




Impact of water temperature of chimarrão on phenolic compounds extraction

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Abstract

This study evaluated the effect of water temperature (65 °C, 75 °C, 85 °C, 95 °C) on the concentration of caffeic acid, 5-caffeoylquinic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid and rutin in *chimarrão* aqueous extracts prepared from two commercial types of yerba mate: traditional and coarse ground. The *chimarrão* preparation simulated the homemade process. Regardless of the temperature, aqueous extracts from the coarse ground had 60% more phenolic compounds than the traditional ones. In the extracts of coarse ground yerba mate the temperature of 95 °C favored the extraction of the compounds in relation to the other temperatures. For the traditional yerba mate extracts there was no difference regarding the temperature. The chlorogenic acids content in the *chimarrão* extracts is significantly higher than in other foods and beverages and represent an excellent contribution for chlorogenic acids intake.

Keywords: chlorogenic acids; *Ilex paraguariensis*; infusion; yerba mate.

Practical Application: Chimarrão consumers will be able to choose the best temperature and type of herb for chimarrão preparation. They will be able to estimate their daily intake of phenolic compounds from the beverage they prepare.

1 Introduction

Yerba mate (*Ilex paraguariensis*), originated from subtropical South America, is widely used in infusions preparation, such as *chimarrão*, *tererê* and mate tea, which are widely appreciated because of their sensorial and stimulant properties (Heck & De Mejia, 2007). Among them, *chimarrão* is a partial infusion prepared by vertically placing the yerba mate into a container called *cuia* and pouring hot water in the gap between the herb and the sidewall of the *cuia*. The beverage is ingested by suction through a metal artifact called *bomba* (Gebara et al., 2017). Successive additions of water are made to the *cuia* so that fresh infusions are prepared from the same herb until the loss of flavor is noticed by the consumer (Meinhart et al., 2010). The market provides yerba mate with different characteristics regarding the origin, composition of stems and leaves, particle size and the addition of other ingredients to the yerba mate. The traditional and coarse ground yerba are the most consumed types of yerba mate for *chimarrão* preparation. Both are made from mixing native and cultivated yerba mate, however, the coarse ground has a larger particle size (about 200 mesh) in comparison with the traditional one (about 500 mesh) (Bizzotto et al., 2012).

The plant has been pointed out as a source of phenolic compounds, particularly the derivative esters of caffeic acid and quinic acid and their mono and dicaffeoylquinic

acid isomers, such as 5-caffeoylquinic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid. Also, yerba mate has a high content of flavonoids, being rutin the predominant flavonoid compound in the plant (Bizzotto et al., 2012).

The aqueous extracts of yerba mate showed high antioxidant capacity, anti-inflammatory, anti-mutagenic, anti-proliferative, cardioprotective and anti-obesity properties (Boaventura et al., 2015; Miranda et al., 2008; Pimentel et al., 2013; Wan et al., 2012). The chlorogenic acids present in high amount in the yerba mate have been associated, in *in vitro* and *in vivo* studies, to several beneficial effects on health, including the ability to modify the glucose metabolism, playing a role in its absorption, oxidative stress attenuation, protection against inflammation in diabetic nephropathy (Peng et al., 2015; Wang et al., 2008) and sensorineural hearing impairment caused by diabetes (Hong et al., 2017), liver protection against the formation of fibrosis induced by CCl₄ or caused by cholestasis (Shi et al., 2016; Wu et al., 2015), inhibiting of the replication of the hepatitis B virus (Wang et al., 2009), reduction of total cholesterol and LDL (low density lipoprotein) and increase levels of HDL (high-density lipoprotein) (Wan et al., 2013), in addition to inhibiting the proliferation of tumor cells in the stomach, colon, (Matsunaga et al., 2002; Shao et al., 2015) and liver (Siswanto et al., 2017; Yan et al., 2017).

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Given the importance of these compounds and the high amount present in yerba mate, it is extremely relevant to study the preparation conditions of the *chimarrão*, in order to enable the maximum extraction of the bioactive compounds for the beverage ingested by consumers. It has been shown that compounds released from the plants may be affected by several parameters, being the temperature of the water used in the infusion preparation a critical parameter (Horžić et al., 2009; Kelebek, 2016). Thus, water temperature increase may favor the transference of the compounds by increasing their solubility, or may promote the degradation of the compounds and decrease their content in the aqueous extracts ingested by the consumer (Horžić et al., 2009; Jeon et al., 2017; Komes et al., 2010). Studies have already been reported evidencing the importance of temperature in the extraction of bioactive compounds during the preparation of infusions (Jeon et al., 2017; Pérez-Burillo et al., 2018), but no studies were found evaluating the effect of this parameter on the concentration of phenolic compounds in the infusions obtained during the consumption of *chimarrão*.

Therefore, this work aimed to study the effect of temperature of the water used for *chimarrão* preparation on the migration of caffeic acid, 5-caffeoylquinic acid (5-CQA), 3,4-dicaffeoylquinic acid (3,4-DQA), 3,5-dicaffeoylquinic acid (3,5-DQA), 4,5-dicaffeoylquinic acid (4,5-DQA) and rutin from the plant to the aqueous extracts ingested by the consumer. Two commercial yerba mate types were used in this study: traditional and coarse ground.

2 Material and methods

2.1 Samples

Chromatography-grade methanol (Merck, Germany) and analytical-grade formic acid (Ecibra, Brazil) were used. Water was purified using a Milli-Q® (Millipore) system. The phenolic compound standards (caffeic acid, 5-CQA, 3,4-DQA, 3,5-DQA, 4,5-DQA, and rutin) were obtained from Biopurify Phytochemicals Ltd. (Chengdu, China). Standard stock solutions were prepared in methanol at a concentration of 1 mg mL⁻¹ and kept at -18 °C for 1 month at most. Organic and aqueous solutions were filtered using polyvinylidene (PVDF) and cellulose membranes, respectively, both with a porosity of 0.22 µm (Millipore).

Yerba mate samples were courteously provided by the Ervateria Vier, located in Rio Grande do Sul, Brazil. The *chimarrão* samples were named as traditional and coarse ground. The herbs were produced with a mixture of leaves and stems (84:26, respectively) of native plants (70%) and cultivated plants (30%). The plants used in the production of the traditional and coarse ground herbs are originated from the same place. The only difference between them was the size of the mill, being 200 mesh for the coarse ground and 500 mesh for the traditional. Ten kilograms of each type of yerba mate were provided in the packages commonly used for commercialization (1000 g). The content of the respective yerba mate packs was mixed and homogenized before being used for the preparation of *chimarrão* infusions.

2.2 Aqueous extracts preparation

The aqueous extracts of yerba mate were obtained according to Da Silveira et al. (2017). Briefly, 85 g of yerba mate were vertically arranged in an average-sized cuia (with total volume of 350 ml of water or about 170 g of yerba mate). The “pump” was positioned and fixated in the remaining space of the cuia and coupled to a vacuum system. Next, the cuia was filled with hot water at 65 °C and kept in a partial infusion for 30 seconds, after which the resulting yerba mate aqueous extract was suctioned with the aid of the vacuum system. These steps were repeated 30 times, which represented the number of successive additions of water usually made by consumers (Meinhart et al., 2010). The extract was collected, the volume registered, filtered in common filter paper, diluted with water to fit the standard curve, filtered using polyvinylidene (PVDF) membranes with 0.22 µm (Millipore) porosity and injected into the HPLC. The same process was repeated using hot water at temperatures of 75 °C, 85 °C and 95 °C. The experiment was carried out in triplicate for each temperature.

2.3 Methanolic extraction of phenolic compounds from yerba mate

An exhaustive extraction of the phenolic compounds was performed using methanol, according to Meinhart et al. (Meinhart et al., 2010). Two grams of ground yerba mate were mixed with 10 mL of methanol for 3 hours, followed by centrifugation for 10 min. at 3500 g. The upper phase was collected and the solid residue was subjected to a new extraction with 10 ml of methanol for 1 hour. The extracts were combined, transferred to a volumetric flask (25 mL), filtered and injected into the HPLC.

The content of phenolic compounds measured in the methanolic extracts (equivalent to 100%) was compared with the amount of these compounds in the aqueous extracts. Thus, it was possible to estimate the percentage of phenolic compounds migrating from the matrix to the aqueous extract during the infusion time (30 seconds) and along the successive extractions (30 times).

2.4 Chromatographic conditions

The chromatographic method optimized and validated, according to International Union of Pure and Applied Chemistry (Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report), 2002), by Da Silveira et al. (Da Silveira et al., 2016) was applied for the quantification of phenolic compounds in both aqueous and methanol extracts of yerba mate. The separation was performed using a HPLC system model 1100 (Agilent Technologies, Santa Clara, CA, USA), coupled with a diode array detector and reverse phase column C18 ACE (Switzerland) that was 100 mm in length and 4.6 mm in diameter with 3 µm particle size. The mobile phase consisted of methanol and an aqueous solution of formic acid 0.1% (v/v). The linear gradient started with 13.6% of methanol and achieved 40% in 39.4 minutes. The detection was recorded at 325 nm. HP-Chemstation software was used for data analysis.

2.5 Statistical treatment

The results obtained were compared by analysis of variance (ANOVA) and Tukey's test in Statistica 6.0 from StatSoft (Tulsa, USA). The samples were considered to be significantly different when $p < 0.05$.

3 Results and discussion

Many factors can alter the amount of bioactive compounds in yerba mate aqueous extracts, such as geographical region, growing and processing conditions (Da Silveira et al., 2016; Isolabella et al., 2010; Thompson et al. 2002). Moreover, factors related to beverage preparation can also affect the concentration of substances in the extracts, including time, the temperature of infusion, the mass ratio of herbs/water volume and particle size (Gebara et al., 2017; Da Silveira et al., 2016). In a previous study (Da Silveira et al., 2017), we demonstrated that the type of yerba mate (different particle size and leaf and stem composition) used to prepare *chimarrão* affects the extraction of chlorogenic acids and rutin. The present approach provides complementary data by demonstrating that in addition to this factor, water temperature may play an important role in the amount of phenolic compounds in aqueous extracts of yerba mate.

Table 1 shows the content of rutin and chlorogenic acids in the methanolic extracts relative to 85 g of yerba mate (amount used in the experiments). The results indicated the predominance of 3,5-DQA (1750.2 mg for traditional and 1704.4 mg for coarse ground) and 5-CQA (1379.5 mg for traditional and 1360.8 mg for coarse ground), which is in agreement with the previously

mentioned literature (Bizzotto et al., 2012; Marques & Farah, 2009). Table 1 also shows the percentage of the compounds that was transferred to the 30 aqueous extracts of *chimarrão*, in the different temperatures studied. It can be observed that the extraction rate varied between 4.0% (for rutin at 65 °C with traditional yerba mate) and 28.3% (for 3,4-DQA at 95 °C with coarse ground yerba mate).

With respect to the type of yerba mate, it can be observed that in all studied temperatures and for all the investigated compounds, the extraction was greater (2.4 times) when the *chimarrão* was prepared with coarse ground yerba mate. This can be observed in the sum of all extracted compounds, considering the average extracted in different temperatures, with the extracts prepared with coarse ground yerba mate giving 17.5% of the content present in the plant, whereas in the extracts prepared with traditional yerba mate, transference of only 7.3% was observed.

The particle size has been reported as the factor with the greatest impact in the extraction of phenolic compounds from yerba mate (Gebara et al., 2017). Meinhart et al. (Meinhart et al., 2010), studying aqueous extracts of coarse ground and traditional yerba mate, reported similar behavior for the extraction of methylxanthines and total phenolic compounds, and suggested that the higher content found in the coarse ground extracts may be linked to the higher penetration of water as a consequence of the superior particle size. Similar findings were also reported by Gebara et al. (Gebara et al., 2017) when comparing the extraction of chlorogenic acids from *chimarrão* (60 mesh) and tererê (30 mesh), with the higher extraction observed for larger particles. Small particles (traditional) form a barrier that hinders

Table 1. Content of the compounds present in the yerba mate and percentage extracted for the beverages of *chimarrão* in different temperatures.

		Coarse ground yerba mate				
Compound	Content in yerba mate (mg 85 g ⁻¹)	% Extracted with 30 extracts				
		65 °C	75 °C	85 °C	95 °C	Average
Caffeic acid	11.4 ^a	15.6 ^a	14.1 ^a	17.1 ^a	27.4 ^a	18.6 ^a
5-CQA	1360.8 ^a	20.0 ^a	14.8 ^a	18.0 ^a	28.1 ^a	20.2 ^a
3,4-DQA	251.6 ^a	18.2 ^a	14.9 ^a	19.1 ^a	28.3 ^a	20.1 ^a
3,5-DQA	1704.4 ^a	13.8 ^a	11.0 ^a	12.9 ^a	18.4 ^a	14.0 ^a
4,5-DQA	454.7 ^a	17.9 ^a	14.9 ^a	17.9 ^a	27.3 ^a	19.5 ^a
Rutin	397.8 ^a	11.8 ^a	9.6 ^a	11.9 ^a	17.6 ^a	12.8 ^a
<i>Average % extracted</i>		16.2	13.2	16.2	24.5	17.5
		Traditional yerba mate				
Compound	Content in yerba mate (mg 85.g ⁻¹)	% Extracted with 30 extracts				
		65 °C	75 °C	85 °C	95 °C	Average
Caffeic acid	11.4 ^a	6.5 ^b	10.3 ^b	8.5 ^b	12.9 ^b	9.5 ^b
5-CQA	1379.5 ^a	6.8 ^b	9.8 ^b	7.2 ^b	8.0 ^b	7.9 ^b
3,4-DQA	256.7 ^a	6.4 ^b	9.1 ^b	7.6 ^b	10.6 ^b	8.4 ^b
3,5-DQA	1750.17 ^a	5.1 ^b	6.7 ^b	5.3 ^b	5.0 ^b	5.5 ^b
4,5-DQA	492.4 ^a	6.5 ^b	9.2 ^b	7.5 ^b	8.1 ^b	7.8 ^b
Rutin	382.9 ^a	4.0 ^b	6.0 ^b	4.3 ^b	3.9 ^b	4.5 ^b
<i>Média % extracted</i>		5.9	8.5	6.7	8.1	7.3

the passage of water and reduces the extraction capacity, while larger particles (coarse ground) provide greater contact and consequently greater extractive capacity. This hypothesis is supported by the average volumes of aqueous extracts collected in each of the 30 successive extractions, which were 25% higher in traditional (95.0 ± 6.7 mL) than in coarse ground (75.0 ± 5.7 mL), showing that larger amounts of water have permeated the coarse ground yerba mate type.

When investigating the effect caused by the temperature difference, in the coarse ground yerba mate the highest extraction percentage for all the compounds occurred with water at 95 °C, allowing the average extraction of 24.5% of the compounds. No significant difference was observed in the extraction between the temperatures of 65 °C, 75 °C, and 85 °C, which correspond to the average values of 16.2%, 13.2% and 16.2%, respectively. However, this extraction percentage is still higher than the extracts obtained from the traditional yerba mate, which had values oscillating between 5.9% and 8.1%. Regarding the traditional yerba mate, the results indicated that the increase in temperature did not affect significantly ($p > 0.05$) the transfer of the phenolic compounds studied to the aqueous extracts of *chimarrão*. The different behavior between the different types of herbs on increasing the temperature may be related to the effect of the greater compaction of the traditional yerba mate because of its smaller particle size, as previously mentioned. It is possible that the greater compaction has hindered the contact of the leaves with the infusion water, reducing the extractability, and, in turn, the effect of the infusion temperature may have been suppressed.

Figure 1 shows the content of the compounds accumulated in the aqueous extracts throughout the extractions, simulating the concentration of the phenolic compounds ingested by consumers. Confirming the results observed, it is apparent that the extracts prepared with coarse ground yerba mate had a higher average amount of phenolic compounds than the ones obtained with traditional yerba mate. In addition, the extracts prepared with coarse ground and water at 95 °C stood out from the others, showing higher amounts ($p < 0.05$) of all phenolic compounds investigated, which could be attributed to the higher solubility of these in boiling or near boiling water temperature (De Maria et al., 1998). Therefore, our results suggest that the preparation of *chimarrão* with coarse-ground yerba mate and infusion at 95 °C is an alternative of a better contribution of phenolic compounds compared with the traditional one. However, in spite of this result, the other temperatures also provided high amounts of these compounds.

Gebara et al. (Gebara et al., 2017) estimated an average daily intake of 648-2160 mL/person of *chimarrão*, which could provide between 512.5-1708.0 mg of chlorogenic acids (mono and dicaffeoylquinic acids). Our results are in accordance with these ranges of volume (30 extracts) and phenolic compounds extraction. Yerba mate is a main source of chlorogenic acids for the diet, along with coffee. One coffee serving corresponding to 200 mL provides between 44 mg and 241 mg of 5-CQA, 0.84 and 13.67 mg of 3,4-DQA, 0.512 and 9.374 mg of 3,5-DQA, and 0.704 and 12.356 mg of 4,5-DQA (Jeon et al., 2017). According to Table 2, with the exception of 5-CQA, these amounts are

obtained with the consumption of 10 extracts and are highly superior if one considers the consumption of 30 extracts (which comprises the average volume/person of *chimarrão*), regardless of the herb or the infusion temperature chosen. In this regard, *chimarrão* extracts can be highlighted as a remarkable source of dicaffeoylquinic compounds. For example, Marques and Farah (Marques & Farah, 2009) studied 14 medicinal plant infusions and found that even though 5-CQA was present in every species, dicaffeoylquinic acids were detectable only in *Baccharis genistelloides* ("carqueja"), seeds of *Pimpinella anisum* (anise), and flowers of *Achyrocline satureioides* ("macelle"), but in amounts still greatly lower than those in *chimarrão* extracts. In another study, Meinhart et al. (2018) investigated the content of chlorogenic acids in 89 plant infusions, and demonstrated that 5-CQA was the most commonly found compound from this family (72% of the samples) in ranges between 0.002 mg.100 mL⁻¹ and 6.349 mg.100 mL⁻¹. The content of dicaffeoylquinic acids found was from traces of 4,5-DQA until 13.334 mg.100mL⁻¹ (3,5-DQA), demonstrating that yerba mate infusions such as *chimarrão* could provide greatly higher amounts of these compounds.

Chimarrão extracts have also been pointed out as a good source of the flavonoid rutin and according to Bizzotto et al. (Bizzotto et al., 2012), yerba mate is one of the richest sources of this compound in nature. The levels of rutin in *chimarrão* extracts found in this study are in accordance with those from Colpo et al. (2016), who found 19 mg and 12.85 mg of rutin in 1000 mL of *chimarrão* extracts (approximately 10 extracts) from coarse ground and traditional yerba mate, respectively, prepared at 75 °C. Jeszka-Skowron et al. (2015) reported that 200 mL of white, black and green tea (*Camelia sinensis*), which are important sources of flavonoids in the diet, supplied on average 0.527 mg, 4.4 mg and 5 mg, respectively, of rutin. Therefore, the content found in 10 extracts of traditional or coarse ground yerba mate, at any studied temperature, provides between 10 and 20 times more rutin than tea.

The beneficial biological effects of the intake of CGAs have already been reported in several studies. For example, according to Wan and colleagues (Wan et al., 2013), the intake of 1 to 10 mg of chlorogenic acids per gram of body weight per day has beneficial effects on lipid metabolism leading to a significant reduction of total cholesterol, LDL and HDL. Considering the data in Table 2, a consumer who ingests, per day, only the first 10 extracts of aqueous *chimarrão* extract, prepared with traditional yerba mate and water at 65 °C (condition with the lowest average content extracted), has the contribution of 159 mg daily (considering only chlorogenic acids) which corresponds to 2.27 mg per kg per day in a 70 kg individual. On the other hand, the consumer who ingests 30 extracts has the intake of 3.30 mg per kg per day. The consumer who ingests the first 10 extracts of *chimarrão*, with coarse-ground yerba mate and water at 95 °C, has a daily intake of 6.74 mg per kg per day, or 12.7 mg per kg per day if the ingestion is of 30 extracts. As seen in Figure 1, the first extracts are the most relevant and represent a large part of the extraction. Consumers who ingest a lower amount of the beverage, such as the first 5 extracts, also have a satisfactory intake. If the first 5 extracts prepared with traditional yerba mate and water at 65 °C are ingested, the intake is 1.76 mg per body weight per day. If the ingestion is of extracts from coarse

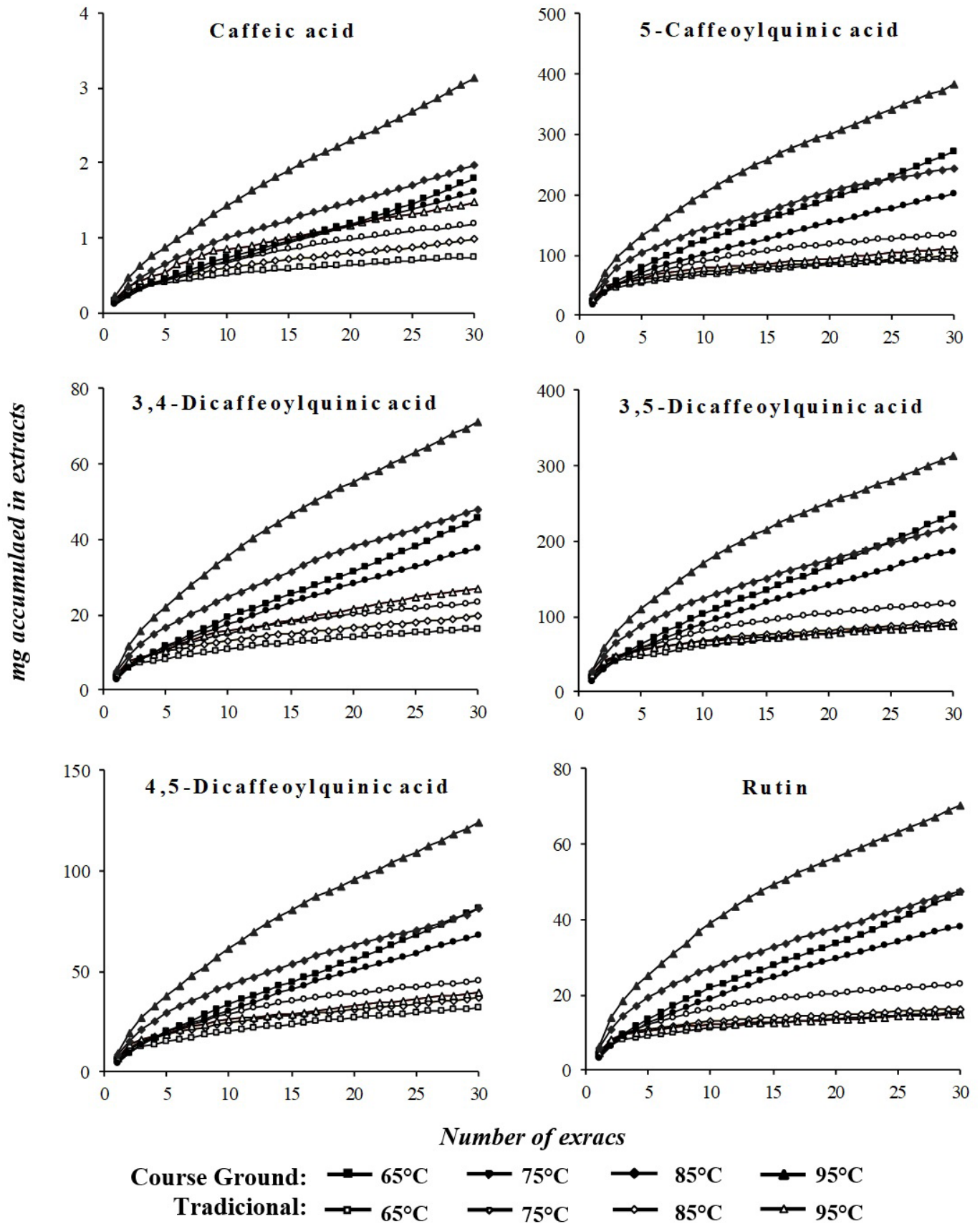


Figure 1. Content of bioactive compounds extracted cumulatively during the extraction of the 30 extracts of *chimarrão*, using different temperatures, in course ground and traditional yerba mate

Table 2. Sum of the content of phenolic compounds in the first 10 *chimarão* extracts (mg) and in 30 *chimarrão* extracts (mg).

Coarse ground yerba mate										
Compound	mg in 10 <i>chimarrão</i> extracts					mg in 30 <i>chimarrão</i> extracts				
	65 °C	75 °C	85 °C	95 °C	Average	65 °C	75 °C	85 °C	95 °C	Average
Caffeic acid	0.7	0.7	1.0	1.4	1.0	1.8	1.6	2.0	3.1	1.8
5-CQA	124.7	101.2	142.6	203.4	143.0	271.6	202.0	244.5	382.4	271.6
3,4-DQA	19.2	17.5	24.6	35.6	24.2	45.7	37.5	48.0	71.3	45.7
3,5-DQA	103.0	90.4	124.6	171.2	122.3	236.0	187.0	220.4	313.2	236.0
4,5-DQA	33.6	30.8	43.1	61.9	42.3	81.4	67.9	81.3	124.1	81.4
Rutin	21.9	19.1	27.2	39.0	26.8	47.1	38.3	47.5	70.2	47.1
<i>Total phenolics</i>	<i>303.1</i>	<i>259.7</i>	<i>363.0</i>	<i>512.5</i>	<i>359.6</i>	<i>683.7</i>	<i>534.3</i>	<i>643.7</i>	<i>964.3</i>	<i>683.7</i>
Traditional yerba mate										
Compound	mg in 10 <i>chimarrão</i> extracts					mg in 30 <i>chimarrão</i> extracts				
	65 °C	75 °C	85 °C	95 °C	Average	65 °C	75 °C	85 °C	95 °C	Average
Caffeic acid	0.5	0.7	0.6	0.8	0.7	0.7	1.2	1.0	1.5	1.1
5-CQA	67.1	90.1	72.9	78.9	77.2	94.2	134.5	98.8	110.7	109.6
3,4-DQA	10.9	15.0	13.1	15.7	13.7	16.3	23.2	19.6	27.2	21.6
3,5-DQA	60.7	81.0	68.3	67.3	69.3	88.6	117.9	92.4	88.1	96.7
4,5-DQA	20.2	29.0	24.5	26.1	25.0	32.2	45.2	36.9	39.8	38.5
Rutin	11.2	16.2	13.0	12.1	13.1	15.4	22.8	16.4	15.0	17.4
<i>Total phenolics</i>	<i>170.7</i>	<i>232.1</i>	<i>192.4</i>	<i>200.9</i>	<i>199.0</i>	<i>247.4</i>	<i>344.9</i>	<i>265.1</i>	<i>282.2</i>	<i>284.9</i>

ground yerba mate with water at 95 °C, the average value is 4.3 mg per day per body weight.

Therefore, our results suggest that all beverages obtained from the two studied yerba mates at all evaluated temperatures may be considered excellent sources of chlorogenic acid intake at concentrations that may have a beneficial biological effect on lipid metabolism, according to the study by Wan and collaborators (Wan et al., 2013). However, it is important to mention that the ingested amount of phenolic compounds does not represent the amount that is bioavailable and that will be biologically active in the body. In this regard, a recent study by Gómez-Juaristi et al. (2018) demonstrated that chlorogenic acids bioavailability (mono and dicaffeoylquinic acids) in humans is low. Thus, further studies are recommended in order to correlate the amounts of chlorogenic acids ingested through mate-based beverages and health-promoting effects.

4 Conclusions

The results showed that the increase in water temperature significantly affected the phenolic compounds content in the aqueous extracts prepared with coarse ground yerba mate, with the extracts prepared at 95 °C showing the highest concentration of all phenolic compounds studied. For the traditional extracts, the difference between temperatures was not statistically significant. In general, irrespective of the water temperature used, the aqueous extracts of *chimarrão* produced from traditional yerba mate presented a lower content of all the phenolic compounds than the coarse ground (60% less on average). However, chlorogenic acids content present in traditional and coarse

ground extracts of yerba mate, regardless of the temperature used, has significantly higher concentrations than other foods and beverages, evidencing *chimarrão* as an excellent alternative for the supply of chlorogenic acids.

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