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Yerba mate (*Ilex paraguariensis* St. Hill.)-based beverages: How successive extraction influences the extract composition and its capacity to chelate iron and scavenge free radicals ☆

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ABSTRACT

Chimarrão or *mate* is a popular beverage from South America that is drank with successive infusions. Although *yerba mate* extracts have been widely studied, few studies have described the extract contents in beverages. Using *yerba mate* samples from Brazil, Argentina, and Uruguay, we examined the extract chromatographic profiles, total polyphenol content and their capacities to chelate iron. In addition, we analyzed antioxidant activity by examining the ability of the extracts to scavenge DPPH and NO. Our results showed that the amount of extracted compound was highest in *yerba mate* extract from Uruguay, followed by Argentina, then Brazil. Herbs from all three areas had a significant capacity to inhibit DPPH and NO free radicals. The Brazilian and Uruguayan herbs had an 80% iron chelation capacity ($p < 0.001$), while the iron chelation capacity of the Argentinean herb was lower but still significant ($p \leqslant 0.05$). We conclude that the compound concentration decreases with successive extractions, while the antioxidant capacity is maintained at significant levels.

1. Introduction

Ilex paraguariensis is a tree from the Aquifoliaceae family, and it has been extensively studied for its beneficial health properties. *Yerba mate* is a main product obtained from the plant. It is consumed in Brazil, Argentina, Uruguay, and Paraguay as a popular beverage. The beverage is known as *mate* or *chimarrão* in Brazil, Argentina, and Uruguay, and it is drank with hot water. In Paraguay, it is drank with cold water and is known as Tererê.

This beverage is prepared in a dried gourd called a "cuia" and drank using a metal straw called a "bombilla". Water is poured over the *yerba mate* multiple times to make a partial infusion. The process of adding water is repeated multiple times using about a liter and half of water (Bracesco, Sanchez, Contreras, Menini, & Gugliucci, 2010; Heck & Mejia, 2007; Meinhart et al., 2010), as demonstrated in Fig. 1.

Many studies have demonstrated that the consumption of *yerba mate* extracts protect against free radical reactivity. This antioxidant activity is primarily attributed to the polyphenol compounds in the extract, which delocalize electrons and form intramolecular hydrogen bonds. However, compounds in *yerba mate* extracts also inhibit chain reactions and repair lesions caused by reactive species (Bastos et al., 2007). Therefore, Schinella, Troiani, Dávila, Buschiazzo, and Tournier (2000) have suggested that the ingestion of mate is an effective and economic way to increase a person's antioxidant defenses.

The phytochemical composition of plants is fundamental in providing antioxidant protection. Previous studies have indicated that many factors affect the characteristics and composition of the final marketed product, including the industrial processing (Esmelindro, Girardi, Mossi, Jacques, & Dariva, 2004) and the conditions under which the plant was cultivated (Cardozo et al., 2007). For example, excessive sunlight can lead to increased polyphenol production (Cardozo et al., 2007; Streit et al., 2007).

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In the current study, we hypothesized that sequential mate extractions that occur during the mate consumption influence the concentration of the substances extracted, and consequently, extract antioxidant and chelating properties.

Using extracts obtained mimicking a legitimate one, we quantified the Total Polyphenol Content (TPC), theobromine, chlorogenic acid, caffeine and caffeic acid concentrations, and analyzed the antioxidant and chelating properties of the extracts. To establish the effect of TPC concentration on extract chelating and scavenger ability, we performed correlation analyses.

2. Materials and methods

Yerba mate samples were purchased in supermarkets. The samples included brands from Argentina, Brazil and Uruguay. The "traditional" presentation form was the preferred choice of *yerba mate*.

Argentinian brands were grown in the northeastern region in the Missiones province. The Brazilian brands were from the northwest and central east region of Rio Grande do Sul. There are no *yerba mate* plantations in Uruguay. The raw material was from Brazil, and in this case, the *yerba mate* was grown in the central east region of Rio Grande do Sul (information from the product package).

To guarantee that all of the herbs had the same characteristics, three packages of each brand were purchased from the same lot, and analysis was performed on all three packages. The traceability of the herbs is ensured by strict legislation and rules that regulate the production of herbs in these three countries.

The herbs were named **Ar 1**, **Ar 2** and, **Ar 3** for the Argentinean brands, **Br 1**, **Br 2**, and **Br 3** for the Brazilian brands and **Uy 1**, **Uy 2**, and **Uy 3** for the Uruguayan brands.

2.1. Obtaining the extracts

Aqueous extracts were obtained by recreating the mate preparation process. Mate was prepared in a medium size gourd, with the *yerba mate* occupying two thirds of the bowl volume (85 g). The free volume was filled with water (70 ml) at 80 °C. The water was remained in contact with the *yerba mate* for one minute. The water was then removed through a pump attached to a suction system (described below). Extracts were successively collected, and infusions numbers 1, 2, 5, 10, and, 15 were stored for analysis. The extraction time was one minute. All other extractions were discarded.

The mate pump was attached to a Kitasato flask by a rubber hose. The bottle cap allowed such a connection because it was a silicone stopper with a hole with a glass tube passing through it. The extract passed through the system and collected into the Kitasato flask, which was connected to a vacuum pump that provided the suction (based on the method described by Meinhart et al., 2010). The extracts were then filtered using filter paper, thickness 205 μm (J.Prolab®, S.J.dos Pinhais, Brazil), and stored in Eppendorf tubes. After extraction, the material was stored and kept in a freezer (−18 °C) until analysis. Analysis of all extracts was performed in triplicate. Fig. 2 shows the suction system that was used.

2.2. Total polyphenol content (TPC) quantification

The total polyphenol in *yerba mate* extracts was determined by UV–visible spectrophotometry using the Folin-Cioacalteau method with modifications (Singleton, Joseph, & Rossi, 1965). TPC was expressed in mg of Gallic acid equivalents (GAE) per ml of aqueous extract, which was analyzed using 0.05 ml extract. Mean and standard deviation (n = 3) were calculated.

2.3. Methylxanthine and phenolic compound quantification

2.3.1. Liquid chromatography apparatus

LC analysis was performed in a Prominence Liquid Chromatography Shimadzu® equipped with an LC-20AT pump, a SIL-20A auto sampler, an SPD-20AT PDA detector, and a CTO-20A column oven (Kyoto, Japan). LC Solution V. 1.24 SP1 system software was used to control the equipment and to obtain data and responses from the LC system.

2.3.2. Chromatographic conditions

LC analysis was conducted using a reverse phase technique. Analyses of the *yerba mate* extracts and theobromine, chlorogenic acid, caffeic acid and caffeine standards were performed in a linear gradient elution mode with a flow rate of 1.0 ml/min, using a mobile phase consisting of (A) water with 0.3% acetic acid and (B) methanol. The run was conducted using the following gradient: 15–20% B for 20 min; 20–85% B for 5 min; 85% B for 5 min (Filip, Lopez, Giberti, Coussio, & Ferraro, 2001). Detection by the PDA system was monitored at 265 nm for caffeine and theobromine and at 325 nm for caffeic and chlorogenic acids. The mobile phase was prepared daily, filtered through a 0.45-μm membrane filter from Millipore (Milford®, MA, USA) and sonicated before use. Measurements were performed on an ODS-Hypersil Thermo Scientific C18 column (250 × 4.6 mm i.d., 5-μm particle size) (Bellefonte, United States). The HPLC system was operated at 25 ± 1 °C. The injection volume was 20 μL.

The reference standards were prepared as 50% hydroethanolic solvents at a final concentration of 50 μg/ml. All solutions, including mates, were filtered through 0.45 mm nylon filters and injected into the HPLC. All samples were run in triplicate. The standards were purchased from Sigma–Aldrich® (St. Louis, MO, USA).

2.4. Determination of extract antioxidant and iron chelating properties

The antioxidant capacity of extracts was measured by the following *in vitro* assays:

2.4.1. Nitric oxide (NO) scavenging assay

NO scavenging was assessed by incubating sodium nitroprusside (SNP) (1 mM) with 0.02 mL extracts at room temperature. After 120 min, 0.25 mL of the incubation solution was sampled and mixed with 0.25 mL Griess reagent. The absorbance was measured at 550 nm.

The values were compared to controls. The percentage of each extract's inhibition of the nitrite reaction with Griess reagent was determined as an index of its NO scavenger activity (Green et al., 1981; Puntel et al., 2008).

To ensure that the extract color would not interfere in the results, color controls were performed for all extracts. A total of 0.25 mL incubation solution was mixed with 0.25 mL Milli-Q water. Tests were performed three times for each brand, and values were expressed as a percentage of the control.

2.4.2. Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical scavenging activity was measured using a method described by Brand-Williams, Cuvelier, and Berset (1995) with minor modifications. A decrease in absorbance was monitored at 517 nm for 30 min using visible spectrophotometry. Tests were performed in triplicate. Ascorbic acid was used as a positive control to determine the maximum decrease in DPPH absorbance.

The values were expressed in the percentage of DPPH absorbance inhibition in relation to control values without extracts (maximal ascorbic acid inhibition was considered 100% inhibition). Calculations were performed using the following formula: