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Polycyclic aromatic hydrocarbons (PAHs) in yerba maté (*Ilex paraguariensis* St. Hil) traditional infusions (*mate* and *tereré*)

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ABSTRACT

This study describes the occurrence of polycyclic aromatic hydrocarbons (PAHs) in traditional yerba maté hot and cold infusions (*mate* and *tereré*), *by* monitoring the content of benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[ah]anthracene, benzo[ghi]perylene and indeno[1,2,3-cd]pyrene (PAH8), that have been chosen as indicators for the occurrence and toxicity of PAHs in food by the European Food Safety Agency. PAH8 content in *mate* and *tereré* was determined by high performance liquid chromatography using fluorescence detection (HPLC-FLD).

PAH8 contents in hot and cold maté infusions ranged from 371.2 to 2438.8 ng/L and from 19.2 to 937.3 ng/L, respectively. Benzo[a]pyrene contents varied between 37.0 and 373.9 ng/L in hot yerba maté infusions and between 7.0 and 92.1 ng/L in cold yerba maté infusions. None of the samples analyzed exceeded the World Health Organization criteria for drinking water, since the maximum level allowed for benzo[a]pyrene is 700 ng/L.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of substances which comprise fused aromatic rings without any heteroatoms or substituent, formed through incomplete combustion or pyrolysis of any kind of organic matter. PAHs are ubiquitous environmental pollutants that have been identified in several matrices such as water, soil, air and food (IARC, 2010). Possible sources of food contamination by PAHs may be through contact with polluted soils, water or air (JECFA, 2005); food processing, particularly smoking or heat treatments at high temperatures (drying, roasting, grilling, frying, etc.); or they can be added to the food matrix by additives (Chung et al., 2011). In the literature, there are many papers reporting on the presence of PAHs in foodstuffs, including vegetables and fruits (Bishnoi, Mehta, & Pandit, 2006; Rojo Camargo & Toledo, 2003), vegetable oils (Ciecierska & Obiedzinski, 2013; Rojo Camargo, Ramos Antoniolli, & Vicente, 2012; Tfouni, Padovani, Reis, Furlani, & Camargo, 2014), meat products (Alomirah et al., 2011; Chung et al., 2011; Roseiro, Gomes, & Santos, 2011), fish and seafood (Serpe, Esposito, Gallo, & Serpe, 2010; Stołyhwo & Sikorski, 2005; Zhang, Xue, & Dai, 2010), coffee (Duedahl-Olesen, Navaratnam, Jewula, & Jensen, 2015; García Falcón, Cancho Grande, & Simal Gándara, 2005a, b; Hischenhuber & Stijve, 1987) and coffee brews (Orecchio, Paradiso Ciotti, & Culotta, 2009; Tfouni et al., 2013), tea (Dravoba et al., 2012; Duedahl-Olesen et al., 2015; García Londoño, Reynoso, & Resnik, 2015; Lin & Zhu, 2004) and tea infusions (Lin, Tu, & Zhu, 2005, Lin, Zhu & Luo, 2006), among others.

The increasing interest of PAHs as food contaminants of the last decades is directly related to food safety since some PAHs have been proved to be carcinogenic, in addition to having other toxic effects (biological, genotoxic and mutagenic) that have also been reported (IARC, 2010). Thus, exposure to PAHs is a significant public health problem. For this reason, these compounds are included in the priority pollutants lists of many international organizations. The two most important lists of PAHs commonly monitored in food and other matrices like water, soil and air are the “16 EPA PAH”, listed by the US Environmental Protection Agency (US EPA) and the “15 + 1 EU priority PAH” defined by the European Union (EU). In 2008, the CONTAM (Contaminants in the Food Chain) Panel of the European Food Safety Authority (EFSA) reviewed all the available data on occurrence and toxicity of PAHs in foodstuffs. After rigorous analysis of all documentation, the CONTAM Panel concluded that the risk characterization of dietary exposure to PAHs should be based upon the eight compounds for which oral carcinogenicity has

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been documented. Thereby, the experts defined a group of eight PAHs, named PAH8, as well as a subset of four PAHs, named PAH4, both suitable indicators for the occurrence and toxicity of PAHs, that should be monitored in food products. The PAH8 compounds are benz[a]anthracene (BaA), chrysene (Chry), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), dibenz[ah]anthracene (DaA), benzo[ghi]perylene (BgP) and indeno [1,2,3-cd]pyrene (IcP). The PAH4 subset consists of BaA, Chry, BbF and BaP (EFSA, 2008).

Ilex paraguariensis St. Hil. is a native South American tree mainly produced and consumed in Argentina, Paraguay and Brazil. The leaves and twigs of *I. paraguariensis* are industrially processed to obtain the final product: yerba maté. During manufacture, freshly harvested branches of yerba maté go through a severe heat treatment. First, they are subjected to direct exposure to flame (*zapecado*), followed by a final drying step where the raw material comes into direct contact with hot air and combustion gases from the burning of wood, wood chips and/or sawdust (Heck & Mejía, 2007; Schmalko & Alzamora, 2001). These processing stages contribute to the formation of PAHs and its subsequent deposition in the branches of yerba maté which offer a large surface area (Vieira et al., 2010; Ziegenhals, Jira, & Speer, 2008).

Yerba maté is traditionally consumed in two basic ways: hot mate or just *mate*, and cold mate or *tereré*. *Mate* is a hot infusion that is prepared by placing 30–50 g of yerba maté in a vessel where a volume of about 30 mL of hot water (70–85 °C) is poured over the material in a systematic way. After each pouring, the water is sucked through a kind of drinking straw that has a filter on the end immersed in yerba maté. *Tereré* is consumed in the same way but pouring cold water (4–8 °C).

Considering the number of reports dealing with PAHs presence in yerba maté and its infusions, the occurrence of these compounds in these matrices has been widely proved (Camargo & Toledo, 2002; García Londoño, Reynoso, & Resnik, 2014; Golozar et al., 2012; Kamangar, Schantz, Abnet, Fagundes, & Dawsey, 2008; Schulz, Fritz, & Ruthenschrör, 2015; Vieira et al., 2010; Ziegenhals et al., 2008; Zuin, Montero, Bauer, & Popp, 2005). Referring specifically to the content of PAHs in the infusions prepared with yerba maté, some researchers conducted studies where they determined the concentration of these compounds in yerba maté hot and cold infusions (Camargo & Toledo, 2002; Kamangar, Schantz, Abnet, Fagundes & Dawsey, 2008; Schulz et al., 2015; Zuin et al., 2005). However, the method used by these researchers to obtain the infusions does not describe entirely the traditional way of preparing and consuming *mate* and *tereré*, differing mainly in the yerba maté/water ratio and the infusion time used.

The aim of this research was to determine the content of PAH8 in hot and cold traditional infusions (*mate* and *tereré*) prepared with commercial brands of yerba maté from Argentina.

2. Materials and methods

2.1. Materials

2.1.1. Samples

Ten packaged commercial brands of yerba maté were purchased from supermarkets in the cities of Posadas and Buenos Aires (Argentina). All the samples were mixed by successive quartering. The samples were hermetically packaged and stored at −18 °C until processing.

2.1.2. Reagents

Ciclohexane, acetonitrile and water used in this study were of HPLC-grade, all obtained from Merck Chemicals (Buenos Aires, Argentina). The anhydrous sodium sulfate was from Merck Chemicals (Buenos Aires, Argentina). PAH standard mixture (BaA, Chry, BbF, BkF, BaP, DahA, Bpe and IcP) was purchased from Supelco (Bellefonte, PA, USA).

2.2. Methods

2.2.1. Infusions (mate and tereré)

Hot and cold yerba maté infusions (*mate* and *tereré*, respectively) were obtained simulating the way in which these beverages are usually prepared and consumed (Hartwig, Brumovsky, & Fretes, 2012). Briefly, 50 ± 0.1 g of yerba maté were placed in a glass recipient and a stainless steel straw was inserted in the material. The straw was connected to a 500 mL *kitasato* flask and this in turn, was connected to a vacuum trap. Approximately 30 mL of distilled water were added to the yerba maté remaining in contact with the material for 20 s. Vacuum was then applied for 20 s, and when the vacuum stopped, aliquot of water was added again. This process was repeated until the recovered volume in the *kitasato* flask reached 500 mL (the overall infusion process lasted between 10 and 15 min). Water temperature for preparing maté was 70 °C and for preparing tereré was 4 °C.

2.2.2. Extraction and clean up

For extraction and clean up procedures, 100 mL of cyclohexane were added to 250 mL of yerba maté infusion (*mate* or *tereré*). The mixture was then stirred in a mechanical shaker for 90 min. The material was transferred into a 500 mL separating funnel. The water phase was discarded and the cyclohexane phase was collected. The extract was dried with anhydrous sodium sulfate and concentrated in a rotary evaporator to 3 mL at 39.5 °C. The concentrated extract (3 mL) was filtered through a commercial solid phase extraction cartridge (Bond Elut SI, 3 mL, 500 mg) (Agilent Technologies, Lake Forest, CA, USA). After conditioning the cartridge with 3 mL of cyclohexane, the extract was applied and eluted with 9 mL of additional cyclohexane. Finally, the extract was evaporated to dryness. The residue was dissolved in 3 mL HPLC-grade acetonitrile using the ultrasonic bath. This solution was filtered through a 0.22 μm nylon syringe filter (Agilent Technologies, Lake Forest, CA, USA) before the injection into the chromatography system. Reagent blank controls were analyzed simultaneously to the extraction and clean up of each group of samples to check the absence of PAHs in the water used to prepare the infusions, and in the solvents used for the extraction and clean up procedures. All samples and blank controls were analyzed in duplicate.

2.2.3. HPLC analysis

A 25 μL aliquot was injected into a HPLC system (Prominence, Shimadzu, Japan) equipped with autosampler (SIL-20A HT), quaternary pump (LC-20A T), thermostated column compartment (CTO-20A C) and a programmable spectrofluorometric detector (RF − 10A XL). For the PAHs separation a Supelcosil™ LC-PAH column (250 × 4.6 mm, 5 μm) (Supelco, Bellefonte, PA, USA) was used. The temperature of the column was kept constant at 30 °C to obtain reproducible retention times. The mobile phase consisted of water and acetonitrile in gradient mode at flow rate of 1 mL/min. The gradient solvent system started with 60% acetonitrile in water (v/v) during 5 min, then increasing linearly to 100% acetonitrile within 30 min. The 100% acetonitrile was maintained for 15 min; when finally returned to the initial conditions in 5 min and allowed to equilibrate the system for another 5 min. The analytes were detected and quantified by monitoring the fluorescence emissions at 430 nm (BaA, Chry, BbF, BkF, BaP, DahA, BPe) and 500 nm (IcP) with one common excitation wavelength of 270 nm.

The identification of PAHs was performed by comparison of

their retention time (t_R, min) with those of the standard PAHs. The external standard plot method was used for quantification. Seven concentrations ranging from 1.2 to 1200 ng/L were measured in triplicate to construct linear regressions curves (peak areas *versus* PAH concentration).

2.2.4. Validation

The proposed method was validated for linearity, limit of detection (LOD) and limit of quantification (LOQ), accuracy and precision according to the International Conference of Harmonization (ICH, 2005) procedures for validation of analytical methods. The linearity of the calibration was verified through determination coefficients of the calibration curves. The LOD and LOQ were determined based on the standard deviation of the response and the slope of the calibration curves. Repeatability was evaluated as relative standard deviation (RSD %) of the peak area of seven concentrations covering the range of the procedure, measured in triplicate during the same day of analysis.

Accuracy of the method was determined using a blank sample as a reference material. In order to obtain blank sample of yerba maté, fresh branches of yerba maté were harvested and then taken to the laboratory (Laboratorio de Yerba Mate – Facultad de Ciencias Exactas, Químicas y Naturales – Universidad Nacional de Misiones – Argentina) where they were manufactured without contact with smoke (*zapecado* with steam and dried with hot air) avoiding any kind of contamination. Then, the yerba maté (approximately 300 g) was ground and aged during 3 months in a chamber at around 60 °C of temperature and 60% of relative humidity. Finally, accuracy was evaluated by performing recovery tests. Yerba maté infusions were obtained as described previously using the reference material. Then, the infusions were spiked with different dilutions of the PAHs standard mixture (40, 100 and 1000 ng/L). The spiked infusions were extracted, purified and analyzed, in the same way as the samples in triplicate.

3. Results and discussion

3.1. Method development, validation and performance

PAHs analysis in food matrices usually requires very laborious and time consuming methods which include several steps, being essential the sample pretreatment and purification procedures (Plaza Bolaños, Garrido Frenich, & Martínez Vidal, 2010). For non-fatty liquid matrices (coffee, tea, juices, and other beverages), the use of liquid–liquid extraction (LLE) with subsequent solid phase extraction (SPE) clean up (silica sorbent) has been widely used (Camargo & Toledo, 2002; Duedahl-Olesen et al., 2015; García-Falcón, Cancho-Grande, & Simal-Gándara, 2005; Lin et al., 2005; Lin et al., 2006; Tfouni et al., 2009, 2013). Methods based in LLE require large amounts of expensive, high purity solvents and are extremely time consuming (Plaza Bolaños et al., 2010). Some authors tested a much more simple technique to determine PAHs in aqueous samples similar to yerba maté infusions using solid phase extraction on C_{18} cartridges (García-Falcón et al., 2005; Kayali-Sayadi, Rubio-Barroso, Cuesta-Jimenez, & Polo-Díez, 1998). Despite the successful results reported by Kayali-Sayadi et al. (1998) when using direct SPE extraction in the determination of PAHs in tea infusions, that option was discarded because of clogging problems described by García-Falcón et al. (2005) when passing the coffee brew sample through the SPE cartridge.

Considering that PAHs are expected to be present at very low concentrations in yerba maté infusions due to their poor solubility in water, an extraction procedure with high pre-concentration capability is required to determine the levels of these compounds in *mate* and *tereré*. Also, because of the complexity of yerba maté infusion matrix, a purification step must be applied in order to remove possible interferences and obtain a clean extract for chromatographic analysis.

The proposed method is a liquid–liquid extraction based technique. Even after the LLE, the extracts showed an intense green color due to the presence of photosynthetic pigments, suggesting that other polar compounds may also be present. For this reason, the inclusion of a purification step was evident. A SPE purification step on a polar support (silica) to trap polar impurities from the extract was included.

All PAHs showed good linearity within the tested concentration range with determination coefficients (R^2) close to 0.999. The relative standard deviation calculated for the tests of repeatability varied from 3.14 to 5.52%. LODs and LOQs of individual PAHs are presented in Table 1. The accuracy of the analytical procedure was evaluated through recovery experiments. Independent triplicates of the spiked samples were analyzed and the obtained results were reported in percent recoveries. Mean recoveries between 91 and 105% with RSD (%) below 4% were obtained for all the compounds studied. LOQ value for BaP, only PAH for which individual maximum levels have been set in various food matrices, did not exceed the value established in the most demanding standards as European Drinking Water Directive, which states that maximum level for BaP in drinking water is 10 ng/L (European Union, 1998). The overall results of the validation indicate that the developed method is suitable for the analysis of PAH8 in the infusions.

Fig. 1 shows a typical chromatogram of a yerba maté hot infusion sample obtained under the elution conditions described above.

3.2. PAHs contents in yerba maté infusions

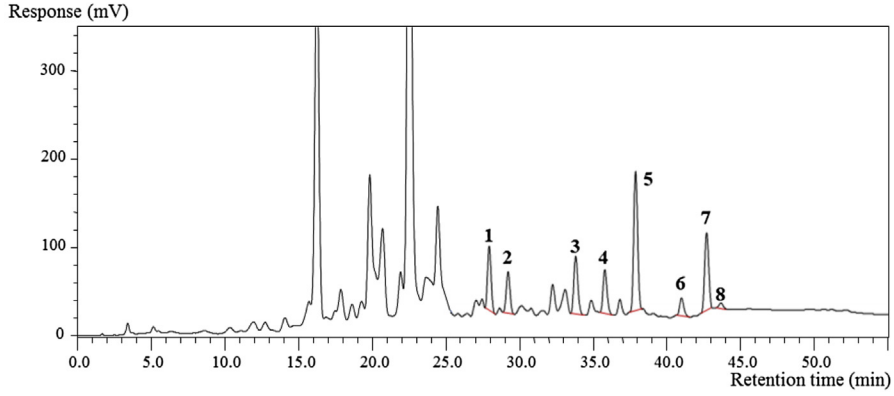
Table 2 presents the PAHs contents determined in hot and cold yerba maté infusions. Some PAH were detected but not quantified, because their concentrations were below the limit of quantification. The concentration of PAH8 in yerba maté hot infusions analyzed ranged from 371.2 to 2438.8 ng/L. BaP was found in all *mate* samples at levels between 37.0 and 373.9 ng/L. For cold infusions, PAH8 levels varied from 19.2 to 937.3 ng/L, and BaP contents were between 7.0 and 92.1 ng/L.

PAHs levels found in the infusions were far lower than those reported by many authors in yerba maté (Kamangar et al., 2008; García Londoño et al., 2014; Schulz et al., 2015; Vieira et al., 2010; Ziegenhals et al., 2008). Because PAHs have high octanol–water partition coefficients (K_{ow}) and low aqueous solubilities (Ma, Lei, Xiao, Wania, & Wang, 2010), especially the higher molecular weight PAHs as those included in this study, their transfer to the infusions is expected to be low. Lin et al. (2006) studied the transfer percentages of individual PAH from various types of tea to tea infusions. They prepared black tea, oolong tea and green tea infusions in the same way in all cases, and found different transfer rates for the different tea varieties. This indicates that other factors, beyond the physicochemical properties of PAHs, are involved on the migration of these compounds into the infusion. Lin et al. (2005), who studied the content of PAHs in tea infusions, suggest that the presence of PAHs in the tea liquor could be related to the presence of essential oils, which may act as co-solvents for many lipophilic substances, and therefore increase the solubility of these compounds in water. Furthermore, it has been suggested that caffeine, substance found in yerba maté infusions, increases the water solubility of PAHs by the formation of a PAH-caffeine complex which facilitates their transfer to the infusion (Hischenhuber y Stijve, 1987; Tfouni et al., 2013). Referring specifically to *mate* and *tereré*, some authors suggest the presence of PAHs in yerba maté infusions can be explained by the passage of yerba maté powder to the

Table 1
Method performance characteristics: Analytical range, limit of detection (LOD), limit of quantification (LOQ), relative standard deviation (RSD) and percent recovery (R).

PAHs	Analytical range (ng/L)	LOD (ng/L)	LOQ (ng/L)	Repeatability test	Recovery test	
				RSD[a] (%)	R[b] (%)	RSD (%)
BaA	3.6–1200	3.6	12.0	4.46	99.8	1.7
CHR	12–1200	18.2	60.6	4.39	95.8	2.9
BbF	3.6–1200	2.5	8.2	3.61	105.1	1.0
BkF	3.6–1200	4.0	13.4	3.24	97.6	3.7
BaP	1.2–1200	1.2	4.0	3.41	99.9	0.7
DhA	8.4–1200	19.7	65.6	4.15	90.9	0.5
BgP	6.0–1200	5.1	16.9	5.52	100.8	2.4
IcP	60.0–1200	25.0	83.5	3.14	91.9	2.6

[a] Mean values of seven concentrations within the analytical range, measured in triplicate.
[b] Mean recovery of three different concentrations in triplicate.



1) benz[a]anthracene; **2)** chrysene; **3)** benzo[b]fluoranthene; **4)** benzo[k]fluoranthene; **5)** benzo[a]pyrene; **6)** dibenz[ah]anthracene; **7)** benzo[ghi]perylene and **8)** indeno[1,2,3-cd]pyrene.

Fig. 1. Typical HPLC-FLD chromatogram of a yerba maté hot infusion sample.

infusion, since the straw does not have a suitable filtration mechanism (Camargo & Toledo, 2002).

As can be seen, the contents of PAHs found in hot and cold yerba maté infusions were highly variable. This wide variation may be due to the heterogeneity in the drying methods of yerba maté, differences in the processing equipments and differences in fuels employed by different industries, which generate different contamination levels of the material. Water solubility of HAPs, like

Table 2
PAHs contents (ng/L) (mean ± standard deviation, n = 2) in yerba maté hot and cold infusions (*mate* and *tereré*).

PAHs	Brands									
	1	2	3	4	5	6	7	8	9	10
Hot infusions										
BaA	164.7 ± 12.0	131.0 ± 10.6	60.1 ± 2.7	308.9 ± 4.2	156.6 ± 2.0	172.8 ± 8.8	65.3 ± 0.2	72.2 ± 2.8	176.3 ± 7.7	398.6 ± 13.3
Chry	278.9 ± 16.4	262.2 ± 11.0	122.6 ± 7.4	541.5 ± 7.8	224.8 ± 1.9	343.7 ± 18.6	133.6 ± 0.4	132.5 ± 5.5	305.6 ± 11.3	78.9 ± 2.3
BbF	167.1 ± 10.3	122.9 ± 8.6	71.6 ± 3.6	277.2 ± 1.3	190.2 ± 5.7	187.7 ± 11.0	72.3 ± 0.4	72.8 ± 5.6	201.9 ± 5.7	464.9 ± 13.6
BkF	55.1 ± 1.4	37.5 ± 1.1	23.4 ± 1.1	89.9 ± 2.5	80.3 ± 2.8	57.2 ± 2.7	18.5 ± 0.1	19.3 ± 0.1	64.7 ± 2.1	163.5 ± 6.0
BaP	106.5 ± 6.1	77.8 ± 5.8	38.5 ± 2.3	221.7 ± 2.4	128.8 ± 1.7	121.1 ± 6.7	40.5 ± 0.5	37.0 ± 3.1	135.0 ± 6.7	373.9 ± 15.7
DaA	125.8 ± 4.8	99.4 ± 4.0	75.0 ± 2.8	258.4 ± 1.8	205.7 ± 1.5	174.6 ± 6.7	68.8 ± 1.0	<LOQ	196.8 ± 4.9	510.5 ± 14.5
BgP	187.3 ± 5.0	143.5 ± 8.4	69.4 ± 3.6	358.6 ± 1.5	185.2 ± 1.4	202.8 ± 12.8	75.2 ± 1.2	37.3 ± 2.5	176.2 ± 6.1	72.9 ± 3.9
IcP	<LOQ	<LOQ	<LOQ	177.8 ± 1.6	112.3 ± 0.4	100.3 ± 4.4	<LOQ	<LOQ	118.6 ± 3.7	375.9 ± 11.4
PAH8	1085.4	874.4	460.7	2234.0	1283.8	1360.4	474.2	371.2	1375.3	2438.8
Cold infusions										
BaA	37.6 ± 2.1	22.3 ± 0.9	<LOQ	48.3 ± 1.5	36.6 ± 0.1	41.2 ± 2.2	24.0 ± 1.3	23.9 ± 1.1	39.6 ± 3.0	93.0 ± 3.4
Chry	63.1 ± 2.8	<LOQ	<LOQ	83.0 ± 3.3	<LOQ	91.3 ± 8.7	<LOQ	<LOQ	70.2 ± 4.3	195.3 ± 6.3
BbF	33.4 ± 3.2	20.4 ± 0.3	12.2 ± 0.5	48.8 ± 2.1	42.9 ± 0.3	47.9 ± 4.2	36.5 ± 1.6	23.6 ± 1.0	39.7 ± 2.2	123.1 ± 4.6
BkF	<LOQ	<LOQ	<LOQ	14.0 ± 0.5	17.1 ± 0.1	<LOQ	<LOQ	<LOQ	<LOQ	35.3 ± 1.1
BaP	20.2 ± 1.0	13.5 ± 0.3	7.0 ± 0.2	40.9 ± 1.6	30.7 ± 0.1	30.3 ± 1.9	16.5 ± 1.1	11.0 ± 0.4	29.8 ± 1.8	92.1 ± 2.2
DaA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	126.9 ± 3.1
BgP	21.2 ± 1.3	26.0 ± 0.6	<LOQ	67.2 ± 3.4	42.7 ± 0.5	54.2 ± 3.8	46.7 ± 3.5	<LOQ	36.5 ± 2.2	175.9 ± 4.7
IcP	<LOQ	ND	ND	<LOQ	<LOQ	ND	<LOQ	ND	<LOQ	95.1 ± 1.7
PAH8	175.5	82.3	19.2	302.2	170.0	265.0	123.7	58.6	215.8	937.3

Table 3
Comparison of PAHs content in yerba maté infusions reported in literature.

Reference	Yerba maté weight	Amount of water	Water temperature	Infusion time	BaP (ng/L)	PAH4 (ng/L)	PAH8 (ng/L)
This study (n = 10)	50 g	~500 mL	Hot: 70 °C	See Section 2.2.1	Hot 37.0 −373.9	Hot 292.8 −1316.3	Hot 371.2 −2438.8
			Cold: 4 °C		Cold 7.0–92.1	Cold 19.2–504.0	Cold 19.2–937.3
Kamangar et al. (2008) (n = 2)	5 g	360 mL	Hot: 80 °C	12 infusion procedures, 5 min duration each	Hot 331.9 −348.6	Hot[a] 1419.4 −1433.5	Hot[b] 2296.7 −2401.5
			Cold: 5 °C		Cold 373.6 −390.3	Cold[a] 1350.0 −1720.8	Cold[b] 2218.9 −2820.8
Camargo and Toledo (2002) (n = 9)	25 g	500 mL	100 °C	5 min	3.5	10.5	19.5[c]
Zuin et al. (2005) (n = 11)	1 g	100 mL	100 °C	5 min	ND – 22.6	7.2–135.9	7.2–184.5
Schulz et al. (2015) (n = 1)	20 g	2000 mL	100 °C	30 min	<5.0	<5.0	<5.0

[a] PAH4 + Triphenylene + Benzo(j)fluoranthene.
[b] PAH8 + Triphenylene + Benzo(j)fluoranthene.
[c] Except IcP.

many other hydrophobic organic compounds, increases with increasing temperature (Reza, Trejo, & Vera-Ávila, 2002). This fact explains the differences in the PAHs contents between *mate* and *tereré* samples.

As mentioned before, considerable research has been conducted on yerba maté and yerba maté infusions contamination with PAHs. However, there is a lack of studies on the water extraction of those PAHs in yerba maté infusions obtained in the way and proportion in which they are usually prepared and consumed. PAHs contents in infusions prepared with yerba maté reported in the literature vary widely. One of the main reasons for this variation is the difference in infusion procedures used by the different authors. This causes a restriction when discussing the results with those obtained in other publications.

Table 3 shows a comparison between the PAHs contents in infusions obtained in the present study and those reported by other authors (Camargo & Toledo, 2002; Kamangar et al., 2008; Schulz et al., 2015; Zuin et al., 2005). In contrast to that observed in this study, Kamangar et al. (2008) found similar PAHs contents in both hot and cold infusions.

Besides water temperature and contamination level of the material used to prepare the infusions, many other variables, directly related to infusion procedures, influence the extent of PAHs transfer to the extract. Lin et al. (2006) studied the effect of solid/water ratio (g/mL) on PAHs transfer when preparing black tea infusions. They demonstrated that the percentage of PAHs transfer to the infusion increased with the decrease of solid/water ratio, and that this effect was more significant for low molecular weight PAHs. The infusion time also has a direct influence in the extent of PAHs transfer to the beverages (Lin et al., 2005, 2006; Kamangar et al., 2008). According to Lin et al. (2005), PAH release from tea leaves to tea liquor increases with infusion time. Note that during the preparation of *mate* and *tereré* samples analyzed in this report, the infusion time between addition and suction of water was relatively short compared with the infusion times of the aforementioned works. This, added to the different water temperatures used to prepare infusions, could explain the differences observed between the results obtained in this study and those reported by other researchers.

Further investigations are needed to completely elucidate the influence of the matrix and other factors related to infusion procedures (especially water temperature and solid/water ratio) in the migration mechanism of PAHs from yerba maté to yerba maté traditional infusions.

3.3. Analysis of the contamination considering the available regulation for foodstuffs and drinking water

The European Communities Regulation 835/2011 (EC, 2011) sets maximum allowable values for BaP and PAH4 in a range of foodstuffs. However, there are categories of food that are not covered by this legislation, especially, dried food products like herbs and spices, and infusions like tea or coffee.

Hot yerba maté infusions showed PAH4 levels ranging from 3.2 to 14.8 μg/kg of the dry mass, and the levels of BaP varied between 0.4 and 4.1 μg/kg of the dry mass. *Tereré* samples showed PAH4 values ranging from 0.2 to 5.5 μg/kg of the dry mass, and BaP values between 0.1 and 1.0 μg/kg of the dry mass. Considering the limits fixed for the types of foods included in the European legislation, all *mate* samples in this study exceeded the minimum value regulated for the sum of BaA, Chry, BbF and BaP (1.0 μg/kg), but only six samples exceeded the most demanding limit for BaP (1.0 μg/kg), both values set for foods for infants and young children. With regard to cold infusions, none of the samples exceeded this maximum for BaP, and six samples showed higher values than allowed for PAH4. These six *tereré* samples were prepared using the same brands of yerba maté used to prepare the six hot infusions whose content exceeded the maximum regulated for BaP (brands 1, 4, 5, 6, 9 and 10; data not shown). Even so, none of the samples analyzed presented higher values than the maximum level regulated by the European Communities for BaP (6.0 μg/kg) and PAH4 (30.0 μg/kg) for other foodstuffs.

Considering that generally infusions are consumed in far higher amounts than other foodstuffs, it might be more appropriate to refer to the PAHs limits set for drinking water. Many international organizations established specific maximum levels for PAHs, especially BaP, in drinking water. The USEPA (2009) set a maximum value for BaP of 200 ng/L and the World Health Organization (WHO, 2011) established a guideline value of 700 ng/L. Given these parameters, all samples in this study (*mate* and *tereré*) did not exceed the guideline value proposed by the WHO for drinking water, and only two hot infusion samples (the ones prepared with the brands 4 and 10) did not meet the requirements set by the US EPA. According to the European Union criteria for drinking water quality (Council Directive 98/83/EC), the maximum level allowed for BaP is 10 ng/L, and the maximum limit for the sum of BbF, BkF, BgP and IcP is 100 ng/L (EU, 1998). Only one of the analyzed samples (*tereré* prepared with the brand 3) did not exceed these quality standards set by the European Communities.

4. Conclusions

This is the first report on the content of eight polycyclic aromatic hydrocarbons (PAHs) in hot and cold infusion prepared with yerba maté from Argentina. *Mate* and *tereré* samples were obtained simulating the way in which they are traditionally prepared and consumed in real life. The eight PAHs studied were identified and

quantified in most of the samples. Despite this, the content of benzo [a]pyrene, the most harmful PAH, did not exceed the maximum level suggested by the World Health Organization for drinking water (700 ng/L) in any of the infusions studied.

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