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The effect of the duration of infusion, temperature, and water volume on the rutin content in the preparation of mate tea beverages: An optimization study

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ABSTRACT

The consumption of tea beverages has increased 30% over the last decade, mainly due to the presence of bioactive compounds. Mate tea, produced by infusing the leaves and stems of *Ilex paraguariensis,* is the most widely consumed beverage in Brazil. The present study employed a central composite experimental design to optimize the transfer of rutin from the leaves and stems to the beverage during the infusion process. The optimum condition was applied to three batches of mate tea beverages from five commercial samples. Analysis of the rutin content was performed by high performance liquid chromatography coupled to a photo diodo array detector. The maximum rutin content in the beverage was obtained when the infusion was performed using 2 g of mate tea added to 100 mL of water at 72 °C and infused for 9 min. The commercial tea beverages prepared under these conditions contained from 0.16 to 1.1 mg of rutin in the ready-to-drink product.

1. Introduction

Tea-based beverages are among the most popular in the world. Their consumption increased by approximately 30% over the last decade; this increase can be primarily attributed to the *in vitro* and *in vivo* studies that demonstrated the inverse relationship between consumption and the risk of degenerative diseases (Bhattacharya, Mukhopadhyay, & Giri, 2011; Mackay & Blumberg, 2002; Martins et al., 2009). The health benefits associated with the intake of these beverages have been correlated with the presence of flavonoids and other phenolic compounds, which are the most important compounds in tea and have potent antioxidant and free radical scavenging activities (Astill, Birch, Dacombe, Humphrey, & Martin, 2001; Atoui, Mansouri, Boskou, & Kefalas, 2005; Matsubara & Rodriguez-Amaya, 2006).

The preparation of mate tea beverages consists of infusing previously ground and roasted leaves and stems of the *Ilex paraguariensis* St. Hill plant, which is native to South America (Souza, 2009). In Brazil, according to the Brazilian Institute of Geography and Statistics (Instituto Brasileiro de Geografia e Estatística – IBGE), the *per capita* consumption of tea in 2008–2009 was 0.500 kg/year, of which 0.482 kg was mate tea (IBGE, 2012). The leaves and stems of *Ilex paraguariensis* are rich in rutin, a flavonoid (flavonol) consisting of a quercetin molecule, linked to a disaccharide, namely, rhamnose and glucose (Pedriali, 2005). This compound is the primary flavonoid in this plant and is found in concentrations ranging from 0.3 mg g^{-1} to 0.5 mg g^{-1} (Filip, Lopéz, Giberti, Coussio, & Ferraro, 2001; Ribani, 2006).

In vitro and *in vivo* studies have shown that rutin has potent antioxidant, anti-inflammatory, and hepatoprotective activities (Ajay, Anwar-Ul, & Mustafa, 2003; Amira, Rotondo, & Mulè, 2008; Kazłowska, Hsu, Hou, Yang, & Tsai, 2010; Martins et al., 2009; Moon & Kim, 2012; Shenbagam & Nalini, 2011; Yang, Guo, & Yuan, 2008; Zhou, Yao, Cao, Jiang, & Xia, 2006). Additionally, several human studies have highlighted the effectiveness of this compound and its derivatives in the treatment of vascular diseases (Petruzzellis et al., 2002; Unkauf, Rehn, Klinger, De La Motte, & Grossmann, 1996). Thus, rutin has been widely used as a component in many drugs intended primarily for patients with vascular disease (Erlund, 2004; Ihme et al., 1996; Jeong et al., 2009).

Several univariate studies have shown that the method of preparing beverages from other teas, like green and white tea, as well as the plant growth and processing conditions, directly alters the chemical composition of teas (Komes, Horzic, Belscak, Kovacevic, & Vulic, 2010; Nishiyama et al., 2010; Rusak, Komes, Likíc, Horzic, & Kovac, 2008). The transfer rate of compounds from the tea to the beverage may be affected by the amount of leaves or stems used, the particle size, the water volume, the temperature, the presence or absence of stirring, the duration of infusion, and the use of additional ingredients, such as sugar or milk. The effect of different preparation methods on the chemical composition of the beverage ingested by the consumer is important because the beneficial properties associated with the consumption of these beverages can be directly correlated to the components extracted from the tea leaves (Astill et al., 2001; Nishiyama et al., 2010).

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Given that mate tea has high levels of rutin, the consumption of beverages prepared from this plant could be an important source of this compound in the human diet. No studies found in the literature discussed optimizing the preparation conditions of beverages produced from the hot infusion of mate tea to achieve the maximum efficiency of rutin extraction in the ready-to-drink product. According to Bruns, Guadagnini, Scarminio, and Barros Neto (1997) and Ballus, Meinhart, Bruns, and Godoy (2011) multivariate experimental designs can be efficiently used to optimize processes and to investigate the effects of independent variables and the interaction effects among them.

Thus, the present work aimed to employ a multivariate experimental design to optimize the preparation conditions for mate tea beverages and achieve the maximum transfer of rutin from the tea to the beverage that is typically prepared for and ingested by the consumer.

2. Materials and methods

2.1. Reagents and samples

The rutin standard was purchased from Sigma Chemical (USA). A stock solution was prepared in methanol at a concentration of 1 mg mL^{-1} and was stored at −18 °C. Analytical grade formic acid was purchased from Ecibra (Brazil), and chromatographic grade methanol was purchased from JT Baker (Germany). The mate tea samples were purchased in supermarkets in the city of Campinas, SP, Brazil.

2.2. Multivariate optimization

The present study used a 2^3 central composite design with 17 trials, including three true replicates at the center point. The effects of three variables were investigated: the duration of infusion of the leaves, the water temperature, and the water volume used for the preparation of the beverages, which were highlighted as important determinants of the component concentration for tea beverages (Astill et al., 2001; Komes et al., 2010; Nishiyama et al., 2010). The ranges studied were from 3.8 to 15.0 min for the duration of infusion (corresponding to levels −1.68 and +1.68), 58.1 to 90 °C for the water temperature, and 100 to 400 mL for water volume. These ranges were selected based on the values usually employed by consumers when preparing the mate tea beverage. The detailed levels are shown in Table 1. The evaluated response was the rutin content in the final, ready-to-drink beverage. All experiments were conducted in random order.

The mate tea (toasted leaves and stems of *I. paraguariensis*) used to prepare the beverages used in the central composite design was obtained from a mixture of either loose or sachet commercial teas from the two most largely commercialized mate tea brands (one unit of loose and sachet tea for every commercial brand). The teas had particle sizes of approximately 500 mesh and were studied at that size to represent the preparation conditions that would be used by the consumer. To prepare the beverages, 2 g of tea was weighed, which is the approximate amount contained in a sachet. Hot water was added to the tea, with the leaves and stems staying totally immersed in hot water, and the infusion remained at rest. After the infusion period, the teas were immediately cooled to room temperature and transferred to volumetric flasks. The contents of the flasks were filtered through filter paper (Whatman n° 1), followed by filtration through Millipore 0.45-μm cellulose filters (Brazil). The filtered solution was injected into the high performance liquid chromatography apparatus to measure the rutin content.

2.3. Analysis of the rutin content by high performance liquid chromatography and validation of the method

A high performance liquid chromatography (HPLC) apparatus (HP 1100, Hewlett Packard, Germany) was used. The HPLC apparatus consisted of a diode array detector, quaternary pump system, autosampler, and column temperature control oven (30 °C). The chromatographic separation was performed on a Microsorb-MV C18 reversed-phase column (5 μm × 250 mm × 4.6 mm, VARIAN, USA). The elution mode was isocratic with a mobile phase consisting of water - aqueous formic acid (0.1% v/v), and methanol (47.5:2.5:50.0 v/v/v). The flow rate was maintained at 1 mL min^{-1} with a run time of 10 min. The injection volume was 50 μL. The rutin in the sample was identified by comparison with the retention time of the standard, the UV-visible absorption spectra of the standard, and co-chromatography. The mobile phases were filtered through 0.45-μm cellulose membranes (Millipore, Brazil). The integrations were performed using Chemstation software (Hewlett Packard, Germany). Quantitation was performed with an external standard calibration detected at 360 nm.

The chromatographic method was validated using the validation rules set by the Brazilian National Health Surveillance Agency (Agência Nacional de Vigilância Sanitária - ANVISA, 2012) with respect to the intra-day precision parameters (10 measurements of the same sample throughout the day), between-day precision (the quantification of the same sample on three different days), linearity (between 5 equidistant points at the concentrations of 0.5 and 5.0 mg mL^{-1}, observing the model-fitting through Analysis of Variance - ANOVA, 95%), and

Table 1
Variables, experimental levels, and concentration of rutin in beverages prepared in the central composite design trials.

Experiment	Codified variables			Decodified variables			Rutin content (mg of rutin in the final beverage)
	Time (x_1) (minutes)	Temperature (x_2) (°C)	Volume (x_3) (mL)	x_1	x_2	x_3	
1	−1	−1	−1	3.8	58.1	160.7	0.192
2	1	−1	−1	12.2	58.1	160.7	0.207
3	−1	1	−1	3.8	81.9	160.7	0.167
4	1	1	−1	12.2	81.9	160.7	0.165
5	−1	−1	1	3.8	58.1	339.3	0,231
6	1	−1	1	12.2	58.1	339.3	0.235
7	−1	1	1	3.8	81.9	339.3	ND [b]
8	1	1	1	12.2	81.9	339.3	ND
9	0	0	0	8	70	250	0.222
10	0	0	0	8	70	250	0.261
11	0	0	0	8	70	250	0.239
12	−1.68	0	0	1	70	250	ND
13	0	1.68	0	8	50	250	0.149
14	0	0	−1.68	8	70	100	0.189
15	1.68	0	0	15	70	250	0.147
16	0	1.68	0	8	90	250	ND
17	0	0	1.68	8	70	400	0.217

[b] ND: content below the detection limit.

detection and quantification limits (estimated at 3 and 10 times the signal-to-noise ratio, respectively).

2.4. Application to the samples

The optimum condition found by the multivariate design was used to prepare the beverages from 2 g of tea. The rutin content was investigated in beverages obtained from three brands of mate tea samples sold in individual sachets; three batches of each brand were studied. Similarly, three batches of beverages obtained from two brands of mate tea sold in bulk (loose leaves and stems) were evaluated for a total of 15 samples.

2.5. Statistical treatment

The data analysis was performed using the Statistica 7.0 software (Statsoft, USA), as well as the calculus of the critical points, which was important to provide the optimal conditions of preparing the mate tea beverages. The multivariate optimization results were analyzed using analysis of variance (ANOVA) to assess the fit of the mathematical model to the experimental data. After the rutin was quantified in the commercial tea samples, the means were compared by ANOVA and Fisher's test to determine whether there were significant differences among the brands, batches, and types of packaging (sachet or loose). The samples were considered significantly different at $p < 0.05$.

3. Results and discussion

3.1. Validation of the chromatographic method

The method for the separation and quantification of rutin was validated and was found to be suitable for quantification according to the ANVISA standards (ANVISA, 2012). For the intra-day precision studies, a relative standard deviation of 2.7% ($n = 10$) was obtained, whereas for the between-day precision, a value of 3.0% ($n = 3$) was obtained. The rutin retention times varied by less than 1.0% ($n = 10$). The method was shown to be linear in the 0.5 to 5.0 mg L^{-1} range ($r^2 = 0.9996$). The analysis of variance revealed that the regression was significant in this range and that the mathematical model showed no lack of fit ($p > 0.05$), demonstrating that this method is suitable for these measurements. The detection and quantification limits were 0.092 mg L^{-1} and 0.3 mg L^{-1}, respectively.

3.2. Optimization of the preparation conditions for mate tea beverages

Table 1 shows the studied variables, the experimental levels, and the concentrations of rutin present in the beverages prepared under the experimental conditions of the central composite design. The rutin concentrations ranged from non-measurable (below the quantification limit of the method) to 0.26 mg of rutin in the ready-to-drink beverage.

The generated regression coefficients of the model of the rutin concentration response are shown in Table 2. The regression for the quadratic model was significant at the 95% confidence level, and the residuals were shown to be random with no evidence of heteroscedasticity. The ANOVA (Table 3) results indicated that the quadratic model showed no lack of fit for the data because the obtained F value (6.34) was lower than the $F_{5,2}$ value (19.3). Thus, the whole mathematical model was used to explain the effects of the variables on the system and to predict the optimum condition for preparing tea beverages to obtain the maximum rutin content.

Table 2
Significant regression coefficients for the coded variables of the quadratic model of the rutin concentration response.

Parameters		Coefficient	Standard error	p^a
b_0	Mean	0.238	0.011	0.002
b_2	Temperature	−0.045	0.005	0.013
b_1^2	Time	−0.043	0.005	0.017
b_2^2	Temperature	−0.043	0.005	0.017
$b_2 \times b_3$	Temperature x Volume	−0.039	0.007	0.029

[a] significant at the 95% confidence level.

Table 3
Analysis of variance of the values for content of rutin of mate tea beverage prepared in the experimental conditions.

Factor	Sum of square	DF	Mean square	F value	p value[a]
Regression	0.0853	9	0.0095	5.2778	0.0241
Residual	0.0124	7	0.0018		
Lack of fit	0.0117	5	0.0023	6.3333	0.1413
Pure error	0.0007	2	0.0004		
Total SS	0.0977	16			

[a] significant at the 95% confidence level.

The negative quadratic effect of the infusion duration indicated that increasing this variable favored rutin extraction up to maximum level, after which there was a decrease. This effect can be observed in the experiments at the central point and the axial points for this variable: the upper axial point, with an infusion duration of 15 min, had half of the rutin concentration obtained at the central point (8 min infusion). Thus, the results showed that prolonged infusion reduced the concentration of rutin in the beverage. Komes et al. (2010) and Perva-Uzunalic et al. (2006) used univariate models to study the effect of the infusion time on the catechin concentration when preparing green tea. Both studies found that longer green tea infusion times favored the transfer of these compounds to the beverage. However, in agreement with the data obtained in the present study, the authors concluded that long infusions (over 20 min) resulted in the degradation of these compounds.

The infusion water temperature was another important variable for the efficient transfer of the rutin from the tea to the beverage. The rutin concentration continued to increase when using temperatures at the lower levels, −1 and 0 (58.1 °C and 70 °C). According to Cacace and Mazza (2003), raising the temperature increases the solubility and the diffusion coefficient of phenolic compounds, thereby providing higher extraction rates.

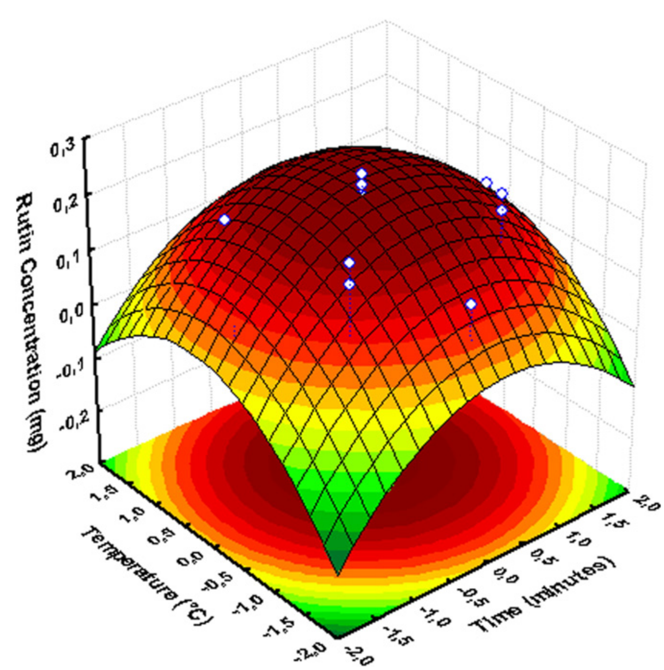

Fig. 1. Response surface for the rutin concentration (mg of rutin in the final beverage). Variables: Time (minutes) × Temperature (°C), with the volume fixed at 100 mL.

Table 4
Concentration of rutin in the commercial samples of mate tea beverages.

Rutin content (mg 100 mL^{-1})			
Brand	Batch	Package	
		Sachet	Loose leaves
A	1	0.130 ± 0.002^{a}	0.119 ± 0.006 ^{b}
	2	0.123 ± 0.003 ^{b}	0.101 ± 0.011 ^{b}
	3	0.118 ± 0.004 ^{c}	0.135 ± 0.003 ^{a}
	Mean	**0.124 ± 0,006**$^{a, \beta}$	**0.11 ± 0.013**$^{a, \upsilon}$
B	1	0.042 ± 0.004 ^{b}	0.047 ± 0.002 ^{b}
	2	0.051 ± 0.008 ^{a}	0.046 ± 0.021 ^{b}
	3	BNF [y]	0.104 ± 0.002 ^{a}
	Mean	**0.045 ± 0,006**$^{b,\gamma}$	**0.046 ± 0.0007** $^{b, \sigma}$
C	1	0.245 ± 0.008 ^{c}	UM[x]
	2	0.593 ± 0.019 ^{a}	
	3	0.540 ± 0.041 ^{b,C}	
	Mean	**0.419 ± 0.246**$^{\alpha}$	

Different lower letters, in the columns, for batches of the same brand, indicate significant difference ($p < 0.05$) between samples.
Different lower letters in italic, in the lines for mean of three batches, indicate significant difference ($p < 0.05$) between different packages of commercialization.
Different Greek e letters, in the columns, for mean of three batches, indicate significant difference ($p < 0.05$) between samples.
[x] unmarketed by the manufacturer.
[y] BNF: Batch not found.

However, when the highest levels of the infusion temperatures, 1.0 and 1.68 (81.9 °C and 90 °C), were used, the concentration of rutin declined and even reached unquantifiable levels. This negative quadratic effect is possibly associated with the degradation of this flavonoid. This interpretation is in agreement with the studies of Zhou, Sun, Du, Liang, and Yang (2000), who evaluated the stability of rutin and found that it begins to degrade at 75 °C.

Regarding the effect of the interaction between the temperature and water volume, it was found that at mild temperatures (levels −1 and 0), increasing the volume of water used to prepare the infusion resulted in a beverage with a higher rutin content. However, when both the temperature and water volume were high, lower rutin concentrations were found in the beverage. This degradation effect can be explained by the slower rate of heat loss in beverages prepared with larger volumes of water (Nishiyama et al., 2010).

After obtaining a mathematical model with a good fit, it was possible to optimize the conditions for preparing the beverage by the infusion of mate tea. Fig. 1 shows the response surface generated for the variables temperature and duration of infusion. The optimum conditions for preparing the beverages were as follows: 9.0 min duration of infusion, water temperature at 72 °C, and water volume of 100 mL. Under these conditions, the model predicted a yield of 0.21 (±0.027) mg of rutin in the beverage. The experimental confirmation was performed in triplicate, and 0.25 (±0.004) mg of rutin was obtained in the beverage. The hypothesis test used to compare the values showed that at a 95% confidence level, there was no significant difference between the value predicted by the model and the value obtained experimentally.

3.3. Application to commercial samples of mate tea

The optimum conditions for preparing mate tea beverages to obtain the maximum efficiency of the rutin extraction were applied to commercial samples, and the results are presented in Tables 4. Fig. 2 shows a chromatography profile of a mate tea beverage prepared under the optimized conditions.

The concentration of rutin in 100 mL of the mate tea beverage ranged from 0.046 to 0.593 mg for the different commercial samples. The results showed significant differences ($p < 0.05$) in the concentration of rutin among beverages prepared from different batches of the same brand. Likewise, the rutin content varied greatly among the beverages obtained from the different brands. The significant differences observed may be attributed to variations in the composition of the plants due to different growing and processing conditions (Astill et al., 2001; Heck & Mejia, 2007; Lin, Tsai, Tsay, & Lin, 2003; Mazzafera, 1997; Pagliosa et al., 2010). Nevertheless, there was no significant difference among the beverages with respect to the type of packaging (sachet or loose).

Compared to other beverages, the level of rutin in 100 mL of mate tea beverages found in the present study is much higher than the contents present in several beverage source of phenolic compounds as Brazilian Syrah wine (0.24 mg 100 mL^{-1}, Ballus, Meinhart, Oliveira, & Godoy, 2012), noni juice (0.188 mg 100 mL^{-1}, Deng, West, & Jensen, 2010), and acerola juice (0.058 mg 100 mL^{-1}, Mezadri, Villaño, Fernandez-Páchon, García-Parrilla, & Troncoso, 2008). These data show mate tea beverage as good source of rutin for human diet. In 100 mL of chimarrao, the main beverage prepared from yerba mate (which consists of dried and ground leaves of *Ilex paraguariensis*), there is approximately 5 mg of rutin (Ribani, 2006). The different processing methods and chemical reactions resulting from roasting the leaves of *Ilex paraguariensis* could explain the observed differences (Bastos, Fornari, Queiroz, & Torres, 2006; Matsubara & Rodriguez-Amaya, 2006; Turkumen Erol, Sari, Çalikoðlu, & Velýoðlu, 2009).

4. Conclusions

The use of response surface methodology with a central composite design was an important tool for evaluating the influence of the studied variables on the concentration of rutin in mate tea beverages and elucidating the effects of the interaction among the variables. Using only 17

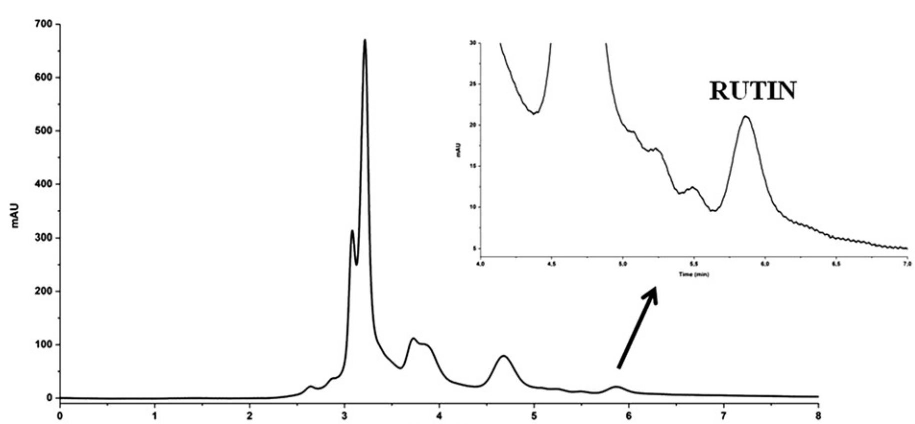

Fig. 2. Chromatography profile of mate tea water extract. (1) Rutin. Chromatographic conditions: C18 (5 μm × 250 mm × 4.6 mm, VARIAN, USA). Mobile phase: water – aqueous formic acid (0.1% v/v), and methanol (47.5:2.5:50.0 v/v/v). In isocratic elution and flow rate = 1 mL min^{-1}.

experiments, it was possible to determine that the optimal conditions for preparing beverages from 2 g of mate tea were as follows: an infusion duration of 9.0 min, water temperature at 72 °C, and water volume of 100 mL. The beverages prepared under these conditions contained between 0.16 and 1.1 mg of rutin in 100 mL.

Among flavonoid-rich foods, beverages prepared from the leaves and stems of mate tea have significant levels of rutin, and consuming these beverages may help supply this substance in the human diet.

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