Food Chemistry 190 (2016) 1159–1167

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Phenolic compounds from yerba mate based beverages – A multivariate optimisation

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ARTICLE INFO

Article history:
Received 27 October 2014
Received in revised form 10 June 2015
Accepted 11 June 2015
Available online 24 June 2015

Keywords:
Chimarrão
Tererê
Multivariate optimisation
Derringer and Suich optimisation

ABSTRACT

This work used a central composite design to optimise a reverse phase high performance liquid chromatographic method for the simultaneous separation of gallic, syringic, 5-caffeoylquinic, caffeic, p-coumaric, ferulic, 3,4-dicaffeoylquinic, 3,5-dicaffeoylquinic, 4,5-dicaffeoylquinc acids, rutin in aqueous extracts of yerba mate (*Ilex paraguariensis*). The effect of the linear gradient time, the initial and the final methanol concentration in the mobile phase on the peak resolution and peak symmetry was evaluated. The 26 responses obtained were simultaneously optimised using the desirability method of Derringer and Suich. According to results, the increasing in the resolution and peak symmetry was achieved by using lesser levels of methanol in both initial and final gradient elution (−1.68, −1), as well as higher gradient times (+1, +1.68). The optimal condition (13.9–40% of methanol in 39.4 min) were successfully applied for analysis of *chimarrão*, *tererê* and mate tea aqueous extracts, which showed as excellent sources of chlorogenic acids.

1. Introduction

Yerba mate is a native plant from South America, whose dry or roasted leaves and branches are widely used to prepare infusions such as *chimarrão*, *tererê* and yerba mate tea. *Chimarrão* is characterised as a partial infusion in which yerba mate is partially immersed in hot water and is prepared in a typical artefact called a *cuia*. *Tererê*, in turn, consists of a traditional infusion in which the yerba mate leaves are totally immersed in cold water, using a *cuia*, jar or common cup. Both beverages are ingested using a metal straw with a flattened bottom extremity containing small orifices for the passage of the liquid (“*bomba*”). However, yerba mate tea is prepared by the total infusion of toasted yerba mate leaves in hot water (Bastos, Oliveira, Matsumoto, Carvalho, & Ribeiro, 2007). These three beverages are widely consumed in South American countries, especially in the South of Brazil, in Argentina, Paraguay, Uruguay and Bolivia, where the estimated daily intake of yerba mate-based beverages is 1 litre per consumer (Bastos et al., 2007).

Yerba mate (*Ilex paraguariensis* St. Hilaire) is a food that has been highlighted as a dietary source of phenolic compounds, especially chlorogenic acids, a family of compounds formed by isomers of esters of caffeic acid with quinic acid (Marques & Farah, 2009; Peres, Tonin, Tavares, & Rodriguez-Amaya, 2013). The literature also reports the presence of gallic acid, caffeic acid, syringic acid, ferulic acid, p-coumaric acid and rutin (Bizzoto et al., 2012; Frizon, Perusselli, Sturion, Fracasso, & Hoffman-Ribani, 2015; Nunes et al., 2015; Pagliosa et al., 2010). These compounds are associated to the health benefits that are possibly promoted by their frequent consumption, including the protection of cell membranes, lipoproteins and DNA (Bracesco, Sanchez, Contreras, Menini, & Gugliucci, 2011; Filip, Lolito, & Ferraro, 2000; Gugliucci, 1996; Heck & Mejia, 2007).

Phenolic compounds in yerba mate extract have been determinated by different separation methods. For instance, Pomilio, Trajtemberg, and Vitale (2002) and Bizzoto et al. (2012) employed capillary electrophoresis (CE) to determinate 1 (5-caffeoylquinic acid) and 3 (rutin, caffeic acid and 3,4-dihydroxybenzoic) phenolic compounds, respectively, in yerba mate aqueous extracts. However, an overview on the related literature indicates that high-performance liquid chromatography (HPLC) is still the most employed technique to determine phenolic compounds in yerba mate extracts, as well as in other food matrix, probably due to some advantages of HPLC over CE. It is known that HPLC presents higher robustness, reproducibility and sensitivity, as well as an

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http://dx.doi.org/10.1016/j.foodchem.2015.06.031
0308-8146/

interface easily coupled to a great range of detectors, such as mass spectrometer (Ibañez, Simó, García-Cañas, & Cifuentes, 2013; Isbell, Strickland, Hitchcock, & McIntire, 2015; Lee & Ong, 2000). Moreover, none of these studies comprised the chlorogenic acid isomers (5-caffeoylquinic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid), which are the most important phenolic compounds related to yerba mate.

The methods of HPLC described in the literature for the determination of phenolic compounds in yerba mate extracts exhibited the simultaneous separation of 2 to 9 compounds with a time of analysis ranging from 20 min (for 2 and 4 separated compounds) to 70 min (for 10 separated compounds). For this type of experiment, the concentration used for the organic solvent (methanol or acetonitrile) in the mobile phase ranged between 40% and 85% (Anesini, Turner, Cogoi, & Filip, 2012; Dugo et al., 2009; Dutra & Ribani, 2010; Filip, Lopéz, Giberti, Coussio, & Ferraro, 2001; Heck, Schmalko, & Meija, 2008; Isolabella et al., 2010; Pagliosa et al., 2010; Peres et al., 2013; Valerga, Reta, & Lanari, 2012; Nunes et al., 2015).

However, according to the consulted literature, to date, the chromatographic conditions for the separation of such compounds have been defined by univariate studies for the optimisation of this method, i.e., by modifying a single chromatographic parameter at a time and evaluating the effect of each variable individually on the studied system. Nevertheless, these univariate methods require a lot of time and a large number of experiments, they are not capable of providing information on the possible iterative effects among the investigated variables and do not allow adequate statistical treatment of the acquired data, which may compromise the reliability of the results for the optimisation (Rodrigues & Iemma, 2005). Furthermore, the optimisations frequently result in chromatographic methods with inefficient separations and a long time for analysis, which results in greater equipment wear and high costs with solvents, human resources and residue treatment (Rodrigues & Iemma, 2005).

A solution that has proven to be efficient for this matter consists of the use of multivariate statistical tools that allow, with a reduced number of experiments, the determination of chromatographic conditions with optimised performances parameters, such as the resolution between peaks, symmetry, their width and height, and the reduction of time for analysis and costs, in addition to decreasing the use of toxic solvents (Ballus, Meinhart, Bruns, & Godoy, 2011).

Yet, in the process of optimising a chromatographic method for many compounds, it is necessary to observe a high number of responses, for which it is very unlikely that the optimum region for all of them will be the same. In this case, the methodology of simultaneous optimisation proposed by Derringer and Suich (1980) becomes a valuable statistical tool because, with the use of mathematical models, it allows the optimisation of a great number of responses and, thus, an appropriate, global answer for the objectives of the research (Ballus et al., 2011).

In this context, the need of exploring multivariate statistical techniques to allow the development of a chromatographic method with optimised criteria for the determination of phenolic compounds in yerba mate-based beverages is of great importance, with special attention drawn to the 3 major dicaffeoylquinic isomers in the plant (3,4-, 3,5- and 4,5-dicaffeoylquinic), which are compounds that are difficult to separate.

Thus, the purpose of the present study was to optimise and validate a method for the separation and quantification of 10 phenolic compounds present in aqueous extracts of yerba mate-based beverages by HPLC. Additionally, the optimised method was employed to determine the content of phenolic compounds present in the aqueous extracts of *tererê*, yerba mate beverage and *chimarrão* that were prepared with yerba mate with different characteristics regarding the composition of branches, leaves and particle size, which were obtained as if the beverage consumer had prepared it.

2. Materials and methods

2.1. Reagents and samples

To prepare the mobile phases, chromatography-grade methanol (Merck, Germany) and analytical-grade formic acid (Ecibra, Brazil) were used. The water was purified using a Milli-Q® (Millipore) system. The standards for the phenolic compounds gallic acid, 5-caffeoylquinic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, rutin and 4,5-dicaffeoylquinic acid were obtained from Biopurify Phytochemicals Ltd. (Chengdu, China). The standard stock solutions were prepared in methanol at a concentration of 1 mg mL^{-1} and kept at −18 °C for a maximum of 1 month. The mobile phases and samples were filtered using polyvinylidene (PVDF) membranes with 0.22 μm (Millipore) porosity for organic solvents and cellulose for aqueous solutions.

To optimise the methodology, 2 units of yerba mate for *chimarrão* were obtained in their traditional and commercial packages, in supermarkets in the metropolitan region of Campinas. The contents of the packages were mixed and homogenised, and the extracts were obtained by preparing a yerba mate infusion according to item 2.5.

2.2. Instrumentation

The separation of the 10 studied phenolic compounds was performed using an Agilent Technologies (Germany) HPLC system model 1100, coupled to a diode array detector, quaternary pump system, HP (Hewlett Packard, Germany) automatic injector and oven for the temperature control. The analytical column used was a reverse phase C18 ACE (Switzerland) that was 100 mm in length and 4.6 mm in diameter with a 3 μm particle size. Detection was performed at 280 nm (for gallic acid and syringic acid) and at 325 nm (for caffeic, 5-caffeoylquinic, p-coumaric, ferulic, 3,4-dicaffeoylquinic, 3,5-dicaffeoylquinic and 4,5-dicaffeoylquinic acids and for rutin). The initial and final concentrations of methanol in the mobile phase and the time of linear gradient (time spent between the initial and final concentration of methanol, in a linear progression rate) were optimised. HP-Chemstation software was used for the data analysis.

2.3. Multivariate optimisation and data treatment

Through a 2^3 central composed design with central and axial points, the effects of the following parameters were investigated: the initial concentration of methanol ranging from 10% to 20% (which correspond to the levels codified from −1.68 and + 1.68); the final concentration of methanol between 40% and 60%; and the linear gradient time between both concentrations, from 20 to 40 min, corresponding to the same coded levels (1.68 and +1.68). These values were limited based on the related literature (Filip et al., 2001; Heck et al., 2008; Marques & Farah, 2009) and preliminary tests performed to determinate the more suitable solvent range to promote the elution of all the phenolics compounds studied.

The tests of the central composed design were performed using aqueous extracts of yerba mate for *chimarrão* added to the standards of phenolic compounds at a concentration of 10 mg L^{-1}. The purpose of this procedure was to verify that the optimum experimental condition obtained for the separation of the

compounds would not be affected by the matrix effect, which could hinder the application of the method in real samples.

Four repetitions were performed at the central point, for a total of 18 experiments. Before the analysis of each of the multivariate design conditions, the chromatographic column was conditioned for 30 min with the initial composition of the mobile phase of its respective experimental level. Between the tests for each experimental condition, the re-equilibrium of the column was performed for 10 min to return to the initial conditions of the gradient. The tests were performed by injecting 30 μL of yerba mate aqueous extract containing the 10 investigated compounds. The elution system was in a linear gradient with a mobile phase composed of methanol and an aqueous solution of formic acid 0.1% (v/v). The flow rate of the mobile phase was 0.8 mL min^{-1}, and the temperature of the column was kept at 30 °C.

As a result, the chromatographic criteria chosen were the resolution (R) (Equation 1) between the adjacent peaks and the symmetry value (directly provided by the HP-Chemstation software). Next, the optimum condition for the separation of the 10 phenolic compounds studied was determined using the technique of simultaneous response optimisation by Derringer and Suich. Hence, the desired values were established for each individual result and combined to establish a global desirability.

$$R = \frac{2(t2 - t1)}{w2 + w1}. \quad (1)$$

where R represents the resolution, t1 e t2 is retention time of adjacent peaks and w1 e w2 the width of them.

The individual desirability were defined to maximise the resolution between the peaks that presented co-elution in the investigated experimental levels, as well as the symmetry, according to Eqs. (2)–(4). Therefore, Rtarget = 3 was defined, a value that already represents an efficient chromatographic separation; for the symmetry, Rtarget = 1, the greatest possible symmetry value for a chromatographic peak. For both of the chromatographic criteria, Rmin was determined as the greatest resolution and the greatest experimentally verified symmetry for the corresponding pair of peaks.

For the pairs of peaks already separated by the experimental conditions of the design, it was established that the resolution could vary freely (in range) between the Rmin and Rtarget values. This means that the algorithm by Derringer and Suich used the mathematical models to indicate the resolution of these pairs of peaks at the chromatographic conditions for which the desired criteria for the symmetry and resolution of the co-eluted peaks were maximised. Thus, the lowest resolution found in the experimental tests for each pair of peaks was considered for Rmin. For Rtarget, in turn, the highest experimentally obtained resolution was taken into consideration. Furthermore, the minimisation of the linear time of the gradient variable was required, with the purpose of achieving shorter times of analysis.

$$di = 0 \text{ se } Ri < Rimin \quad (2)$$

$$di = \left(\frac{Ri - Rimin}{Rtarget - Rimin}\right), \text{ se } Rimin \leqslant Ri \leqslant Rtarget \quad (3)$$

$$di = 1 \text{ se } Ri > Rtarget \quad (4)$$

where di corresponds to desirability i between 0 and 1, Ri are the predicted values by the models for i resolutions or symmetry, Rtarget are the maximum values for resolution or symmetry, Rimin is the minimal value for resolution or symmetry.

The mathematical methods were evaluated by the variance analysis, and the optimisation of the chromatographic conditions was performed using the algorithm by Derringer and Suich from the Design Expert 6.0 (Stat-Ease, Minneapolis, USA) program with 95% reliability.

2.4. Evaluation of the figures of merit from the chromatographic method

Once the method was optimised, its figures of merit were evaluated according to the recommendations of the Harmonised Guide and the National Health Surveillance Agency (Agência Nacional de Vigilância Sanitária – ANVISA) (BRASIL & de 29 de maio de, 2003; IUPAC, 2002). The limits of detection and quantification were determined by the successive dilution of the standard solutions and were estimated as 3 and 5 times the signal/noise ratio, respectively. The established ground for the limit of quantification was the lowest concentration level with an acceptable relative coefficient of variation for the evaluated concentration (20%). Analytical curves, including 8 concentration levels for each studied compound, were randomly created at different equidistant concentration ranges, in triplicate. The linearity of the analytical curves was evaluated, and the models were validated by analysis of variance (ANOVA) with the respective verification of the adjustments. The method's precision was assessed by injecting a standard solution containing the 10 compounds at concentrations that probe the linear interval of the method, at three different concentrations for each compound, including the limit of quantification, the centre of the analytical curve and its maximum concentration. To evaluate the repeatability, 10 determinations were performed in the same day (n = 10). The intermediate precision was evaluated by five determinations at the same concentration levels for the repeatability, on three different days (n = 3).

2.5. Acquisition of the samples and preparation of the aqueous extracts

The optimised, validated method was applied for the determination of phenolic compounds in yerba mate-based beverages, including aqueous extracts of *chimarrão, tererê* and yerba mate tea.

The yerba mate samples used to obtain *chimarrão* and *tererê* were politely provided by Ervateria Vier, located in Rio Grande do Sul, Brazil. The samples were named according to their different characteristics regarding their percentage composition in branches, native and cultivated leaves, sugar and different particle sizes, including smooth, native, traditional, coarse grinded and yerba mate for *tererê*. The latter contained only *I. paraguariensis* leaves and stems (with no addition of other plants). Ten kilograms of each type of yerba mate were provided in the packages commonly used for commercialisation (500 g for *tererê* and 1000 g for *chimarrão*). The contents of the respective yerba mate packages were mixed and homogenised before being used for the preparation of *chimarrão* or *tererê* infusions.

The yerba mate aqueous extracts were obtained from commercialised in-bulk tea (loose leaves) from 3 different brands, acquired in supermarkets in the metropolitan region of Campinas. The teas had a particle size of approximately 500-mesh and were studied in its grain size to represent the conditions of preparation by the consumer.

The production of the *chimarrão* and *tererê* aqueous extracts was performed according to Meinhart et al. (2010), who reproduced the manner in which such beverages are usually prepared by consumers. Hence, for *chimarrão*, 85 g of yerba mate was vertically disposed in a *cuia* of average size (approximately 250 mL), occupying nearly 2/3 of its volume. The "bomba", the equipment used to suction the beverage, was positioned and fixed in the remaining space of the *cuia* and coupled to a vacuum system. Next, the *cuia* was filled with water at 75 °C and kept in a partial infusion for 30 s, after which the resultant yerba mate aqueous extract was suctioned with the vacuum system. The extract was

collected, chilled, filtered in common filter paper, diluted and injected in the HPLC.

For the preparation of the *tererê*, 50 g of yerba mate for *tererê* was weighed, followed by the addition of 180 mL of water at 11 ± 2 °C. The herb was kept in total immersion for 30 s. The extracts were collected and prepared for analysis in an analogous way for *chimarrão*.

The mate tea beverages were prepared according to the instructions from the manufacturer's label, weighing 2 g of tea, which is the amount corresponding to a sachet. Next, 300 mL of water at 90°C was added, and the infusion was left to rest for 4 min, resulting in the yerba mate aqueous extract, representing the beverage that would normally be ingested by the consumer. The extracts were then transferred to volumetric flasks and brought to a total volume of 500 mL with water. The content of the flask was filtered with common filter paper, followed by filtration in cellulose paper with a porosity of 0.22 μm (Millipore, Brazil), and then injected into the HPLC for determining the content of the studied compounds.

All of the beverages were prepared in triplicate.

2.6. Statistical treatment of the data

The obtained results were compared by analysis of variance (ANOVA) and Tukey's test in Statistica 6.0 (Statsoft Inc., 2001). The samples were considered to be significantly different when $p < 0.05$.

3. Results and discussion

3.1. Multivariate optimisation

From the execution of the multivariate design, 26 answers were obtained by considering the values of the resolution among the compounds of interest and also between them and the interferent peaks: gallic acid/interferent 1 (GAL/INT1), interferent 2/5-caffeoylquinic acid (INT2/5CQ), 5-caffeoylquinic acid/interferent 3 (5CQ/INT3), interferent 3/caffeic acid (INT3/CAF), caffeic acid/syringic acid (CAF/SIR), syringic acid/p-coumaric acid (SIR/CUM), p-coumaric acid/ferulic acid (CUM/FERR), ferulic acid/3,4-caffeoylquinic acid (FERR/3,4DQ), 3,4-caffeoylquinic acid/3,5-dicaffeoylquinic acid (3,4DQ/3,5DQ), 3,5-dicaffeoylquinic acid/rutin, (3,4DQ/RUT), rutin/4,5-dicaffeoylquinic acid (RUT/4,5DQ), and 4,5-dicaffeoylquinic acid/interferent 4 (INT4/4,5DQ). Additionally, the value of the 14 symmetries referent to each one of the peaks was also used as an answer. Table 1 summarises the results obtained in each experimental condition.

Linear and quadratic models were created for each of the responses. The simplest model that presented proper adjustment was used to perform the prediction of the optimal conditions by using Derringer and Suich. Table 2 presents the significant coefficient in each model and their respective ANOVA results, as well as the adjustment test of each of the models.

All the responses presented a highly significant regression (Bruns, Barros Neto, & Scarminio, 2010) with the exception of 5CQ/INT3, SIR/CUM resolution and gallic acid (GAL), rutin (RUT) and 4,5-dicaffeoylquinic acid (4,5DQ) peak symmetry. There was no evidence for a lack of fit of the models ($p > 0.05$), except for the peaks 4,5DQ/INT4. However, the diagnostic residuals vs. predicted value graph for this response did not present evidence that the residual behaviour was not normal or suffered from heteroscedasticity. Moreover, the ANOVA indicated that the quadratic mean caused by pure error value (MSpe) for this pair of peaks resulted in a much lower value (0.009) in comparison to the MSpe for the other pairs of peaks. So, the *F* value for the model fit

Table 1
Levels of the studied variables and experimental responses.

Experiment	Variables and codified levels			Responses for resolution											Responses for simmetry										
	Initial concentration of methanol in the gradient	Final concentration of metanol in the gradiente	Time (min)	Galic/ Interferent 1	Interferent 2/5-Cafeoylquinic	5Caffeoylquinic/ Interfernt 3	Interferent 3/ Caffeic	Caffeic/ Siryngic	Siryngic/ p-coumaric	p-coumaric/ Ferulic	Ferulic/3,4-Dicaffeoylquinic	3,4dicaffeoyl-quinic/ 3,5dicaffeoyl-quinic	3,4dicaf-feoylquinic/ Rutin	Rutin/ 4,5dicaf-feoylquinic	4,5dicaf-feoylquinic/ interferent 4	Galic	Interferent 1	Interferent 2	5caffeoyl-quinic	Interferent 3	Caffeic	Siryngic	*p*-coumaric	Ferulic	3,4dicaf-feoylquinc	3,5dicaf-feoylquinic	Rutin	4,5dicaf-feoylquinic	Interferent 4
1	12.04	44.04	24	12.8	21.2	5.7	5.2	5.5	19.6	7.6	18.2	1.6	7.4	11.5	7.2	0.43	0.70	0.55	0.67	0.73	0.76	0.85	0.81	0.86	0.83	0.67	0.69	0.70	0.68
2	17.97	44.04	24	7.2	14.6	4.8	6.4	4.1	20.2	6.7	17.9	1.6	7.3	13.8	7.6	0.49	0.72	0.52	0.52	1.33	0.72	0.79	0.80	0.81	0.77	0.61	0.80	0.69	0.92
3	12.04	55.95	24	12.6	20.1	5.5	5.9	4.7	18.6	6.2	12.4	1.5	7.9	8.6	8.4	0.43	0.72	0.57	0.69	0.84	0.81	0.80	0.80	0.83	0.89	0.67	0.74	0.73	0.84
4	17.97	55.95	24	7.0	13.7	4.5	6.0	3.4	18.9	5.7	12.7	1.4	7.3	9.3	7.7	0.50	0.65	0.50	0.52	1.34	0.67	0.72	0.78	0.80	0.90	0.61	0.72	0.71	0.84
5	12.04	44.04	35.9	13.0	22.4	5.1	4.2	6.9	20.2	9.3	25.3	1.8	6.9	16.4	7.0	0.42	0.70	0.54	0.63	0.40	0.80	0.84	0.85	0.84	0.80	0.67	0.84	0.75	0.85
6	17.97	44.04	35.9	6.9	14.4	4.9	6.5	4.3	20.0	7.8	24.2	1.7	6.4	17.7	6.3	0.50	0.63	0.48	0.52	1.18	0.71	0.72	0.79	0.84	0.78	0.60	0.85	0.73	0.87
7	12.04	55.95	35.9	12.8	21.4	5.4	4.9	5.7	19.8	8.0	19.7	1.7	7.2	12.3	7.8	0.42	0.70	0.56	0.66	0.68	0.78	0.82	0.82	0.87	0.82	0.67	0.71	0.71	1.01
8	17.97	55.95	35.9	7.2	14.5	4.8	6.4	4.0	19.7	6.8	18.4	1.6	7.1	13.4	7.2	0.49	0.68	0.49	0.52	1.30	0.72	0.74	0.78	0.81	0.84	0.61	0.75	0.70	0.87
9	10	50	30	15.8	23.2	5.2	4.2	6.2	19.5	8.1	19.1	1.6	7.4	11.6	7.3	0.44	0.74	0.58	0.68	0.52	0.78	0.83	0.81	0.85	0.83	0.68	0.68	0.71	0.66
10	20	50	30	5.3	11.7	4.6	6.3	3.1	18.9	6.0	16.9	1.5	6.8	12.9	7.2	0.47	0.67	0.48	0.47	1.39	0.66	0.74	0.77	0.79	0.77	0.57	0.69	0.66	0.86
11	15	40	30	9.3	18.5	5.8	6.3	5.4	20.5	8.5	24.6	1.8	6.8	17.2	6.8	0.51	0.69	0.52	0.58	0.70	0.72	0.82	0.84	0.86	0.80	0.65	0.85	0.74	0.85
12	15	60	30	9.1	17.4	5.0	6.2	4.4	19.1	6.6	15.0	1.5	7.6	10.5	7.6	0.52	0.69	0.52	0.61	0.97	0.73	0.76	0.81	0.84	0.86	0.63	0.76	0.69	0.70
13	15	50	20	9.2	17.1	4.9	6.1	4.3	19.4	6.0	12.5	1.5	7.7	9.2	8.6	0.52	0.71	0.53	0.62	1.20	0.80	0.81	0.78	0.82	0.88	0.66	0.72	0.71	0.88
14	15	50	40	9.1	18.3	5.7	6.3	5.3	20.3	8.3	23.6	1.8	6.9	16.4	6.8	0.55	0.70	0.51	0.58	0.69	0.72	0.82	0.83	0.85	0.78	0.63	0.82	0.70	0.86
15	15	50	30	8.6	17.6	5.3	6.4	4.9	19.6	7.4	18.9	1.6	7.2	12.7	7.8	0.56	0.69	0.51	0.57	0.81	0.76	0.77	0.81	0.84	0.78	0.64	0.70	0.69	0.95
16	15	50	30	9.1	18.0	5.4	6.6	4.8	19.3	7.4	19.0	1.6	7.4	13.2	7.7	0.52	0.69	0.52	0.60	0.78	0.77	0.75	0.80	0.84	0.79	0.65	0.79	0.71	0.95
17	15	50	30	9.2	16.7	5.1	6.2	4.7	18.9	7.4	19.2	1.6	7.0	12.6	7.7	0.42	0.69	0.49	0.48	0.82	0.73	0.73	0.78	0.82	0.78	0.65	0.70	0.70	0.95
18	15	50	30	9.6	17.9	5.4	6.5	4.8	19.2	7.3	19.0	1.6	7.3	13.1	7.5	0.42	0.69	0.52	0.60	0.75	0.76	0.75	0.80	0.84	0.80	0.65	0.80	0.70	0.95

Table 2
Statistical model coefficients and F-distribution parameters for model validation.

Responses	Indicated model	Intercept	Coefficients									
			A	B	C	A^2	B^2	C^2	AB	AC	BC	Regression significance ($p<0.05$)	Model fit ($p>0.05$)
GAL/INT1	Quadratic	9.11	−2.96			0.58						<0.0001	0.6275
INT2/5CQ	Linear	17.70	−3.46	−0.35	0.37							<0.0001	0.9140
5CQ/INT3	Linear	5.18	−0.28									0.0129	0.1070
INT3/CAF	Quadratic	6.43	0.64			−0.45			−0.21	0.30		0.0002	0.2012
CAF/SIR	Linear	4.80	−0.90	−0.34	0.36				0.13	−0.19		<0.0001	0.2478
SIR/CUM	Quadratic	19.22		−0.40	0.29		0.20	0.21			0.21	0.0033	0.7673
CUM/FER	Quadratic	7.37	−0.55	−0.58	0.70	−0.09	−0.05	−0.08	0.07	−0.16		<0.0001	0.0951
FER/3.4DQ	Quadratic	19.04	−0.44	−2.82	3.30	−0.37	0.27	−0.34		−0.32		<0.0001	0.0558
3.4DQ/3.5DQ	Quadratic	1.61	−0.03	−0.08	0.08	−0.01	0.01			−0.01		<0.0001	0.7876
3.5DQ/RUT	Linear	7.19	−0.17	0.20	−0.26							<0.0001	0.8675
RUT/4.5DQ	Quadratic	12.89	0.56	−1.98	2.10	−0.24	0.32					<0.0001	0.4384
4.5/INT4	Linear	7.44		0.32	−0.41							0.0003	0.0289
GAL	a	a										a	0,9330
INT1	a	a										a	0,0033
INT2	Linear	0.52	−0.03		−0.01							<0.0001	0.7462
5CQ	Linear	0.58	0.07									<0.0001	0.9946
INT3	Quadratic	0.79	0.29	0.07	−0.11	0.07		0.06				<0.0001	0.0645
CAF	Linear	0.75	−0.03									0.0014	0.2498
SIR	Quadratic	0.75	−0.03	−0.01				0.02				0.0034	0.4231
CUM	Linear	0.8	−0.01		0.01							0.0013	0.3702
FER	Linear	0.83	−0.01		0.01							0.0003	0.1911
3.4DQ	Linear	0.79		0.02	−0.02		0.01	0.01			−0.01	0.0017	0.0960
3.5DQ	Quadratic	0.65	−0.03			−0.01						<0.0001	0.3946
RUT	Quadratic	0.74		−0.03	0.02							0.0563	0.8689
4.5DQ	Quadratic	0.70	−0.01				0.01					0.0552	0.1013
INT4		0.95				−0.05						0.1484	<0.0001

GAL: galic acid; INT1: interferent 1; INT2: interferent 2; 5CQ: 5-caffeoylquinic acid; INT3: interferent 3; CAF: caffeic acid; SIR: syringic acid; CUM: *p*-coumaric acid; FER: ferulic acid; 3,4DQ: 3,4-dicaffeoylquinc acid; 3,5DQ: 3,5-dicaffeoylquinic acid; RUT: rutin; 4,5DQ: 4,5-dicaffeoylquinic acid; INT4: interferent.
a: regression non significative, at 95% of confidence level.

Table 3
Desirability conditions employed for the simultaneous optimisation of phenolic compounds resolutions and simmetry. Predicted responses by the models and values experimentally observed.

Variable/Response	Goal	Inferior limit	Superior limit	Importance	Predicted	Observed
% initial MeOH	In range	−1.68	1.68	1	–	–
% Final MeOH	In range	−1.68	1.68	1	–	–
Time	Minimise	−1.68	1.68	3	–	–
GAL/INT1	In range	5	30	1	10.71	11.11
INT2/5CQ	In range	2	30	1	20.13	20.61
5CQ/INT3	In range	5	30	1	5.60	5.08
INT3/CAF	In range	5	30	1	4.89	4.67
CAF/SIR	In range	4	30	1	6.46	6.37
SIR/CUM	In range	3	30	1	20.99	20.49
CUM/FER	In range	5	30	1	9.67	9.83
FER/3.4DQ	In range	5	30	1	29.38	31.27
3.4DQ/3.5DQ	Maximise	1.9	3	5	1.94	1.97
3.5DQ/RUT	In range	5	30	1	6.50	5.95
RUT/4.5DQ	In range	5	30	1	20.31	19.79
4.5/INT4	In range	5	30	1	6.29	5.57
INT2	Maximise	0.4	1	3	0.52	0.53
5CQ	Maximise	0.4	1	3	0.58	0.60
INT3	Maximise	0.4	1	3	0.51	0.42
CAF	Maximise	0.4	1	3	0.75	0.75
SIR	Maximise	0.4	1	3	0.84	0.83
CUM	Maximise	0.4	1	3	0.86	0.83
FER	Maximise	0.4	1	3	0.83	0.87
3.4DQ	Maximise	0.4	1	3	0.64	0.79
3.5DQ	Maximise	0.4	1	3	0.98	0.65

GAL: galic acid; INT1: interferent 1; INT2: interferent 2; 5CQ: 5-caffeoylquinic acid; INT3: interferent 3; CAF: caffeic acid; SIR: syringic acid; CUM: *p*-coumaric acid; FER: ferulic acid; 3,4DQ: 3,4-dicaffeoylquinc acid; 3,5DQ: 3,5-dicaffeoylquinic acid; RUT: rutin; 4,5DQ: 4,5-dicaffeoylquinic acid; INT4: interferent 4.

(Table 2) of this response could be overestimated as a result of underestimation of its MSpe value, resulting in the observed lack of fit (Ballus et al., 2011). Since it did not adversely affect the search for the optimum conditions and there was a good agreement between the predicted and observed values, we decided to use this model to perform the prediction using Derringer and Suich optimisation.

According to the Table 1 and Table 2, it was verified that the linear and quadratic increase of the initial methanol percentage parameter (coefficient of regression A) caused the reduction of the symmetry and of the resolution between peaks. Thus, the use of lower concentrations from the experimental region (−1.68, −1) was indicated to promote a higher efficiency of separation and higher symmetry. Similarly, for the final methanol

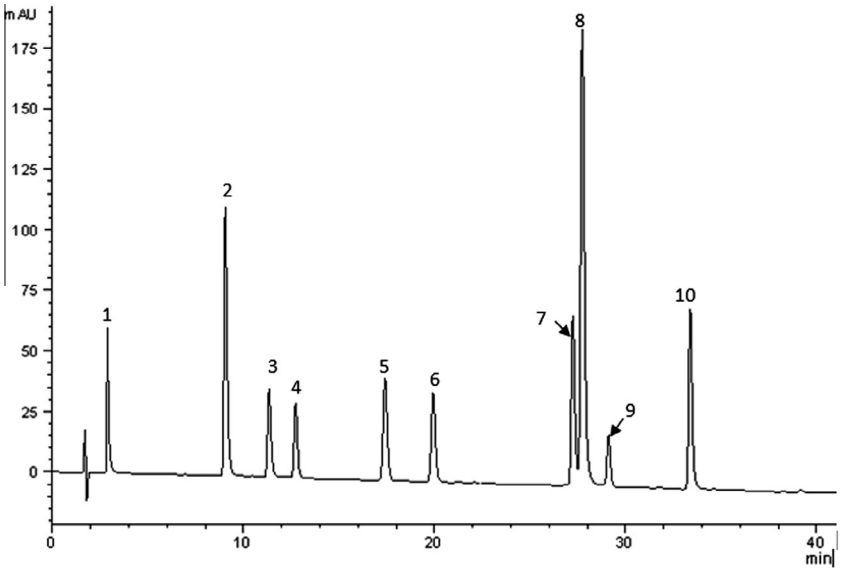

Fig. 1. Chromatographic profile of phenolic compounds standards for aqueous extracts of *chimarrão*, *tererê* and mate tea after method optimisation and validation. (1) Galic acid, (2) 5-caffeoylquinc acid, (3) caffeic acid, (4) syringic acid, (5) *p*-coumaric acid, (6) ferulic acid, (7) 3,4-dicaffeoylquinic acid, (8) 3,5-dicaffeoylquinic acid, (9) rutin, (10) 4,5-dicaffeoylquinic acid.

Table 4
Figures of merit for method validation.

Compounds	Limit of Detection (mg L^{-1})	Limit of quantification (mg L^{-1})	Linearity			Precision[a]		
			Linear range (mg.L^{-1})	R^2	Model Fit ($p > 0.05$)	Concentration (mg L^{-1})	Repeatabilityn = 10	Intermediate precision $n = 3$
Galic acid	0.003	0.015	0.1–7.0	0.9992	0.2650	0.015	21.29	17.47
						4.0	0.57	0.77
						7.0	0.72	0.36
5-CQ acid	0.013	0.063	0.3–25.0	0.9991	0,8542	0.063	19.21	9.87
						12.0	0.56	0.94
						21.0	0.33	0.30
Caffeic acid	0.013	0.063	0.1–7.0	0.9989	0.7781	0.063	4.52	17.99
						4.0	0.50	0.78
						7.0	0.25	0.13
Siyringic acid	0.025	0.125	0.1–7.0	0.9994	0.6671	0.125	9.08	6.96
						4.0	1.13	0.70
						7.0	0.56	0.16
p-Coumaric acid	0.025	0.125	0.1–7.0	0.9985	0.9747	0.125	12.86	8.44
						4.0	0.44	0.74
						7.0	0.27	0.30
Ferulic acid	0.006	0.030	0.1–7.0	0.9991	0.7961	0.030	13.49	12.61
						4.0	0.25	0.84
						7.0	0.36	0.28
3.4 DQ acid	0.006	0.030	0.2–14.0	0.9983	0.7064	0.030	12.19	8.7
						8.0	0.62	1.02
						14.0	0.46	0.54
3.5-DQ acid	0.013	0.063	0.5–35.0	0.9979	0.9724	0.063	12.26	20.68
						20.0	0.21	0.59
						35.0	0.34	0.48
Rutin	0.013	0.063	0.1–7.0	0.9984	0.4530	0.063	19.32	18.41
						4.0	1.08	1.88
						7.0	0.90	0.11
4.5-DQ acid	0.006	0.030	0.2–14.0	0.9983	0.9692	0.030	18.80	18.25
						8.0	0.72	0.85
						14.0	0.75	0.80

R^2 = determination coefficient. 5-CQ acid: 5-caffeoylquinic acid; Ácido 3,4DQ: 3,4-dicaffeoylquinic acid, 3,5DQ acid: 3,5-dicaffeoylquinic acid; 4,5DQ acid: 4,5-dicaffeoylquinic acid.

[a] Data for precision are expressed in terms of variation coefficient of área under the peak (%).

concentration variable, the negative coefficients (B) of linear regression indicated that the increase of this variable caused decrease of the resolution between peaks.

Regarding the effect of the linear time of the gradient of the mobile phase, represented by C, the coefficients in general presented a positive signal, indicating that the increase in this variable

Table 5
Content of phenolic compounds present in aqueous extracts of mate tea beverage. "*tererê*" and "*chimarrão*" prepared from yerba mate with different composition in branches and leaves.

Infusion	Type of herb/ Brand	Concentration (mg.100 mL^{-1})[A]								
		Leaves of native plants	Leaves of cultivated plants	Branches	Sugar	5-caffeoylqyinic acid	Caffeic acid	3.4-dicaffeoylquinic acid	3.5-dicaffeoylquinic acid	Rutin	4.5-dicaffeoylquinic acid
Chimarrão	Course- ground	60%	40%	26%	–	26.88 ± 4.36^{a}	0.15 ± 0.03^{a,b}	4.29 ± 0.78^{a}	21.82 ± 3.86^{b}	4.49 ± 0.73^{a}	6.99 ± 1.13^{c}
	Traditional	70%	30%	27%	–	25.10 ± 1.93^{a}	0.19 ± 0.05^{b}	4.52 ± 0.67^{a}	22.57 ± 0.94^{b}	4.99 ± 1.26^{a}	6.98 ± 0.42^{c}
	Smooth	70%	30%	24%	10%	27.95 ± 5.82^{a}	ND	4.83 ± 0.81^{a}	35.4.47 ± 8.76^{a}	5.82 ± 1.79^{a}	10.84 ± 2.29^{b}
	Native	100%	-	27%	-	24.62 ± 3.29^{a}	0.21 ± 0.01^{a}	6.43 ± 1.51^{b}	38.19 ± 2.29^{a}	4.47 ± 0.61^{a}	18.31 ± 1.78^{a}
Tererê	*Tererê*	-	100%	27%	-	3.66 ± 0.16^{c}	0.04 ± 0.005^{c}	0.70 ± 0.007^{b}	7.16 ± 0.51^{c}	1.93 ± 0.07^{b}	1.80 ± 0.03^{d}
Mate tea	Brand A		NI[B]			4.9 ± 0.16^{d}	ND[C]	0.61 ± 0.02^{b}	0.69 ± 0.07^{d}	NQ[D]	1.27 ± 0.06^{e}
beverage	Brand B					4.61 ± 0.2^{d}	ND	0.57 ± 0.12^{b}	0.61 ± 0.03^{d}	NQ	1.22 ± 0.1^{e}
	Brand C					0.45 ± 0.05^{e}	ND	0.06 ± 0.004^{c}	0.06 ± 0.006^{e}	NQ	0.09 ± 0.007^{f}

[A] Equal lower letters in the same column represent samples that do not differ at a 95% confidence level.
[B] Not informed by manufacturer.
[C] ND: not detected.
[D] NQ: Not quantified.

resulted in the increase of resolution, most likely because a longer linear gradient time favoured a higher interaction between the compounds and the chromatography column. For the pair of peaks 3,4DQ/3,5DQ that presented the most difficulty of separation, the results demonstrated that the resolution between these peaks was increased by the use of a lower initial percentage of solvent (A) and longer linear time of gradient (C). This adjustment resulted in a greater retention and number of interactions of such compounds with the stationary phase, which positively affected its efficiency for separation.

After the simultaneous optimisation of the responses employing the algorithm by Derringer and Suich, the optimum chromatographic conditions found were an initial methanol concentration in the gradient of 13.9%, final concentration of 40% and linear gradient time of 39.4 min. The values for the desirability conditions and the responses predicted by the models for the resolutions and symmetries presented good concordance with the values experimentally obtained, as can be observed in Table 3.

Hence, with only 18 experiments, it was possible to establish the optimised chromatographic conditions, which resulted in the efficient separation of the 10 phenolic compounds studied. Peaks 3,4DQ/3,5DQ, which presented greater difficulty of separation because they are isomers, were also efficiently separated. In comparison to the methods reported in the literature, for the same number of compounds, the optimum chromatographic conditions obtained in this study resulted in a chromatographic method with 50% shorter time of analysis and 50% less final concentration of organic solvent in the mobile phase. Therefore the use of the present chromatographic method for determination of phenolic compounds in aqueous extracts of yerba mate will result in a reduction of the analysis costs, in minor use of organic solvent and lesser generation of toxic residues. Fig. 1 presents the chromatographic profile of the phenolic standards after the optimisation.

3.2. Figures of merit from the chromatographic method

The method was successfully validated, and the figures of merit for the linearity, precision and limits of detection and quantification are presented in Table 4.

The analysis of variance showed that there was significant linear regression in the studied concentration ranges and that the regression equations did not present a lack of fit ($p > 0.05$), proving to be appropriate for quantifications. The results indicated that the developed chromatographic method was adequate for the determination of the 10 phenolic compounds of the study, in accordance with the validation requirements (BRASIL, 2003; IUPAC, 2002).

3.3. Application in samples

In Table 5, the composition and characteristics of the studied yerba mate samples can be observed, as well as the concentrations of the phenolic compounds present in the yerba mate tea beverages and aqueous extracts prepared from yerba mate for *chimarrão* and *tererê*.

It was observed that for all of the samples, derivatives of caffeic acid (caffeoylquinic and dicaffeoylquinic acids) were major compounds in the composition of the aqueous extracts, of which 5-caffeoylquinic acid and 3,5-dicaffeoylquinic acid demonstrated two of the highest concentrations, in agreement with the literature on the subject (Bastos, Fornari, Queiroz, Soares, & Torres, 2005; Marques & Farah, 2009).

The contents of all of the compounds present in the aqueous *chimarrão* and *tererê* extracts were markedly superior ($p < 0.05$) to those observed in mate tea beverage, as reported by Marques and Farah (2009) and Bastos et al. (2005). These divergences may be linked to the amount of herb used to prepare such beverages

(85 g is used for *chimarrão* prepare, approximately 2 g for mate tea beverage). Additionally, regarding the rutin content, Silveira, Meinhart, Ballus, and Godoy (2014) determined that infusion temperatures of the mate tea beverage above 80 °C promote the degradation of this compound, which may have contributed to their non-quantifiable levels in the beverages analysed in this study, as they were prepared with water at 90 °C.

Among the yerba mates used for *chimarrão* (traditional, coarse grinded, smooth and native herbs), the results indicated the absence of significant differences ($p > 0.05$) between the content of 5-caffeoylquinic acid and the rutin. In turn, the extracts obtained from yerba mate Native (constituted only by native leaves) presented the highest levels of caffeic, 3,4-dicaffeoylquinic, 3,5-dicaffeoylquinic and 4,5-dicaffeoylquinic acid. These results are in contrast with those obtained by Meinhart et al. (2010), who observed that the content of total phenolic compounds did not differ significantly between the aqueous *chimarrão* extracts prepared from herbs with characteristics similar to the ones from this study.

Moreover, the aqueous *tererê* extracts presented significantly lower concentrations ($p < 0.05$) of all of the tested compounds in comparison to the *chimarrão* extract. This finding may be linked to the fact that *tererê* is prepared with cold water (11 °C). Thus, the water used at higher temperatures for the preparation of the other beverages may have favoured the transfer of the compounds from the plants to the infusion water, especially that of the chlorogenic acids, whose transfer is favoured at temperatures close to 100 °C (Farah & Donangelo, 2006; Cacace & Mazza, 2003).

In comparison to other beverages, a cup of coffee (100 mL) contains 30 mg of 5CQ acid, 2.4 mg of 3,4DQ acid, 1.62 mg of 3,5DQ acid and 1.45 mg of 4,5DQ acid (Duarte & Farah, 2011). The same volume of *chimarrão* extract contains approximately the same concentration of 5-CQ acid, twice the concentration of 3,4DQ acid, 15 times more 3,5DQ acid and six times more 4,5DQ acid. In turn, for yerba mate tea, the consumption of 300 mL may correspond to the levels of chlorogenic acids present in 50 mL of coffee (Duarte & Farah, 2011; Tagliazucchi & Verzelloni, 2012).

Moreover, it is important to highlight that the data in Table 5 refer to the consumption of only 100 mL of *chimarrão*. However, an assiduous consumer of this beverage ingests approximately one litre of *chimarrão* or *tererê* every day, resulting in the ingestion of such compounds in high concentrations. Thus, this study corroborates that yerba mate-based beverages are superior sources of dietary phenolic compounds, especially for chlorogenic acids.

4. Conclusions

The multivariate statistical techniques employed proved to be efficient tools because they promoted the maximisation of the resolution and symmetry of the peaks of interest, as well of the maximisation of the resolution of the time of analysis. The obtained mathematical methods were useful to describe the modifications in the resolution and in the symmetry of the studied compounds that were caused by changes in the investigated variables. The multivariate optimisation performed in the food matrix added to the standard proved to be a good alternative to guarantee the applicability of the method in a real sample.

The optimum separation condition was an initial methanol concentration in the gradient of 13.9%, a final concentration of 40% and a linear gradient time of 39.4 min. The method presented time of analysis and methanol concentration in the mobile phase lower than the one found in other methods from the literature. The validation study presented satisfactory results, with the conclusion that the method is adequate for the determination of the 10 phenolic compounds studied in yerba mate beverages.

The present study reported novel unpublished data on the chemical composition of *chimarrão, tererê* and yerba mate tea aqueous extracts, demonstrating that the methods used by consumers to prepare these beverages favour the production of beverages with high concentrations of phenolic compounds, especially chlorogenic acids.

Acknowledgments

We would like to thank FAPESP – Fundação de Amparo à Pesquisa do Estado de São Paulo (process no. 2012/12565) and CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior for their financial support of this study.

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