theobromine ratio. Besides the populational variability, a high variability among plants was also observed. Supposing high heritability to the methylxanthines content, the results highlight the possibility of selecting plants with low caffeine accumulation.

**RESUMEN.** “Diversidad Poblacional en los Tenores de las Metilxantinas de Yerba Mate (*Ilex paraguariensis* A. St. Hil. Aquifoliaceae)” Este trabajo tuvo como objetivo la verificación de la variabilidad en el perfil cuantitativo de las metilxantinas de diferentes poblaciones de la especie, originarias de cuatro estados brasileños: Mato Grosso do Sul (MS), Paraná (PR), Santa Catarina (SC) y Rio Grande do Sul (RS), incluyendo cinco poblaciones. Fueron observadas diferencias significativas entre poblaciones en lo que se refiere a los tenores de cafeína y teobromina, y en el tenor de metilxantinas totales. Los contenidos de cafeína variaron desde 0.04 mg/g hasta 9.60 mg/g y los de teobromina desde 0.001 mg/g hasta 2.60 mg/g. Fueron observadas también diferencias entre poblaciones en la relación cafeína:teobromina. Junto a la variabilidad entre las poblaciones se verificó una gran variabilidad en las plantas de una misma población. Suponiendo una heredabilidad significativa para estos caracteres, los resultados sugieren una interesante posibilidad de selección de plantas con bajos tenores de cafeína.

**INTRODUCTION**

The mate (*Ilex paraguariensis* A. St.-Hil.) is a species native to South America, where it is known as “erva-mate” or “yerba mate”, and widely used as a raw material in stimulant beverages. Its chemical composition includes saponins ¹⁻⁴, flavonoids ⁵, and methylxanthines ⁶⁻⁷.

Maté derived products are consumed in many countries in South America and in other parts of the world ⁸. Besides its stimulating properties, the maté may be an important vitamin C source, a recognized fact since the early XX century ⁹⁻¹⁰. Recently, there is a worldwide interest in the biological effects of maté, e.g., concerning its antioxidant effects ¹¹, anti-topoisomerase activity ¹² and inhibition of proteasome activity ¹³.

The annual consumption is nearly 500 thousand tons, which makes its production an important crop in meridional South America. In spite of its economic importance, research on its chemical composition has only recently intensified. Information about chemical variability from populations of this species is scarce.

The goal of our investigation was to obtain a first insight concerning the populational variability of maté methylxanthine contents from samples of native populations, under extractivism, from five different regions, situated in four Brazilian States: Mato Grosso do Sul, Paraná, Santa Catarina and Rio Grande do Sul. These States constitute the main region of geographical distribution of the species in Brazil, and the sampled sites represent a transect between the

**KEY WORDS:** Caffeine, *Ilex paraguariensis*, Maté, Populational variability, Theobromine.

**PALABRAS CLAVE:** Cafeína, *Ilex paraguariensis*, Mate, Teobromina, Variabilidad poblacional.

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extreme latitudes of its occurrence. It is important to establish that the present knowledge on the methylxanthines content of *maté* is based mostly on a limited number of plant specimens.

**MATERIALS AND METHODS**

**Plant material**

The leaves of *I. paraguariensis* were gathered separately from each tree and stored in paper bags. Only fully expanded leaves were included. The plant material was dried on a ventilated stove for seven days at 40 °C. A total of 38 plants were analysed. Geographical location and data collection are shown in Table 1.

**Methylxanthine extraction**

An extract from 3.0 g of crushed leaves was prepared from each sample. The material was boiled with H2SO4 (20%, aq.) for 10 minutes and filtered. After cooling, the filtrate was neutralized with NH4OH (50%, aq.) and extracted with chloroform:isopropanol (3:1 v/v, 3 × 10 mL). The resultant solution was dried out in Na2SO4 and evaporated to dryness under reduced pressure.

**Caffeine and theobromine determination**

The qualitative analysis by TLC was performed according to Reginatto. The quantitative HPLC determination was carried out according to Peterman & Baumann, except for the mobile phase flow rate (1.0 mL/min.). The methylxanthine residue was submitted to HPLC analysis by dilution in the mobile phase MeOH:H2O (4:6 v/v), with concentration in the range of 30 to 50 μg/ml. The analytical conditions were described in detail by Athaye et al.

The statistical analysis was performed through one-way ANOVA and pairwise Tukey comparisons (α = 0.05). The data were transformed by the equation \[ v^\prime = \text{arsen} P \], except the data of the caffeine/theobromine ratio.

**RESULTS AND DISCUSSION**

Caffeine and theobromine were detected in all samples. Theophylline, whose presence was reported by some authors, but rarely mentioned in the majority of literature, was not observed.

The populations were different in caffeine content (ANOVA, P = 0.0003). A higher caffeine content was observed in the populations from Iguatemi (Mato Grosso do Sul) and Pinhão (Paraná) (Table 2). Nevertheless, the average caffeine content of the Pinhão population (Paraná), would be 1.85 mg/g, in the case of exclusion of a single plant with exceptionally higher caffeine content (9.60 mg/g).

There were also significant differences among populations in the theobromine content (ANOVA, P = 0.0060). The relative position order for the populations was modified: the populations from Ilópolis (Rio Grande do Sul) and Pinhão (Paraná), presented the highest values, while Iguatemi (Mato Grosso do Sul) presented the lowest values.

Relative to the total methylxanthine contents, there were also significant differences among

<table>
<thead>
<tr>
<th>State</th>
<th>County</th>
<th>Date</th>
<th>Nr. of plants</th>
<th>Coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mato Grosso do Sul</td>
<td>Iguatemi</td>
<td>February 1997</td>
<td>8</td>
<td>23° 40' S, 54° 28' W</td>
</tr>
<tr>
<td>Paraná</td>
<td>Pinhão</td>
<td>February 1997</td>
<td>7</td>
<td>25° 20' S, 51° 11' W</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25° 26' S, 51° 31' W</td>
</tr>
<tr>
<td></td>
<td>Guarapuava</td>
<td>February 1997</td>
<td>3</td>
<td>25° 27' S, 51° 31' W</td>
</tr>
<tr>
<td>Santa Catarina</td>
<td>Catanduvas</td>
<td>February 1997</td>
<td>10</td>
<td>27° 03' S, 51° 40' W</td>
</tr>
<tr>
<td>Rio Grande do Sul</td>
<td>Ilópolis</td>
<td>February 1998</td>
<td>10</td>
<td>28° 55' S, 52° 50' W</td>
</tr>
</tbody>
</table>

**Table 1.** Sampled populations with the geographical location, date and number of plants.

<table>
<thead>
<tr>
<th></th>
<th>Caffeine</th>
<th>Theobromine</th>
<th>Total methylxanthines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iguatemi - Mato Grosso do Sul</td>
<td>2.91 ± 0.36 a</td>
<td>0.35 ± 0.28 a</td>
<td>3.26 ± 0.28 b</td>
</tr>
<tr>
<td>Guarapuva - Paraná</td>
<td>0.08 ± 0.03 c</td>
<td>0.42 ± 0.36 ab</td>
<td>0.50 ± 0.39 a</td>
</tr>
<tr>
<td>Pinhão - Paraná</td>
<td>2.96 ± 2.97 ab</td>
<td>1.05 ± 0.58 b</td>
<td>4.01 ± 3.35 b</td>
</tr>
<tr>
<td>Catanduvas - Santa Catarina</td>
<td>2.12 ± 1.01 ab</td>
<td>0.77 ± 0.51 ab</td>
<td>2.89 ± 1.10 b</td>
</tr>
<tr>
<td>Ilópolis - Rio Grande do Sul</td>
<td>1.41 ± 1.26 bc</td>
<td>1.07 ± 0.63 b</td>
<td>2.48 ± 1.06 b</td>
</tr>
</tbody>
</table>

**Table 2.** Caffeine, theobromine and total methylxanthine contents (mg/g) from different populations of *maté* (*Ilex paraguariensis*). The letters indicate differences according to the Tukey test (P < 0.05).
populations (ANOVA, \( P = 0.0008 \)), similarly to what was observed for caffeine contents. The populations from Iguaí (Mato Grosso do Sul) and Pinhão (Paraná), showed the highest values, while the population from Guaraupuava (Paraná), presented the lowest value (Table 2).

Considering the results for each individual plant (results not shown), it is possible to notice a large range of contents in the analyzed populations (Table 3).

The mean theobromine values may be close to the caffeine values, as is the case of the Ilópolis population (Rio Grande do Sul), in which more than half of the sampled plants presented higher contents in theobromine than in caffeine. In the Catanduvas population (Santa Catarina), only one plant showed theobromine content higher than caffeine and in Guaraupuava (Paraná), two plants presented the same feature. There were no plants with more theobromine than caffeine in the Pinhão (Paraná) and Iguaí (Mato Grosso do Sul) populations.

Elevated values of theobromine were also reported by Coelho et al. \(^{17}\), when analysing \( I. \) \textit{paraguariensis} var. \textit{vestita} Loes. from the Ivaí region (Paraná State, Brazil, February, 2000), as 50% of the plants sampled on that study presented a higher theobromine than caffeine contents. This set of data indicates a different character to \( I. \) \textit{paraguariensis} as a species, regarding the literature references \(^{16,18,19}\), generally pointing out much higher caffeine than theobromine contents. The widespread use of chloroform as the only solvent in the methylxanthine extractions should be emphasized as an important methodological aspect, which could have determined the predominant view that mate is a species with a much higher caffeine content relative to theobromine, as theobromine is poorly soluble in this solvent. The mixture chloroform: isopropanol (3:1, v/v) is more satisfactory for the methylxanthine extraction \(^{20,21}\).

In \textit{Camellia} and \textit{Coffea}, the caffeine is synthesized from xanthosine, via 7-methylxanthosine, 7-methylxanthine and theobromine, theobromine methylation being the last step on this route \(^{22,23}\). The studies of Ashihara \(^{24}\) on the mate pointed out a similar biosynthesis pathway. In \textit{Coffea arabica}, plants with caffeine-synthetic genetic variants showed lower caffeine accumulation, with higher theobromine contents, as compensation \(^{25}\). The results obtained with mate indicate that the theobromine accumulation is relatively independent from the caffeine accumulation, in a populational context. Accordingly, plants with low caffeine content could also present low theobromine content, as observed in the Guaraupuava population.

The populations differed in relation to the caffeine:theobromine ratio (ANOVA, \( P = 0.0002 \)). The Iguaí (Mato Grosso do Sul) population presented the highest mean value (Table 4). Considering the ratio caffeine:theobromine, the ranking is not the same as the ranking concerning the total methylxanthine contents, but would be the same if the one plant with exceptional high values for caffeine contents (9.6 mg/g) from Pinhão (Paraná), was excluded. The much higher caffeine content of this plant could be related not only to genetic factors, but also to environmental interference. In \textit{Camellia sinensis}, pathogenic fungus attacks cause an increase in caffeine concentration \(^{26}\).

A correlation between caffeine and theobromine concentration was not observed, analysing the total set of plants (\( n=38, \) \( y =

<table>
<thead>
<tr>
<th>Population</th>
<th>Caffeine</th>
<th>Theobromine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iguaí-Mato Grosso do Sul</td>
<td>2.40 - 3.60</td>
<td>0.13 - 0.90</td>
</tr>
<tr>
<td>Guaraupuava-Paraná</td>
<td>0.05 - 0.10</td>
<td>0.01 - 0.56</td>
</tr>
<tr>
<td>Pinhão-Paraná</td>
<td>1.00 - 9.60</td>
<td>0.001 - 0.17</td>
</tr>
<tr>
<td>Catanduvas-Santa Catarina</td>
<td>0.66 - 3.76</td>
<td>0.28 - 1.90</td>
</tr>
<tr>
<td>Ilópolis-Rio Grande do Sul</td>
<td>0.04 - 3.30</td>
<td>0.20 - 2.60</td>
</tr>
</tbody>
</table>

Table 3. Range of caffeine and theobromine contents (mg/g) from different Brazilian mate populations (\textit{Ilex paraguariensis} A. St.-Hil.), February.

<table>
<thead>
<tr>
<th>Population</th>
<th>Caffeine/ Theobromine ratio</th>
<th>± s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iguaí-Mato Grosso do Sul</td>
<td>13.80</td>
<td>± 8.17 a</td>
</tr>
<tr>
<td>Guaraupuava-Paraná</td>
<td>1.69</td>
<td>± 2.69 b</td>
</tr>
<tr>
<td>Pinhão-Paraná</td>
<td>2.96</td>
<td>± 1.72 b</td>
</tr>
<tr>
<td>Catanduvas-Santa Catarina</td>
<td>4.09</td>
<td>± 3.84 b</td>
</tr>
<tr>
<td>Ilópolis-Rio Grande do Sul</td>
<td>2.62</td>
<td>± 3.87 b</td>
</tr>
</tbody>
</table>

Table 4. Ratio caffeine:theobromine mean values from mate (\textit{Ilex paraguariensis} A. St.-Hil.) Brazilian populations, February. Different letters indicate differences according to the Tukey test (\( P < 0.05 \)).
0.0034x + 0.0778, x represents caffeine and y represents theobromine content, \( r^2 = 0.0001, P = 0.955 \). To some extent, these data oppose the expectation derived from the knowledge of the caffeine and theobromine metabolic pathway, from which one could expect an increase of theobromine accumulation concomitant to a decrease in caffeine. Similarly, Scherer \(^7\), analysing different maté populations, verified a significant negative correlation between caffeine and theobromine. The diversity of environmental conditions of the populations addressed in the present study could have avoided this phenotypic correlation between the content of these two substances. Therefore, the interference of ecological factors in the methylxanthine contents deserves more attention, since significant influences were observed in other species such as *Camellia sinensis* \(^26\) and *Coffea arabica* \(^25\).

*I. paraguariensis* is a species with high genetic \(^30\)\(^-\)\(^32\) and morphologic \(^35\) variability, which could be explained by its dioecy. The data obtained indicate significant phenotypic differences in the methylxanthine contents among populations. On the other hand, the high degree of differences among plants of the same population should be stressed. In the Ilópolis population the extreme values of caffeine content differ by a factor of 80.

Studies have been carried out in *Coffea* varieties, aiming to the selection of low caffeine content plants, including intra- and inter-specific hybridization, and genotype selection \(^5\). The elevated variability of chemical profiles of maté plants and populations suggests a wide range of possibilities of selecting plants with low caffeine content, in the same manner that was reported to *Coffea arabica* \(^25\), or even plants with low total methylxanthines accumulation, since there are maté plants and populations with concurrent low amounts of caffeine and theobromine.

In conclusion, the set of data suggests the following comments: a) there are phenotypic differences among populations with respect to caffeine and theobromine contents, and to the caffeine:theobromine ratio, b) although the caffeine content tends to be higher than the theobromine content, a considerable number of plants (approximately 20%) present higher values of theobromine content; the population with the highest number of plants with this feature was in Ilópolis, Rio Grande do Sul State, c) the dissimilarities amongst populations could determine quality variations in the maté commercial products, and d) the elevated intrapopulational variability highlights the possibility of selecting plants with low methylxanthine accumulation.

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**REFERENCES**