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Comparison of Caffeine Content in Coffee and Yerba Mate Prepared According to Traditional Consumption Methods in Argentina

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Highlights

- Caffeine content was quantified in coffee, yerba mate, and mate cocido prepared according to traditional Argentine consumption methods.
- Espresso-based preparations showed the highest caffeine concentration among coffee beverages.
- Yerba mate without stems showed more sustained caffeine extraction across successive servings.
- HPLC-DAD provided a reliable approach to compare caffeine exposure across commonly consumed infusions.
- The findings are relevant to dietary caffeine intake, sleep, and sports performance.

Abstract

The amount of caffeine provided by a beverage depends not only on its source but also on the preparation method and the amount consumed. In Argentina, coffee and yerba mate are habitual sources of caffeine, yet their actual contribution according to preparation method has not been clearly established. This study quantified and compared the caffeine content of beverages commonly consumed in the country, including espresso and filter coffee, instant coffee, mate cocido, and yerba mate with and without stems across 15 consecutive servings under conditions simulating habitual consumption. Caffeine was determined by high-performance liquid chromatography with diode-array detection (HPLC-DAD). The highest caffeine concentrations were observed in beverages prepared with an espresso machine (225.0–533.9 mg/100 mL), followed by filter coffee (60.2–119.0 mg/100 mL), instant coffee (39.9–60.2 mg/100 mL), and mate cocido (17.3–18.4 mg/100 mL). In yerba mate, total caffeine extraction across 15 servings was 243 mg for samples with stems and 273 mg for stem-free samples. The first four servings provided an amount of caffeine comparable to that of a single espresso. Several preparations exceeded 200 mg per intake, whereas two cups of mate cocido could reach the suggested limit for the pediatric population. These findings show that the preparation method substantially modifies caffeine exposure.

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Keywords: caffeine; coffee; yerba mate; mate cocido; HPLC-DAD; beverage composition; dietary exposure; ergogenic aid

1. Objective

To quantify and compare the caffeine concentration and caffeine dose delivered by coffee, yerba mate, and mate cocido infusions prepared according to traditional consumption methods in Argentina, and to contextualize these values in relation to external dietary safety and sports-nutrition reference intakes.

2. Introduction

Caffeine (1,3,7-trimethylxanthine) is a psychoactive alkaloid naturally present in plants such as coffee, tea, cocoa, guarana, and yerba mate [1,2]. Synthetic caffeine can also be added to beverages and supplements with the aim of promoting stimulation, alertness, energy, and an elevated mood [3]. It is estimated that approximately 80% of the world population consumes some products containing caffeine on a daily basis [4]. Habitual sources of caffeine include coffee, tea, yerba mate, and cola-based soft drinks [2]. At room temperature (25 °C), its solubility in water is approximately 2 g/100 mL. This capacity increases exponentially as the thermal energy of the system rises; at 80 °C, solubility increases to approximately 18 g/100 mL and reaches 66 g/100 mL at the boiling point (100 °C) [5]. From the standpoint of chemical stability, caffeine is a notably robust compound. It possesses a purine ring that gives it strong resistance to thermal degradation during normal infusion and roasting processes. This stability ensures that the amount of caffeine extracted from coffee beans or yerba mate leaves remains unchanged in the final beverage, even when the beverage is held at high temperatures for extended periods [6].

Current dietary consumption patterns, accompanied by stress and certain social habits, may lead to high caffeine consumption, which could trigger adverse effects such as anxiety, irritability, and palpitations. In the long term and under certain conditions, it may also lead to dependence and tolerance [7].

The European Food Safety Authority (EFSA) [8] indicates that, in healthy adults, single doses of up to 200 mg of caffeine (3 mg/kg) do not pose a risk to health. In addition, Health Canada [9] and the FDA [10] consider total daily intakes of up to 400 mg to be safe, although single doses of 100 mg (1.4 mg/kg) may alter sleep duration and quality if consumed close to bedtime. During pregnancy and lactation, the limit considered safe by the EFSA is 200 mg per day, although the Canadian government allows up to 300 mg [9]. In children and adolescents, the maximum limit according to the EFSA [8] is 3 mg/kg, as in adults, due to comparable pharmacological responses, whereas Health Canada [9] proposes a limit of 2.5 mg/kg and warns that the available data supporting this value are still insufficient.

Regarding the use of this alkaloid in the sports context, in 2004 the International Olympic Committee (IOC) and the World Anti-Doping Agency (WADA) allowed the use of caffeine in competition by removing the substance from the Prohibited List, although it remains included in WADA's Monitoring Program [11,12]. It is currently considered that this type of supplement can improve sports performance in endurance and team sports, as well as in strength and power sports [13,14]. According to the Australian Institute of Sport (AIS) and the International Society of Sports Nutrition (ISSN), a minimum dose of 2 mg/kg of body weight can be effective for improving sports performance [15,16]. Despite this, in several clinical and meta-analytic studies, caffeine is considered ergogenic (potentially performance-enhancing) at doses between 3 mg/kg and 6 mg/kg of body weight [13,15,16].

Very high doses of caffeine (9 mg/kg of body weight) have been associated with a high incidence of side effects and do not appear to be necessary to produce an ergogenic effect [16].

This study presents the analysis and evaluation of caffeine consumption in different infusions as they are prepared and consumed in Argentina, replicating, as closely as possible, habitual preparation and consumption practices. Considering recommended maximum intakes as well as doses related to improved sports performance, there is a need to know caffeine concentration in different infusions such as mate and coffee and the caffeine intake represented by each type of preparation.

2.1. Coffee-Based Infusions

In recent years, coffee has gained popularity, especially in major Argentine cities such as Buenos Aires, Córdoba, and Rosario, because of the growing supply of specialty coffee shops and the diversification of home preparation methods. Nevertheless, annual coffee consumption in Argentina is low (1.4 kg per capita) compared with other countries in the region, such as Brazil and Colombia, where consumption reaches 4.8 kg and 2.8 kg per capita, respectively [17,18].

Different coffee infusions are mainly prepared from beans of two plant species: Arabica and Canephora, the latter being better known by the name of one of its varieties, Robusta.

Arabica coffee contains less caffeine, is more delicate, requires growth at higher altitudes, and has the potential to achieve great organoleptic qualities in the cup. In contrast, Robusta coffee contains a higher caffeine content and is more resistant to pests but has more limited flavor and aroma profiles. Consequently, it is often used in blends with Arabica beans. These blends are common in more economical coffee presentations such as torrado or instant coffees.

In addition to coffee varieties (Arabica or Robusta) and the way the product is commercially acquired (beans, ground, torrado, or instant), the way each infusion is prepared affects the degree of caffeine extraction and its final concentration in the beverage. This means that aspects such as water temperature and pressure, contact time, and grind size are considered key factors during preparation [19,20].

Commercially, Arabica coffee is most commonly available as whole beans to be ground and brewed. Ground coffee can also be purchased ready for infusion, with a medium grind size, which makes it more versatile for different traditional preparation methods (espresso or drip coffee maker). Finally, instant coffee can be purchased in powder or granulated form and is prepared by adding water above 80 °C without the need to use a filter.

These three types of commercial presentations of solid coffee can be found in decaffeinated form (less than 0.1% caffeine), and regardless of this, they may be purchased as torrado, a process in which the bean is roasted with the addition of sugar, or as non-torrado, roasted without any added substances. Torrado coffee is usually applied to beans of low organoleptic quality to conceal defects or undesirable characteristics.

2.2. Yerba Mate-Based Infusions

Yerba mate (*Ilex paraguariensis*) is a plant traditionally used in South America and widely consumed in countries such as Argentina, Brazil, Paraguay, and Uruguay. In Argentina, mate occupies a central place in culture and everyday life as reflected in its legal recognition: two laws declare mate the national infusion and establish the "National Mate Day", respectively [21,22]. According to official data, yerba mate consumption in the country reaches 6.2 kg per inhabitant per year, and it is present in more than 90% of households [23].

Yerba mate is consumed in various ways, with the hot infusion (also known as "mate") and mate cocido being the most representative. Mate is prepared by placing between 25 and 50 g of yerba in a container (also called "mate"), to which hot water is added (generally between 70 °C and 80 °C), and it is consumed through a metal straw. This process is repeated several times with the same portion of yerba mate, and each repetition is called a "cebada" (serving of hot water). As this process progresses, a depletion effect known as "mate lavado" (washing out) becomes evident, in which each serving loses flavor and color. Mate cocido, in turn, is obtained by infusing the yerba, which may be contained in tea bags or infused directly with boiled water and then filtered, in a manner similar to other infusions such as tea.

According to its composition, yerba mate can be classified mainly into two variants. On the one hand, yerba mate with stems contains at least 65% dried leaves and no more than 35% stems and splinters, which contributes to a milder and more balanced flavor in the infusion. On the other hand, despalada, or stem-free yerba mate, contains a minimum of 90% leaves, limiting stem content to a maximum of 10%, thus generating a beverage with a more intense and longer-lasting flavor [24,25].

Given that several studies have shown that yerba mate consumption can modulate oxidative, inflammatory, and metabolic parameters linked to physical performance [26–30], its use in the athletic population has attracted growing interest. In this context, precisely determining the amount of caffeine present in a real portion of infusion becomes especially relevant for understanding the ergogenic potential of this food.

In Argentina, mate cocido is among the caffeinated beverages consumed by children and adolescents [31–33]. Depending on the type of yerba and the preparation method, the caffeine content in each cup of mate cocido may vary between 13 and 55 mg [34,35]. Determining the concentrations of this compound is relevant from a nutritional and toxicological point of view for the most sensitive populations.

2.3. General Procedure

This work evaluated the final caffeine concentration in different infusions prepared from different coffee presentations (beans, ground, and instant), considering the most common commercial varieties (non-torrado, torrado, 100% Arabica, and decaffeinated) and the traditional or recommended preparation methods for each case (espresso, lungo, ristretto, or filter coffee maker).

In parallel, the caffeine concentration during a typical mate-serving process was studied by comparing the two most consumed yerba varieties (with and without stems). Finally, the caffeine content in the infusion known as mate cocido was evaluated.

The caffeine contributions of each beverage were compared considering the typical serving volume for each preparation.

3. Materials and Methods

3.1. Selection of Coffee, Yerba Mate, and Mate Cocido Samples

For the coffee study, different brands and presentations were selected based on availability in local shops. In addition, two brands of mate cocido tea bags and the same yerba mate brand in two presentations to be compared (with and without stems) were selected. The aim was to select widely consumed brands that represented the consumption habits of the Argentine population. Table 1 shows the presentations used in the study, their preparation method, and the description on the label. Caffeine quantification was performed on the solid coffee, yerba mate, and mate cocido samples as commercially acquired, as well as on the infusions prepared from them.

Table 1. Presentations used in the study, brand distinction, preparation method, and label description.

Presentation	Brand	Preparation Method	Label Description
Coffee beans, non-torrado	Brand 1	Espresso machine (Espresso, ristretto, and lungo)	Roasted coffee—beans—100% Arabica—Colombia
Coffee beans, non-torrado	Brand 1	Filter coffee maker	
Coffee beans, non-torrado	Brand 2	Espresso machine (Espresso, ristretto, and lungo)	Roasted coffee—beans—100% Arabica—Brazil and Colombia
Coffee beans, non-torrado	Brand 2	Filter coffee maker	
Ground coffee, non-torrado	Brand 1	Filter coffee maker	Roasted coffee—ground—100% Arabica—Colombia
Ground coffee, non-torrado	Brand 2	Filter coffee maker	Roasted coffee—ground—made in Brazil
Ground torrado coffee	Brand 1	Filter coffee maker	Sugar-roasted coffee—ground—Uruguayan industry
Ground torrado coffee	Brand 2	Filter coffee maker	Coffee roasted with white sugar—ground—Arabica and Robusta blend—Argentine industry
Instant coffee, non-torrado	Brand 1	Dissolution in hot water	Roasted coffee—instant—Arabica and Robusta blend—Brazil
Instant coffee, non-torrado	Brand 2	Dissolution in hot water	Roasted coffee—instant
Instant torrado coffee	Brand 1	Dissolution in hot water	Sugar-roasted coffee—instant—Brazil
Instant torrado coffee	Brand 2	Dissolution in hot water	Sugar-roasted coffee—instant—Argentine industry
Instant Arabica coffee	Brand 1	Dissolution in hot water	Roasted coffee—freeze-dried instant—Arabica—Colombia, Brazil, Honduras, Vietnam and Indonesia
Instant Arabica coffee	Brand 2	Dissolution in hot water	Roasted coffee—granulated instant—100% Arabica—Argentine industry
Instant decaffeinated coffee	Brand 1	Dissolution in hot water	Roasted decaffeinated coffee—Instant—Arabica and Robusta blend—Colombia, Brazil, Honduras, Vietnam and Indonesia
Instant decaffeinated coffee	Brand 2	Dissolution in hot water	Roasted decaffeinated coffee—freeze-dried instant—Colombia
Yerba mate with stems	Brand 1	Consecutive servings	Yerba mate with stems—low dust content—*Ilex paraguariensis*—Corrientes
Yerba mate without stems	Brand 1	Consecutive servings	Stem-free yerba mate—low dust content—*Ilex paraguariensis*—Corrientes
Mate cocido tea bags	Brand 1	Infusion	Mate cocido in tea bag—Yerba mate—Corrientes
Mate cocido tea bags	Brand 2	Infusion	Mate cocido in tea bag—Yerba mate—Corrientes

3.2. Infusion Preparation Methods

For preparation with the espresso machine, a professional La Valente Athina 2-group machine (La Valente, Avellaneda, Buenos Aires, Argentina) was used (Figure 1). To grind the coffee beans, a La Valente M20 grinder (La Valente, Avellaneda, Buenos Aires, Argentina) was used (Figure 1). Preparation parameters were calibrated through sensory evaluation by a certified Q Grader, who adjusted variables such as grind size, coffee dose, and extraction time to achieve a balanced beverage. Using this preparation method, three coffee versions were prepared: espresso, ristretto, and lungo. These differ from one another in the coffee-to-water ratio and the contact time between coffee and water. In the double portafilter, a dose of 18 g of

coffee was used, and a ratio of 2.5 was applied for espresso coffee (18 g of coffee to obtain 45 g of beverage), 1.4 for ristretto coffee (18 g of coffee to obtain 25 g of final beverage), and 5 for lungo coffee (18 g of coffee to obtain 90 g of final beverage).



Figure 1. Representative equipment used for espresso-based coffee preparation. (**Left**) Professional espresso machine used to prepare espresso, ristretto, and lungo coffee infusions. (**Right**) Electric coffee grinder used to grind whole coffee beans before espresso extraction. AI-generated illustrative images created using OpenAI ChatGPT Images 2.0 and 2.5. The authors reviewed and edited the generated images; no third-party copyrighted material was reproduced or adapted.

For preparation with the electric filter coffee maker, an Ultracomb Vienna coffee maker (First Rate S.A., Buenos Aires, Argentina), an Ultracomb Vienna coffee maker and a 1Zpresso Q2S manual grinder (1Zpresso Co., Ltd., New Taipei City, Taiwan) were used (Figure 2). The preparation ratio was 16, using 35.6 g of coffee and 570 g of filtered water. It is important to note that, in preparation with the espresso machine, the ratio relates the weight of the final beverage to the weight of coffee used, whereas in the electric filter coffee maker it represents the relationship between the weight of water added and the weight of coffee used.



Figure 2. Representative equipment used for filter coffee preparation. (**Left**) Domestic electric filter coffee maker used for drip coffee extraction. (**Right**) Manual coffee grinder used to grind whole coffee beans before filter coffee preparation. AI-generated illustrative images created using OpenAI ChatGPT Images 2.0. The authors reviewed and edited the generated images; no third-party copyrighted material was reproduced or adapted.

Preparation of instant coffee: in all cases, 1.5 g of instant coffee was weighed and dissolved in 100 g of water at 100 °C. This ratio was the one most commonly suggested in the instructions on the product labels.

Preparation of the mate cocido samples was carried out following the manufacturers' instructions: each tea bag, containing 3 g of mate cocido, was infused in 230 g of water at 100 °C for 5 min. Once the contact time had elapsed, the bags were squeezed once and removed.

For caffeine determination during the cebada process, a device was specifically designed to simulate traditional mate preparation and consumption (Figure 3). The system

consisted of a gourd mate with a stainless-steel straw directly connected to a Kitasato flask coupled to a vacuum pump. Then, 30 g of yerba mate was weighed and placed in the gourd, a quantity selected to reflect typical consumption practices. Water was heated to 80 °C and transferred to an insulated thermos in order to maintain a constant temperature throughout the trial. The temperature was checked at the beginning and at the end of the experiment to confirm its preservation. Fifteen consecutive servings were carried out: 50 mL of water was used in the first one, whereas 40 mL was used in the following ones. This procedure was carried out identically for the two types of yerba mate samples, with and without stems. A larger volume was used in the first serving due to the initial absorption of the dry yerba. In each serving, the water–yerba contact time was standardized to 20 s. After this time, the vacuum pump was activated to draw the liquid through the straw, simulating the suction exerted by a person during the consumption of this beverage. The extract obtained in each serving was collected in the Kitasato flask and then transferred to a graduated cylinder to record its volume. This procedure was repeated identically for all 15 servings.



Figure 3. Experimental device to simulate the serving of each mate and its subsequent collection. From left to right: glass graduated cylinder for volume measurement, timer to record contact time, vacuum pump to simulate suction, Kitasato flask for collection of each serving, mate with straw containing the yerba, and adiabatic thermos to maintain water temperature.

3.3. Determination of Caffeine by HPLC-DAD and Measurement of Solids

Caffeine was quantified in solid samples and prepared infusions (except yerba mate servings) according to ISO 10727:2002 [36]. This procedure consisted of an aqueous extraction at 100 °C for 1 h with the addition of magnesium oxide to prevent acidification of the extract and possible degradation of caffeine. The extract obtained was brought to volume and then filtered for subsequent analysis by HPLC with a diode-array detector (DAD). Hereafter, the ISO method refers to caffeine determination using this extraction procedure.

To simplify the analysis of the simulated servings obtained from yerba mate, these were analyzed directly by HPLC without carrying out the prior extraction indicated in ISO 10727:2002 [36]. The percentage recovery of this simplified method was evaluated, yielding a recovery of 85.9%, which was considered sufficient to correct each measurement made using this method. Hereafter, the simplified method refers to this procedure.

All analytical determinations were performed using samples at room temperature. The HPLC system used in this study consisted of a Shimadzu LC-20 series instrument with an SPD-M20A photodiode array detector (Shimadzu Corporation, Kyoto, Japan) configured

at 272 nm. Chromatographic separation was achieved using a Kromasil 100-5-C18 column (250 × 4.6 mm, 5 µm; AkzoNobel, Arlöv, Sweden). Column temperature was maintained at 40 °C. Elution flow was set at 1.0 mL/min, injection volume was 10 µL, and caffeine elution time was 4.4 min. The mobile phase consisted of a mixture of HPLC-grade acetonitrile and ultrapure water in a proportion of 20:80 (*v*/*v*). Quantification was carried out using a caffeine calibration curve in a range from 5 µg/mL to 500 µg/mL. These standards were prepared from solid caffeine material (CAS No. 58-08-2; Sigma-Aldrich, St. Louis, MO, USA) through serial dilution to working concentrations. Ethanol:water (1:3 *v*/*v*) was used as a solvent. Quantification was performed by comparing the sample peak area against the nearest point on the calibration curve.

The linearity of the caffeine calibration curve was evaluated, obtaining an R^2 value of 0.99998. The limit of detection (LOD) and limit of quantification (LOQ) were established based on the signal-to-noise ratio, with values of 0.09 mg/100 mL and 0.30 mg/100 mL, respectively. Method precision was evaluated through repeatability and intermediate precision, obtaining relative standard deviations (%RSD) of 0.74% and 0.76%, respectively.

To express caffeine content on both a mass and volume basis, the density of all infusions and yerba mate servings obtained during the study was measured at room temperature (20 °C).

The percentage of solids in each prepared coffee infusion was determined using a DiFluid R2 refractometer (Shenzhen Digitizing Fluid Technology Co., Ltd., Shenzhen, China). In the case of mate cocido infusions and simulated yerba mate servings, an ATAGO N1 handheld refractometer (ATAGO Co., Ltd., Tokyo, Japan) was used. Measurements were carried out in all cases at 25 °C.

4. Results

The results of the duplicate measurements by the ISO method for the solid coffee, mate cocido, and yerba mate samples are shown in Table 2.

Table 2. Mean result of caffeine concentration in the dry, non-infused coffee, yerba mate, and mate cocido samples, expressed as mg of caffeine/100 g of sample. Measurement performed in duplicate using the ISO method. The error of the result was calculated considering the standard deviation of the duplicate measurement.

Type of Sample	Sample	Caffeine Content in the Non-Infused Sample (mg/100 g)
Coffee beans	Coffee beans, non-torrado Brand 1	1229 ± 3
	Coffee beans, non-torrado Brand 2	1298 ± 0
Ground coffee	Ground coffee, non-torrado Brand 1	1250 ± 2
	Ground coffee, non-torrado Brand 2	1907 ± 0
	Ground torrado coffee Brand 1	1890 ± 23
	Ground torrado coffee Brand 2	1319 ± 5
Instant Coffee	Instant coffee, non-torrado Brand 1	3404 ± 76
	Instant coffee, non-torrado Brand 2	3019 ± 22
	Instant torrado coffee Brand 1	2429 ± 36
	Instant torrado coffee Brand 2	2504 ± 7
	Instant Arabica coffee Brand 1	2878 ± 23
	Instant Arabica coffee Brand 2	3753 ± 21
	Instant decaffeinated coffee Brand 1	101 ± 1
	Instant decaffeinated coffee Brand 2	160 ± 2

Table 2. *Cont.*

Type of Sample	Sample	Caffeine Content in the Non-Infused Sample (mg/100 g)
Yerba mate	Yerba mate with stems	1239 ± 30
	Yerba mate without stems	1318 ± 6
Mate cocido	Mate cocido tea bags Brand 1	1674 ± 12
	Mate cocido tea bags Brand 2	1455 ± 11

The caffeine concentration, expressed in mg/100 mL, obtained by the ISO method for each prepared coffee and mate cocido infusion is presented in Table 3. In this case, the determination for each sample was performed in triplicate.

Table 3. Caffeine concentration in the different prepared coffee and mate cocido infusions, expressed as mg/100 mL. Caffeine content is the mean of the assay performed in triplicate using the ISO method. The error of the result was calculated considering the standard deviation of the triplicate measurements.

Preparation Method	Sample	Caffeine Content in the Infusion (mg/100 mL)
Espresso machine	Coffee beans, non-torrado Brand 1—Espresso preparation	381.4 ± 18.4
Espresso machine	Coffee beans, non-torrado Brand 2—Espresso preparation	366.7 ± 20.2
Espresso machine	Coffee beans, non-torrado Brand 1—Ristretto preparation	533.9 ± 1.0
Espresso machine	Coffee beans, non-torrado Brand 2—Ristretto preparation	488.0 ± 2.0
Espresso machine	Coffee beans, non-torrado Brand 1—Lungo preparation	235.5 ± 0.6
Espresso machine	Coffee beans, non-torrado Brand 2—Lungo preparation	225.0 ± 1.1
Filter coffee maker	Coffee beans, non-torrado Brand 1	79.1 ± 0.6
Filter coffee maker	Coffee beans, non-torrado Brand 2	87.9 ± 0.1
Filter coffee maker	Ground coffee, non-torrado Brand 1	79.0 ± 0.2
Filter coffee maker	Ground coffee, non-torrado Brand 2	119.0 ± 0.3
Filter coffee maker	Ground torrado coffee Brand 1	60.2 ± 0.7
Filter coffee maker	Ground torrado coffee Brand 2	79.2 ± 0.3
Dissolution	Instant coffee, non-torrado Brand 1	55.9 ± 0.5
Dissolution	Instant coffee, non-torrado Brand 2	48.1 ± 0.2
Dissolution	Instant torrado coffee Brand 1	40.3 ± 1.0
Dissolution	Instant torrado coffee Brand 2	39.9 ± 0.3
Dissolution	Instant Arabica coffee Brand 1	45.6 ± 0.1
Dissolution	Instant Arabica coffee Brand 2	60.2 ± 0.1
Dissolution	Instant decaffeinated coffee Brand 1	1.75 ± 0.1
Dissolution	Instant decaffeinated coffee Brand 2	2.60 ± 0.1
Infusion	Mate cocido tea bags Brand 1	18.4 ± 0.0
Infusion	Mate cocido tea bags Brand 2	17.3 ± 0.1

Caffeine concentration in the servings obtained from yerba mate with and without stems was measured in triplicate using the simplified method. Table 4 presents the mean results obtained for each serving, ordered according to the sequence in which they were obtained.

Table 4. Caffeine concentration in servings obtained through the simulation of yerba mate consumption with and without stems, expressed as mg/100 mL. Caffeine content is the mean of the assay performed in triplicate using the simplified method.

	Yerba with Stems		Yerba Without Stems	
Serving No.	**Serving Volume (mL)**	**Caffeine Content (mg/100 mL)**	**Serving Volume (mL)**	**Caffeine Content of Yerba Without Stems (mg/100 mL)**
1	22	67.3 ± 0.5	25	67.6 ± 0.1
2	29	69.6 ± 0.2	32	70.6 ± 0.3
3	34	65.8 ± 0.1	32	69.4 ± 0.5
4	37	65.3 ± 0.0	36	65.6 ± 0.5
5	38	60.2 ± 0.1	38	62.7 ± 0.5
6	38	49.9 ± 0.1	38	58.3 ± 0.2
7	38	44.9 ± 0.1	38	56.5 ± 0.1
8	38	42.7 ± 0.1	38	50.0 ± 0.1
9	38	40.7 ± 0.3	38	45.0 ± 0.0
10	39	36.9 ± 0.1	39	43.1 ± 0.2
11	38	35.7 ± 0.0	38	41.4 ± 0.2
12	39	29.9 ± 0.0	38	36.6 ± 0.1
13	40	29.3 ± 0.1	39	35.7 ± 0.6
14	40	27.1 ± 0.1	38	32.6 ± 0.0
15	39	23.3 ± 0.1	38	30.0 ± 0.3

Considering the volume of each serving as well as its concentration, it is possible to determine the absolute contribution of each one. Figure 4 shows these results during the progression of the serving simulation.

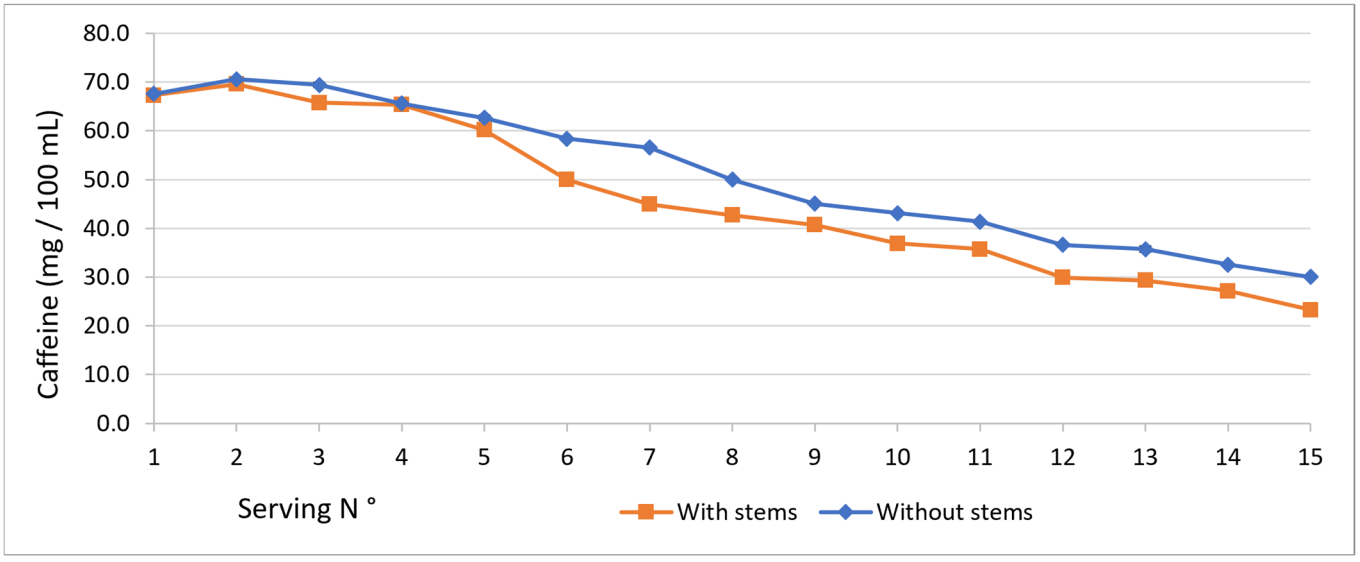


Figure 4. Caffeine concentration in mg/100 mL in each serving obtained during the simulation of mate preparation using yerba mate with and without stems.

If we consider the habitually consumed portion of each beverage and the caffeine concentration experimentally obtained in this work, the caffeine dose provided by that consumed volume can be calculated. For example, in the case of coffee prepared with an espresso machine, a portion of single espresso (21.35 mL) and double espresso (42.7 mL), single ristretto (11.85 mL) and double ristretto (23.7 mL), and single lungo (45.35 mL) and double lungo (90.7 mL) were considered. In the case of coffee prepared with a filter coffee maker, instant coffee, and mate cocido, a habitual portion of 250 mL was considered. In the simulation of yerba mate servings, the portion considered was the total volume obtained in each case. The comparative results are reflected in Figure 5, where the 100 mg and 200 mg doses were also incorporated as references, representing, respectively, a dose that could affect sleep quality if consumed close to bedtime and a dose considered safe in single administrations for adults according to the EFSA [8]. The value represented in each bar is the mean of the results between Brand 1 and Brand 2 for each case studied, except for the beverages prepared from coffee acquired in the ground non-torrado presentation. In this case, the doses delivered by the beverages prepared with Brand 1 and Brand 2 were represented individually because not only was a 41.6% difference observed between the two infusions, but also only the Brand 1 package declared the variety with which it was prepared (100% Arabica). The bars were ordered in descending order from the beverage that provides the highest caffeine dose (filter coffee, non-torrado, Brand 2) to the lowest (decaffeinated instant coffee).

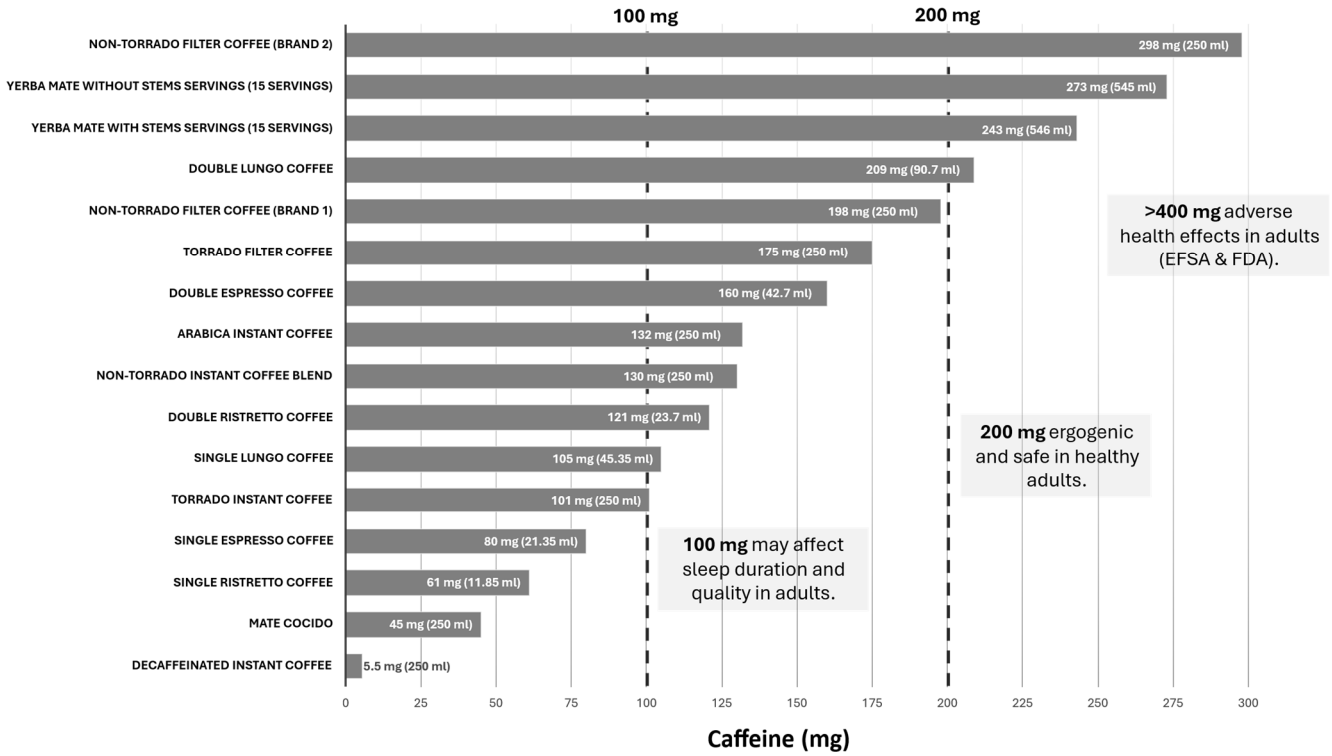


Figure 5. Comparison of the caffeine dose (mg) provided by each beverage as a function of its portion size (mL).

To evaluate the extraction yield in each type of preparation, the caffeine content in the total volume produced for each type of beverage was considered with respect to the caffeine content present in the portion of coffee, mate cocido, or yerba mate used for that preparation. The results obtained are reflected in Table 5.

Table 5. Mean caffeine extraction yields according to prepared beverage. It is important to consider that the ratio used for preparations with the espresso machine is not directly comparable with the ratio of preparation with the filter coffee maker.

Prepared Beverage	Dose (g)	Ratio	Time (s)	Extractable Caffeine (mg)	Extracted Caffeine (mg)	Extracted Caffeine (%)
Double ristretto (mean—Brand 1)	18	1:1.3	11.8	221.2	129.1	58
Double espresso (mean—Brand 1)	18	1:2.3	21.2	221.2	164.7	74
Double lungo (mean—Brand 1)	18	1:5	38.8	221.1	208.6	94
Ground filter coffee (mean preparation with coffee beans Brands 1 and 2)	35.8	1:16	360–720	452.5	409.2	91
Yerba mate without stems (sum of 15 servings)	30	-	300 (20 per serving)	396.6	273.1	69
Yerba mate with stems (sum of 15 servings)	30	-	300 (20 per serving)	371.8	242.9	65
Mate cocido (mean)	3	-	300	46.9	44.8	96

5. Discussion of Results

The final caffeine content in each beverage evaluated is related to its concentration in the coffee or yerba mate used to prepare it, as well as to extraction yield during the preparation process. Parameters during this process, such as water temperature and contact time with the samples, are critical for extracting the greatest number of soluble compounds, including caffeine. The increase in water temperature drastically accelerates the extraction kinetics of chemical compounds, since it increases molecular kinetic energy, improving diffusion and solubility while disrupting cellular structures to release solutes. As for contact time, several studies have shown that equivalent caffeine extraction concentrations can be reached under contrasting conditions of temperature and infusion duration. For example, Fuller and Rao [37] reported that preparation by the French press method with water at 98 °C for 6 min extracts a caffeine concentration comparable to that obtained by the cold brew method (cold extraction), using water at 25 °C for a period of 7 h. The contact surface between water and food particles also plays a key role because it exposes more molecules to interaction and increases the transfer rate of solutes to the aqueous phase. For this reason, the greater the degree of grinding of the solids, the more caffeine can be extracted, as well as the rest of the total dissolved solids in the final beverage [19,20]. These concepts are consistent with the results obtained during coffee preparation using the espresso machine, where shorter water-to-coffee contact times, as in the case of ristretto coffee preparation, showed a lower extraction yield (58%). On the other hand, at longer contact times, as in lungo coffee preparation, the extraction percentage reached 94% (Table 5). As expected, intermediate contact times, as in espresso coffee preparation, represented an intermediate yield (74%).

It should be emphasized that this preparation method differs substantially from the rest of the methods tested because, in addition to using water above 90 °C, it operates at 9 bars of pressure, which implies that caffeine is extracted at a pressure nine times higher than that used in the filter coffee maker method, resulting in high yields in considerably shorter times.

With respect to beverages prepared with the filter coffee maker, in addition to using commercially acquired ground coffee, the samples acquired as beans were also ground and used. The coffee-to-water ratio used in this type of preparation was 1:16, which represents between 3 and 12 times more water per gram of coffee than in espresso machine preparations.

This produces a dilution effect of caffeine in the final beverage, which is evidenced in the concentration range obtained (60.2–119.0 mg/100 mL). This range is considerably below that of beverages prepared with the espresso machine (225.0–533.9 mg/100 mL). Despite this difference between beverage types, it should be noted that the portion of filter-prepared coffee is 250 mL, whereas the portion of a double lungo is 90.7 mL. This implies that the caffeine dose consumed according to the type of coffee may be similar if this difference in the size of the portions habitually consumed is considered. By way of example, and considering Figure 5, it could be said that a similar amount of caffeine is incorporated by consuming a 250 mL cup of torrado coffee prepared with the filter coffee maker (175 mg) and by consuming a cup (42.7 mL) of double espresso coffee (160 mg). Another important difference in the results obtained is found between the caffeine provided by the infusion prepared with the filter coffee maker using ground non-torrado coffee Brand 1 (197.6 mg) and that prepared with ground non-torrado coffee Brand 2 (297.6 mg). Although it is the same coffee presentation, the product obtained with Brand 2 produced a beverage with 50% more caffeine. This suggests that this difference could be due, among other factors, to the coffee variety contained in each package. In the case of Brand 1, the package explicitly stated that it was a coffee made with 100% Arabica beans, whereas that information was not found on the Brand 2 package. From this analysis, it may be inferred that Brand 2 ground coffee was made with a blend of Arabica and Robusta bean varieties, the latter being responsible for considerably increasing the caffeine content of the product. Regarding beverages prepared with ground torrado coffee, the results were not proportional to the caffeine content in the solid products. This finding suggests that, although Brand 1 contained more caffeine than Brand 2 (1890 mg/100 g and 1319 mg/100 g, respectively), the beverage prepared from Brand 1 had a lower caffeine concentration than that prepared from Brand 2 (60.2 and 79.2 mg/100 mL, respectively). This difference could be due to the grind size of each product, assuming it is finer for Brand 2, which would imply smaller particles and a greater capacity for caffeine extraction [19,20]. This hypothesis would be consistent with the results for total solids measured in each beverage, which were 1.05 g/100 mL for the Brand 1 infusion and 2.17 g/100 mL for Brand 2.

If we analyze the results for caffeine concentration in the solid instant coffee samples, we find that the non-torrado presentations showed values of 3404 mg/100 g for Brand 1 and 3019 mg/100 g for Brand 2. If we compare these results with the evaluated instant torrado coffee brands, we see that the versions with added sugar contained 2429 mg/100 g for Brand 1 and 2504 mg/100 g for Brand 2. If we compare the average of each presentation, we can observe that instant torrado coffee has 23.2% less caffeine than the non-torrado alternative. If we consider that the Argentine Food Code (Art. 1166) [24] establishes a maximum added sugar level of 10%, this difference could be due in part to an amount of added sugar greater than that allowed or the use of a higher percentage of Robusta coffee in the composition of the non-torrado product. The caffeine values of the 100% Arabica instant coffee presentations were 2878 mg/100 g and 3753 mg/100 g. Comparatively, these values are high if one considers that the Arabica variety usually contains 50% less caffeine than the Robusta variety. These results could indicate that differences exist during the production of this type of instant coffee, relative to torrado and non-torrado alternatives, that could contribute to this variability in caffeine content. Decaffeinated instant coffee showed concentrations between 101 mg/100 g and 160 mg/100 g. The Argentine Food Code (Chapter XV—Art. 1174bis) [24] establishes that the maximum permitted for this type of decaffeinated product is 300 mg/100 g; therefore, the measured concentration is consistent with what would be expected.

If we analyze the results of beverages prepared with instant coffee, it is important to clarify that, in this case, it is not preparation by an extractive process but rather a solubilization in hot water of the totality of the powder used. For this reason, discussion

of yield will be omitted for this type of preparation, given that all the caffeine in the solid product is transferred to the final beverage. Without considering the samples prepared with decaffeinated instant coffee, all beverages obtained with the other instant varieties (non-torrado, torrado, and 100% Arabica) ranged from 39.9 mg/100 mL to 60.2 mg/100 mL. Comparatively, beverages prepared with the filter coffee maker showed higher caffeine concentrations, with 60.2 mg/100 mL and 119.0 mg/100 mL as the lower and upper limits, respectively. It is important to emphasize that, although solid instant coffee samples showed a higher caffeine content than ground coffee samples, the proportions used of each to prepare their respective beverages are responsible for the range of concentrations of instant beverages being below that of filter coffee beverages.

The mate cocido tea bag samples analyzed showed concentrations of 1674 mg/100 g for Brand 1 and 1455 mg/100 g for Brand 2. These caffeine content values were reflected in the beverages prepared from them, with contents of 18.4 mg/100 mL and 17.3 mg/100 mL, respectively. If one considers that each tea bag contained 3 g of mate cocido and the final volume of each prepared beverage, it can be calculated that the caffeine extraction yield was 91.6% for Brand 1 and 99.7% for Brand 2. This difference could be due to factors such as leaf size, number of stems, or dust content within each brand's tea bags. It is important to consider that, on average, in a traditional 250 mL portion of this beverage, 45 mg of caffeine would be consumed. Considering that its consumption is usually common among Argentine children from an early age, these values take on special importance in contexts in which the effects of caffeine on the health of children and adolescents are evaluated, particularly in relation to sleep, attention, and modulation of the central nervous system. It is important to bear in mind that daily caffeine doses considered free of harmful effects for adults (3 mg/kg body weight/day) can also be applied to children, since the rate at which children and adolescents process caffeine is at least the same as that of adults, and the available studies on the acute effects of caffeine on anxiety and behavior in children and adolescents support this level [8]. In fact, even lower doses (1.4 mg/kg) can increase sleep latency and reduce sleep duration in some children and adolescents, as well as in adults [8]. Therefore, for a child weighing 30 kg, two cups of mate cocido per day equal the maximum level of daily caffeine intake suggested by the EFSA [8] for that body weight (90 mg).

The caffeine obtained by the ISO method in the yerba mate without stems sample was 6.4% higher than in the sample with stems (1318 mg/100 g vs. 1239 mg/100 g). This difference could be related to the lower stem content and the higher proportion of leaves in the stem-free product, since caffeine is mainly found in the leaves [38,39]. This difference was also reflected in the total caffeine extracted during the serving simulation, with 273 mg obtained from stem-free yerba mate and 243 mg from yerba mate with stems. Under the conditions evaluated, the stem-free yerba mate sample yielded 12.3% more caffeine than the sample with stems. This difference could be associated not only with the initial caffeine content of each product but also with differences in extraction related to leaf size and the presence or absence of stems (Table 5). Considering the caffeine concentration in the progression of servings (Figure 4), it can be observed that the highest concentration is obtained in both cases in serving number 2. This could be because, in the first serving, the water used cools rapidly upon contact with the dry yerba, something that is usually perceived sensorially when consumed. From the peak in the second serving onward, a gradual decrease in caffeine concentration is observed in each subsequent serving. In the case in which yerba mate with stems is used, serving number 15 contained a concentration three times lower than serving number 2. When using stem-free yerba mate, this decrease is smaller, with the last serving being 2.3 times less concentrated than the second. Under the conditions of this simulation, the stem-free yerba mate sample maintained higher caffeine concentrations across successive servings.

It is important to highlight that, during the simulation with stem-free yerba, the first five servings accounted for 40% of the total mass of caffeine extracted. At the same point in the simulation with yerba with stems, this percentage reached 43%. This is particularly relevant given that mate is a social infusion in which the same mate and its successive cebadas are typically shared by several people. In fact, just the first four servings reach a caffeine contribution (85.3 mg) equivalent to a single espresso coffee (80 mg). This could be especially relevant in athletes who use mate consumption as an ergogenic aid but also share it as part of traditional social practice. According to the International Society of Sports Nutrition [16], the minimum effective doses of caffeine are still not clear, but they could be as low as 2 mg/kg of body mass. This would mean that, for a 70 kg athlete, the minimum intake for sports purposes could begin at 140 mg. These values are easily reached by consuming the first 7 mate servings under the conditions of the present study, representing an intake of between 235 mL and 240 mL of mate. Comparatively, reaching this caffeine value by consuming a double espresso (42.7 mL) represents an intake volume 5 times lower. When comparing the results of the present study with the data summarized by Torterolo et al. [40], relevant differences are observed both in the simulation design and in the kinetics of caffeine extraction. In the cited work, mate preparation is described from 50 g of yerba, with 100 mL water servings at 80 °C until completing 1000 mL of infusion and a higher water:yerba ratio than that employed in the present study. Under those conditions, the authors indicate that approximately 70% of total caffeine is extracted in the first 400 mL of the simulation, whereas in our model cumulative extraction reached 67% at the end of the simulation, with a total volume of 546 mL. This may be explained, at least in part, by the different proportions of yerba and water used, as well as by differences not detailed in the operational variables of the serving process. In this regard, it is worth noting that the review by Torterolo et al. does not specify the contact time between the water and the yerba in each extraction. Unlike that scheme, the present study was designed to reproduce as faithfully as possible the habitual conditions of mate preparation and consumption in Argentina.

As a final analysis of the doses provided by all types of prepared beverages relative to the maximum values recommended by the EFSA to avoid adverse effects (Figure 5), it can be mentioned that nly non-torrado filter coffee Brand 2, double lungo coffee, and the cumulative volume of mate servings exceeded the single dose of 200 mg. If we consider the social habit of sharing mate, it is difficult to assume how much caffeine each participant is consuming, especially considering that caffeine content depends on the number of servings that are shared. On the other hand, the coffees that exceed this value coincide with those that achieve better caffeine extraction yields and represent a larger beverage volume. Furthermore, except for single espresso coffee, single ristretto, mate cocido, and decaffeinated coffee, all beverages exceeded 100 mg of caffeine, a level that is not recommended before sleep. Finally, it is very important to consider that, although no single beverage exceeded 400 mg of caffeine, repeated consumption throughout the day of this type of infusion or the addition to the diet of certain dietary supplements could make this value easily attainable.

6. Limitations

This study has some limitations that should be considered when interpreting the findings. First, only selected commercial brands and presentations available in the local market were analyzed, so the results may not represent the full variability of coffee, yerba mate, and mate cocido products available in Argentina or other countries. Second, caffeine concentrations were determined under specific preparation conditions chosen to reflect common consumption practices; therefore, variations in water temperature, contact time, grind size, product composition, and serving size could lead to different results under

other real-world conditions. Third, the simulation of successive cebadas was designed to reproduce habitual mate consumption as closely as possible, but it cannot capture all the variability associated with individual preparation and drinking behaviors. Finally, the nutritional, toxicological, and ergogenic interpretations presented here are based on the caffeine doses delivered under the experimental conditions evaluated and should therefore be understood within that context.

7. Conclusions

The present study shows that caffeine concentration in traditional beverages prepared and consumed in Argentina depends not only on the caffeine content of the raw materials but also on a complex interplay among extraction variables, including water proportion, temperature, contact time, pressure, and grind size. The results confirm that high temperatures and prolonged contact times, as in filter coffee preparation, favor caffeine extraction and diffusion, whereas high-pressure methods achieve higher extraction yields in markedly shorter times.

Regarding yerba mate, under the specific conditions evaluated in this study, the stem-free sample yielded a higher total caffeine content and showed a more sustained extraction pattern across successive servings than the sample with stems. In addition, this study, conducted under specific conditions, provides relevant information on how caffeine concentration changes with serving number, an aspect that has received little attention in the available literature. Specifically, caffeine concentration peaked in the second serving and then declined gradually through the fifteenth. These findings are particularly relevant to sports nutrition, as they suggest that, although mate may serve as an ergogenic aid, the volume of liquid required to achieve optimal doses could limit its practicality for athletes.

For mate cocido, two 250 mL servings would provide approximately 90 mg of caffeine under the preparation conditions evaluated. When interpreted against the EFSA reference value of 3 mg/kg/day, this amount would correspond to the suggested daily caffeine intake for a 30 kg child. This comparison should be understood as a dietary exposure estimate based on external reference values and not as a direct assessment of health effects in children or adolescents.

8. Future Perspectives

The growing diversification of the market for coffee-containing products, such as coffee capsules and ready-to-drink refrigerated beverages, highlights the need for further research to quantify the actual caffeine contribution of these products to the habitual diet. In this context, it is important to broaden the analysis to include new commercial formats, considering variables such as serving size, declared concentration, and preparation conditions.

Likewise, traditional preparation methods such as the Italian coffee maker and the French press offer an opportunity to examine the extent of caffeine extraction achieved by these methods and the final caffeine concentration in the beverages they produce, thereby expanding the range of traditional extraction methods evaluated in this study.

Given the considerable caffeine contribution of the beverages analyzed in this study, future research should also examine the contribution of caffeine to other commonly consumed products, such as energy drinks, sodas, and dietary supplements. This line of research is particularly relevant in countries in the region, such as Argentina and Uruguay, where high mate consumption is accompanied by a substantial intake of caffeinated carbonated beverages and by a sustained increase in coffee consumption in recent years.

Likewise, the results of this study also open the possibility of developing new ergogenic formulations based on yerba mate or coffee infusions, given their high caffeine content and potential applicability.

A comprehensive approach would allow a more precise estimation of total caffeine exposure across different population groups and provide background information for future nutritional and regulatory recommendations.

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