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Physicochemical composition, methylxanthine and phenolic compound content of byproducts from the yerba mate agroindustry

Composição físico-química, teor de metilxantinas e compostos fenólicos em subprodutos da produção de chá-mate

José Marcos Hammerschmidt[1]*; Roger Raupp Cipriano[2]; Cristiane Vieira Helm[3]; Katia Christina Zuffellato-Ribas[4]; Cicero Deschamps[5]

Highlights

Methylxanthine content is influenced by the yerba mate byproduct and harvesting time.
Caffeine and theobromine levels are higher in green *cancheada* and torrefied chile.
Average protein content is higher in yerba mate from April to August.
Phenolic compound contents are higher in the grounds and torrefied chile byproducts.

Abstract

Yerba mate tea is sold in liquid form after packaging or fresh after torrefaction. Both processes produce byproducts with potential for different applications, depending on their biochemical characteristics. This study aimed to assess the physicochemical composition and methylxanthine and phenolic compound content of byproducts produced by the yerba mate agroindustry during packaging or torrefaction. The yerba mate byproducts used were obtained from producers in Paraná and Santa Catarina states, with harvesting times from September 2020 to March 2021 (spring/summer) and April to August 2021 (fall/winter). The samples were assessed fresh (*cancheada*), after separating the stalks and leaves (green *chile* and green stalk), after torrefaction (torrefied *chile*) and after packaging (grounds). The experiment was conducted in a completely randomized design, comparing harvesting times and byproduct types, with three repetitions. Means were compared by Tukey's test at 5% probability. Green *cancheada* (10.57%)

[1] Master's Degree in Agronomy, Graduate Program in Agronomy, Universidade Federal do Paraná, UFPR, Curitiba, PR, Brazil. E-mail: josemarcosh@gmail.com
[2] PhD in Agronomy, Ecophysiology Laboratory Technician, UFPR, Curitiba, PR, Brazil. E-mail: rogerraupp@gmail.com
[3] PhD Researcher in Embrapa Forests, Empresa Brasileira de Pesquisa Agropecuária, EMBRAPA, Colombo, PR, Brazil. E-mail: cristiane.helm@embrapa.br
[4] PhD Prof[a]. in Plant Physiology, Researcher in the Graduate Program in Agronomy, UFPR, Curitiba, PR, Brazil. E-mail: kazu@ufpr.br
[5] PhD Prof. in Plant Physiology, Researcher in the Graduate Program in Agronomy, UFPR, Curitiba, PR, Brazil. E-mail: cicero@ufpr.br
* Author for correspondence

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and torrefied *chile* (11.9%) exhibited higher caffeine and theobromine contents when harvested from April to August. The highest protein content was recorded in toasted *chile* grounds (16.10% in fall/winter and 13.20% in spring/summer) and ashes (6.57% fall/winter and 6.29% summer/winter), and green powder (6.65% fall/winter and 6.62% spring/summer), for both harvesting times. Average protein content was higher in yerba mate grounds harvested from April to August (16.10%). In regard to phenolic compounds, grounds exhibited the highest 3-caffeoylquinic (33.62%), 4-caffeoylquinic (32.09%) and 3,4-dicaffeoylquinic acid contents (20.57%), and the torrefied *chile* samples the highest levels of 5-caffeoylquinic (46.09%) and 3,5-dicaffeoylquinic acid (31.64%), and grounds and torrefied *chile* the highest 4,5-dicaffeoylquinic acid concentrations, at 30.40 and 26.28%, respectively. Methylxanthine content was influenced by the type of sample and harvesting time, while phenolic compound content varied primarily as a function of type of byproduct and was higher in the grounds and torrefied *chile* samples.
Key words: *Ilex paraguariensis.* Bioactive compounds. Caffeine. Caffeoylquinic acid.

Resumo

O chá de erva-mate é comercializado na forma líquida após embalagem ou fresco após torrefação. Ambos os processos produzem subprodutos com potencial para diversas aplicações, dependendo de suas características bioquímicas. Este estudo teve como objetivo avaliar a composição físico-química e o teor de metilxantina e compostos fenólicos de subprodutos produzidos pela agroindústria da erva-mate durante a produção de chá-mate envasado ou torrefação. Os subprodutos da erva-mate utilizados foram obtidos de produtores dos estados do Paraná e Santa Catarina, com épocas de colheita de setembro de 2020 a março de 2021 (primavera/verão) e de abril a agosto de 2021 (outono/inverno). As amostras foram avaliadas *in natura* (cancheada), após separação dos talos e folhas (pimentão verde e talo verde), após torrefação (pimentão torrado) e após embalagem (moagem). O experimento foi conduzido em delineamento inteiramente casualizado, comparando épocas de colheita e tipos de subprodutos, com três repetições. As médias foram comparadas pelo teste de Tukey a 5% de probabilidade. A cancheada verde (10,57%) e a chile torrada (11,9%) apresentaram maiores teores de cafeína e teobromina quando colhidas de abril a agosto. O maior teor de proteína foi registrado em borra de chile torrada (16,10% no outono/inverno e 13,20% na primavera/verão) e cinzas (6,57% outono/inverno e 6,29% verão/inverno) e pó verde (6,65% outono/inverno e 6,62% primavera/verão), nas duas épocas de colheita. O teor médio de proteína foi maior nas borras de erva-mate colhidas de abril a agosto (16,10%). Em relação aos compostos fenólicos, o pó apresentou os maiores teores de ácido 3-cafeoilquínico (33,62%), 4-cafeoilquínico (32,09%) e ácido 3,4-dicafeoilquínico (20,57%), e as amostras de chile torrado apresentaram os maiores teores de 5-cafeoilquínico. (46,09%) e ácido 3,5-dicafeoilquínico (31,64%), e a chile moída e torrada apresentaram as maiores concentrações de ácido 4,5-dicafeoilquínico, 30,40 e 26,28%, respectivamente. O teor de metilxantina foi influenciado pelo tipo de amostra e época de colheita, enquanto o teor de compostos fenólicos variou principalmente em função do tipo de subproduto e foi maior nas amostras de chile moída e torrada.
Palavras-chave: *Ilex paraguariensis.* Compostos bioativos. Cafeína. Ácido cafeoilquínico.

Introduction

Yerba mate is a plant species native to the subtropical and temperate regions of South America and can be found in Brazil, Paraguay and Argentina. It is a socioeconomically important plant in Brazil and can be formally cultivated or produced by extractivism, with two main harvesting times, from September to March (spring/summer) and April to August (fall/winter) (Mesquita et al., 2021; Ohtaki et al., 2023; Simeão et al., 2002; Carvalho, 2003).

The species *Ilex paraguariensis* A.St.-Hil holds significant economic and cultural importance in certain regions of South America (Ohtaki et al., 2023), particularly for agroindustry, since the plant is primarily used in a drink known as *chimarrão* or *tereré,* typically consumed several types a week (Mesquista et al., 2021; Verbeke & Vackier, 2005; Ohtaki et al., 2023). However, it can also be used in medications, to recover degraded areas, restore riparian forests, and to form agroforestry systems in conjunction with other perennial and annual species (Veiga et al., 2021; Antoniazzi et al., 2018).

Interest in yerba mate is largely due to the presence of caffeine alkaloids, theophylline and theobromine, which are used as tranquilizers, sedatives, stimulants and analgesics (Comert et al., 2020; Bordim et al., 2021; Rosa, 2020; R. B. Costa et al., 2005), and because of its antioxidant properties through phenolic compounds, known for their antioxidant capacity, direct participation in regulating metabolic energy, and anti-inflammatory and anticancer properties (Mitterer-Daltoé et al., 2021; Bracesco et al., 2011).

Yerba mate processing (thermal bleaching, known as *sapeco* in Portuguese, followed by drying, and chopping or *cancheamento*) produces a large amount of waste, making it relevant to assess the possibility of using these materials in different applications and technologies (Lermen et al., 2021). It is important to reuse plant organic matter waste (biomass) to generate clean energy through gases released during combustion (Vasconcellos et al., 2004) and torrefaction (Bortoluzzi, 2019).

The waste produced during yerba mate processing is used to generate energy in boilers, as mulch (Lourenço et al., 2001; Mosele, 2002), charcoal for the adsorption of chemical solutions (Gonçalves et al., 2007), in ceramic materials (Scharnberg et al., 2019), agglomerates (Guiotoku et al., 2008), MDP boards (J. T. Souza, 2016), fertilizers (Krepki, 2018), soil conditioners (Sousa, 2013), and absorption dyes (Rossa et al., 2017).

Using yerba mate waste is important for sustainability, mitigating environmental impacts and contributing to the reuse of byproducts (Cunha et al., 2022). Strategies for reusing yerba mate are relevant due to environmental concerns, reducing waste accumulation, fostering sustainability and the economy, and lowering costs, since these materials would otherwise be discarded (Rosa, 2020).

Furthermore, the notion of reusing yerba mate waste is evident in agroecological practices, generating sustainably certified products, fostering and valuing the reduction of waste and environmental pollution, generating income and promoting the circular economy. As such, it is a promising strategy for stimulating sustainability (Rzasa-Duran et al, 2022).

Given this context, the present study aimed to assess the physicochemical composition and methylxanthine and phenolic compound content of byproducts from the yerba mate agroindustry.

Material and Methods

The experiment was carried out from September 2022 to August 2023 at the UFPR Ecophysiology Laboratory in Curitiba, and the Non-Timber Forest Products Laboratory of EMBRAPA Florestas in Colombo, both located in Paraná state, Brazil.

The dried samples (chopped yerba mate) were obtained from 28 rural producers, 14 in Paraná (cities: Espigão Alto do Iguaçu, Paula Freitas, Turgo, Fernandez Pinheiro) and 14 in Santa Catarina state (cities: Concórdia, Bituruna), with harvest times from September 2020 to March 2021 (spring/summer) and April to August 2021 (fall/winter). After using these raw materials in the industrial yerba mate production process, the separate byproducts were denominated green *chile* (*chile* refers to a specific yerba mate cutting technique), green stalk, green powder, torrefied *chile* and grounds (leaves and stalks), and assessed for physicochemical composition and methylxanthine and phenolic compound contents in two harvesting times.

The byproducts were ground into a fine homogeneous powder. For the aqueous extract, 250 mg of the ground sample was added with 50 mL of ultrapure water (concentration of 5 mg.mL-1) and shaken in a vortex shaker for 30 seconds, with three repetitions. Extractions were performed in a boiling water bath for 30 minutes and the samples shaken every 10 minutes. The extracts were filtered through filter paper, and then a 0.22 µm-mesh nylon filter, and used to determine methylxanthine (caffeine and theobromine) and caffeoylquinic acid content.

Chromatographic analyses were performed in a Shimadzu® liquid chromatograph (UFLC) controlled by LC Solution software and equipped with an automatic injector and UV detector (SPD-20A). The compounds were separated using a Shim-Pack CLC-ODS analytical column (M)® (250 x 4.6 mm, Ø 5 µm) with a ShimPack CLC G-ODS® precolumn (10 x 4.0 mm, Ø 5 µm), both Shimadzu®. The separation conditions for the compounds in the aqueous extract (20 µL of injection) were: 30 °C with an eluent flow rate of 0.5 mL.min-1 and mobile phases A (H2O:Alphatec® acetic acid – 99.9: 0.1 v/v) and B (Merck® acetonitrile - 100%). The wavelength used for compound detection was 280 nm (fixed). The gradient elution program was 0-15 min (3%B), 15-20 min (3-20% B), 20-40 min (20% B), 40-45 min (20-30% B), 45-55 min (30-100% B), 55-75 (100% B), 75-80 (100-3% B) and 80-95 (3% B). The methylxanthines caffeine (1,3,7-trimethylxanthine) and theobromine (3,7-dimethylxanthine) were identified and quantified using the analytical curves obtained with Sigma® standards, between concentrations of 0 to 1.0 mg.mL-1 and 0 to 0.5 mg.mL-1 for caffeine and theobromine, respectively. The caffeoylquinic acids 3-caffeoylquinic (3-ACQ), 4-caffeoylquinic (4-ACQ) and 5-caffeoylquinic acid (5-ACQ) were identified using the Sigma® standard and the three compounds quantified according to 3-ACQ. Quantification was based on isomers, which have the same molar absorptivity, obtained from an analytical curve between 3-ACQ concentrations of 0 and 10 mg.mL-1. The results were expressed in mg of compound per gram of sample (mg.g-1).

Protein content was determined by adding a 0.2g sample (triplicate) to a solution containing 12.6g of sodium selenite, 8.0g of copper sulfate, and 97g of sodium sulfate, and 5ml of sulfuric acid. After 24h in an exhaust fume hood, the samples were placed in an oven at 100ºC for one hour, and the temperature increased by 50ºC every hour until 350ºC, which was maintained for 3h. Reading was performed in a nitrogen distiller after adding the samples with 0.5ml of 4% boric acid solution and filtering (Gaivani & Gaertner, 2006).

Total phenolic compound content was analyzed using a 0.5g sample (duplicate) in a Falcon tube (50 mL), added with 45 mL of heated pure water and placed in an ultrasound device for 30 minutes. The solution was then filtered, and the volume of the tube topped up with 45ml of pure water. Next, a 100uL aliquot of extract was removed and added with 5 ml of pure water and 500uL of Folin & Ciocalteu's phenol reagent (F9252), then homogenized in a vortex shaker for 1 minute and added with 2 ml of sodium carbonate (Na2Co3 15%) (Veber et al., 2015).

Moisture content was identified by weighing 1.5g of sample (triplicate), drying in an oven at 100ºC for 24h, followed by 3h of cooling and then weighing to determine moisture content. Ash content was determined using ash samples (triplicate) obtained in the previous drying process, washed at 550ºC for 5h, and the results expressed in g of compound / 100g of dry matter (Araújo et al., 2006).

The results, expressed on a dry basis, were submitted to analysis of variance (ANOVA) and the means compared by Tukey's test at 5% probability, using the SISVAR program (Ferreira, 2019).

Results and Discussion

The methylxanthine content and physicochemical composition results of the fresh samples (green *cancheada*) and yerba mate byproducts are presented in Table 1.

In regard to methylxanthines, the highest caffeine contents were recorded in the torrefied *chile* samples in the second harvest (fall/winter), followed by torrefied chile in the first harvest (spring/summer) and grounds in the second. The green *cancheada* samples obtained intermediate results (Table 2). Theobromine content varied from 0.3 to 1.85%, with higher values in the green *cancheada* samples and no difference ($p \leq 0.05$) in torrefied *chile,* both in the second harvest.

Methylxanthines are the main purine alkaloids found in yerba mate leaves (Meinhart et al., 2010). These components are largely responsible for the stimulating activity of the central nervous system and the increased use of fat as an energy source (Silva et al., 2011), with muscular, cardiac and renal functions (C. A. L. Souza et al., 2021).

Table 1
Methylxanthine content and physicochemical content of fresh yerba mate (green *cancheada*) and byproducts of yerba mate production after two harvests

Type of sample	Number of samples	Harvest time*	Caffeine**	Theobromine**	Protein**	Ash**
Green *cancheada*	14	1	7.97% cd	1.46% bcd	11.08% c	5.86% bc
	14	2	10.57% ab	1.85% a	11.77% bc	6.08% bc
Green *chile*	14	1	8.14% c	1.19% de	12.66% bc	6.13% bc
	14	2	9.44% bc	1.10% e	13.47% c	6.40% ab
Green stalk	14	1	1.56% f	0.30% g	4.55% d	3.47% f
	14	2	1.90% f	0.35% g	2.78% d	3.89% f
Green powder	14	1	4.15% e	0.66% f	11.25% c	6.62% a
	14	2	6.36% d	1.06% e	12.27% bc	6.65% a
Torrefied *chile*	14	1	10.08% b	1.50% bc	13.42% b	6.29% ab
	14	2	11.90% a	1.60% ab	12.68% bc	6.57% a
Grounds	14	1	8.41% c	1.29% cde	13.20% b	5.51% d
	14	2	10.18% b	1.35% bcde	16.10% a	4.94% e

* Harvest time: spring/summer – September 2020 to March 2021 (1) and fall/winter – April to August 2022 (2)
*Means followed by the same letter in the column do not differ statistically according to Tukey's test at 5% probability.

Yerba mate leaves contain high levels of caffeine (Melo et al., 2020), increasing linearly in soil with a high nitrogen content, regardless of genotype. Average caffeine concentrations in green yerba mate vary between 1.74 and 3.01% (Gerhardt et al., 2017). However, in the present study, concentrations greater than 10% were observed in the torrefied *chile* (11.9%), grounds (10.18%) and green *cancheada* (10.57%) samples (April to August). This finding may be linked to the climate conditions in Paraná, since the period from April to August is cooler, with an average temperature of 19ºC (Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA], 2012). The climate in the state is predominantly wet subtropical, with an average annual temperature between 14 and 27°C, and colder in the southern portion of inland plateaus (Paraná, 2023). The climate in Santa Catarina state is similar to that of Paraná, with the coldest temperatures in fall/winter (average of 12 to 21°C), increasing to an average of 26°C in the hottest season (spring/summer) (Instituto Nacional de Meteorologia [INMET], 2022; Wrege et al., 2012).

In regard to caffeine content in the two yerba mate harvest times, although there was no difference ($p \leq 0.05$) in the green *chile* and green stalk samples, the second harvest produced the highest caffeine levels. The predominant climate in southern Brazil is classified as subtropical, with well-defined seasons and large temperature variations.

For most of the samples, caffeine content was higher in the second harvest (fall/winter) when temperatures were lower, including episodes of frost (EMBRAPA, 2012).

Considering the average protein content of the samples, grounds exhibited higher concentrations in the second harvest than the other samples. There was no difference ($p \leq 0.05$) in sample protein content between harvest times, with lower values only recorded in the green stalk samples. Similar protein content results were reported by (Barbosa et al., 2018; Santos et al., 2017) in yerba mate.

According to (Lobo, 2020), yerba mate has high total phenolic compound, protein and caffeine content and antioxidant activity, which vary according to climate conditions. In this study, ash content did not differ ($p \leq 0.05$) between harvesting times (green *cancheada* 5.86% spring/summer and 6.08% fall/winter; green *chile* 6.13% spring/summer and 6.40% fall/winter; green stalk 3.47% spring/summer and 3.89% fall/winter; green powder 6.62% spring/summer and 6.65% fall/winter; torrefied *chile* 6.29% spring/summer and 6.57% fall/winter; and ground 5.51% spring/summer and 4.94% fall/winter). The green powder samples in both harvests and torrefied *chile* in the first harvest obtained the highest ash content, with no difference ($p \leq 0.05$) only for green *chile* in the second harvest and torrefied *chile* in the first. Similar values were obtained by (Croge et al., 2021) in yerba mate leaf samples and after processing, with average values of 5.57 and 6.62%, respectively.

For phenolic compound content, 3-caffeoylquinic, 5-caffeoylquinic, 4-caffeoylquinic, 3,5-dicaffeoylquinic, 3,4-dicaffeoylquinic and 4,5-dicaffeoylquinic acid were identified in all the yerba mater samples regardless of harvest time (Table 2). According to (Melo et al., 2020), caffeoylquinic acid derivatives such as 3-caffeoylquinic, 4-caffeoylquinic and 5-caffeoylquinic acid are the major compounds in yerba mate leaf tissue.

Table 2
Phenolic compound (caffeoylquinic acid) content of fresh yerba mate (green *cancheada*) and byproducts of yerba mate production after two harvests

Type of sample	Number of samples	Harvest time*	3 caffeoylquinic acid	5 caffeoylquinic acid	4 caffeoylquinic acid	3,5 dicaffeoylquinic acid	3,4 dicaffeoylquinic acid	4,5 dicaffeoylquinic acid
Green cancheada	14	1	18.65% d	14.70% d	13.57% de	6.38% gh	5.42% de	8.76% e
	14	2	19.10% d	15.93% d	14.90% d	7.97% fg	6.08% d	11.86% d
Green chile	14	1	30.46% b	41.79% bc	26.58% c	27.87% bc	15.72% c	25.26% c
	14	2	26.86% c	38.51% c	25.92% c	24.65% d	14.82% c	26.28% bc
Green stalk	14	1	15.45% e	13.21% d	9.59% f	9.33% ef	4.51% ef	9.08% e
	14	2	17.85% de	16.54% d	11.74% fg	11.47% e	5.58% de	11.47% de
Green powder	14	1	6.40% f	6.50% e	5.72% g	3.33% i	2.68% g	5.01% f
	14	2	7.27% f	8.26% e	7.01% g	4.55% hi	3.64% fg	5.34% f
Torrefied chile	14	1	31.09% ab	46.09% a	29.18% b	31.64% a	18.75% b	30.87% a
	14	2	28.93% bc	45.52% a	31.01% ab	29.50% ab	18.21% b	31.35% a
Grounds	14	1	33.62% a	41.60% bc	29.68% ab	26.88% cd	20.57% a	28.63% ab
	14	2	33.62% a	43.30% ab	32.09% a	25.74% cd	19.36% ab	30.40% a

* Harvest time: spring/summer – September 2020 to March 2021 (1) and fall/winter – April to August 2022 (2)
*Means followed by the same letter in the column do not differ statistically according to Tukey's test at 5% probability.

Among the phenolic compounds studied, the highest overall average content in the samples was recorded for 5-caffeoylquinic acid. In the byproducts, the torrefied chile samples differed ($p \leq 0.05$) for the two harvest times (41.79% spring/summer and 38.51% fall/winter) and the grounds in the second harvest (43.30%). The second highest level in the samples was recorded for 3-caffeoylquinic acid, particularly grounds at both harvests (33.62% spring/summer and 33.62% fall/winter) and torrefied *chile* in the first harvest (31.09%). These results may be associated with the higher concentration of these compounds at the end of the yerba mate production process (Duarte, 2020).

The total phenolic compound content in yerba mate results in greater antioxidant activity due to caffeoyl derivatives (Filip et al., 2000). Thus, high caffeoyl acid concentrations are important in producing different tonics, anticellulite and anti-aging products, and in choleretic, hypocholestremic, hepatoprotective, glycogenolytic and lipolytic activity, as well as bitterness. (Canterle, 2005; Gao et al., 1999; Bermudez, 2020) found that the presence of bioactive compounds with antioxidant activity means that yerba mater extract can be incorporated in sheep feed to improve meat quality, demonstrating that phenolic compounds are important in animal nutrition.

The results obtained in this study corroborate those of (Filip et al., 2000), who reported a difference ($p \leq 0.05$) in caffeoyl acid derivatives in *Ilex paraguariensis* A.St.-Hil, especially during periods with lower temperatures, similar to the samples harvested from April to August (fall/winter) in the present study.

According to (Silva et al., 2011), yerba mate with higher polyphenol levels shows greater antioxidant activity, largely due to polyphenols, chlorogenic acid and its derivatives 3,4-dicaffeoylquinic, 3,5-dicaffeoylquinic, and caffeic acid (Heck & Mejia, 2007). The polyphenol content of yerba mate has been compared to that of red wine and green tea, making it a relevant source of flavonoids (Dudonné et al., 2009).

It is important to note that the antioxidant compounds (total phenolic compounds and caffeoylquinic acids) and stimulants (caffeine and theobromine) in yerba mate are essential for use of the plant and its byproducts (Tomasi et al., 2021). As such, depending on the amounts involved, these compounds can be obtained from yerba mate byproducts during production, ensuring better use of the raw material in the industrial process. For example, (Vieira et al., 2009) observed high total polyphenol levels in that yerba mate powder, representing an alternative to add value to the food industry.

Conclusions

It was concluded that methylxanthine content was influenced by the type of sample and harvesting time, while phenolic compound content varied primarily as a function of type of byproduct.

Methylxanthine (caffeine and theobromine) content was higher in the byproducts green *cancheada* and torrefied *chile* in the fall/winter harvest, while in spring/summer grounds exhibited the highest protein content and levels of the phenolic compounds 3-caffeoylquinic, 4-caffeoylquinic and 3,4-dicaffeoylquinic acid, and the torrefied *chile* samples 5-caffeoylquinic and 3,5-dicaffeoylquinic acid. The highest 4,5-dicaffeoylquinic acid content was recorded in grounds and torrefied *chile.*

While methylxanthine content was influenced by the type of sample and harvesting time, phenolic compound content varied mainly as a function of type of byproduct and was higher in the grounds and torrefied *chile* samples.

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