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Relation among taste-related compounds (phenolics and caffeine) and sensory profile of erva-mate (*Ilex paraguariensis*)

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Abstract

Erva-mate (*Ilex paraguariensis* St. Hil.) is a native species from temperate regions of South America, such as Brazil, Paraguay, and Argentina, that is consumed as a beverage known as mate. The objective of this research was to determine the content of caffeic acid, catechin, chlorogenic acid, caffeine, and gallic acid in mate to explain their influence in beverage taste and sensory differences between native and reforested plants, as well as between beverages from plants of different regions of Brazil (Santa Catarina and Rio Grande do Sul states). Compounds were determined by HPLC and results were related to a sensory evaluation performed by trained tasters. Tasters considered the beverage from reforested plants to be more bitter than the beverage from native plants. Beverages from reforested plants had significantly higher caffeic acid and lower catechin, chlorogenic acid, caffeine, and gallic acid content than native plants. Beverages from plants of Santa Catarina state had significantly higher catechin, caffeine, and gallic acid content than plants from Rio Grande do Sul state.

Keywords: *Ilex paraguariensis*; Mate; Sensory analysis; Chromatography

1. Introduction

Ilex paraguariensis Saint Hilaire (Aquafoliaceae) is known popularly as erva-mate. It is native to temperate climate regions, resists to low temperature, and its natural occurence area is restricted to Brazil, Paraguay, and Argentina (Anuário Brasileiro da erva-mate, 1999). It is a stimulant beverage similar to green tea and is consumed traditionally in South America. This beverage is prepared by the infusion of green or dry *Ilex* leaves from South America, in particular, *Ilex paraguariensis* (Clifford & Ramirez-Martinez, 1990). Erva-mate may show variations in quality and physico-chemical characteristics due to the influence of some factors such as age of the tree and leaves, harvest time, kind of herb (native or reforested), cultivation system, producer region, processing, and storage (SAAP, 1997).

Polyphenols are the most abundant compounds in the tea leaves. Among them, flavanols (catechins) constitute above 30%, and have an important contribution especially to the bitter and astringent taste of green tea (Finger, Kuhr, & Engelhardt, 1992). Catechins are found in green tea together with chlorogenic acids, such as 5-caffeoylquinic acid (5-CQA), which is the major phenolic found in coffee among other compounds (Kilmartin & Hsu, 2003). Caffeic acid is a bitter taste compound, that is usually

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found in small quantities in the processed arabic coffee (Variyar, Ahmad, Bhat, Niyas, & Sharma, 2003).

Ilex paraguariensis is known for its characteristic bitter taste. Nowadays, it is used in commercial herbal preparations such as a tonic, against cellulite and aging. Some of these pharmacological activities are attributed to the high cafeoil and flavonoid content (Adzet, Camarasa, & Laguna, 1987). Research has revealed that erva-mate has a significant content of various chlorogenic acids (CGA) (Clifford & Ramirez-Martinez, 1990).

Some flavonoids are commonly found as polymers such as tannins. While monomeric flavonoid compounds are more bitter than astringent, the polymeric compounds are more astringent than bitter (Robichaud & Noble, 1990). Tannins are compounds of high molecular weight, which give the sensation of astringency to food, and are classified into two groups based on their structural types: hydrolysable tannin and condensed tannin. The first group has a glucose central nucleus or a polyhydric alcohol esterified with gallic or elagic acid, and it is easily hydrolyzed by acids, bases, or enzymes. The second group includes catechin polymers and/or leucoanthocyanidins that are not easily hydrolyzed by acid treatment (Soares, 2002).

Caffeine is neutral compound when in water solution, and its molar sensory perception is 7.0×10^{-4} (Pfaffmann, Bartoshuk, & McBurney, 1971). Caffeine is a smell free alkaloid with a distinct bitter taste. Several studies tried to establish a correlation between the bitter characteristics of coffee as a beverage and its caffeine content, but with little success. As a matter of fact, it has been found that caffeine contributes only with a small proportion (ranging from 10% to 30%) of the detected bitter taste of coffee (Clarke & Macrae, 1985; Viani, 1988).

Under stress conditions, some plants have the capability of increasing alkaloid production (Hoft, Verpoorte, & Beck, 1996). Moreover, an increasing trend on alkaloid production according to the degree of shade was observed in shade-tolerant plants (Hoft, Verpoorte, & Beck, 1998). The increase of defense substances against defoliating insects and fungi (ex. caffeine and theobromine) on plants under a higher degree of shade may be a way to guarantee leaves longevity. Therefore, the leaves would stay on the plant for longer to perform photosynthesis, thus compensating the biologic investment necessary to the construction of these organs.

The objective of the present research was to evaluate taste-related compounds (caffeic acid, catechin, chlorogenic acid, caffeine, and gallic acid) and the sensory profile of erva-mate infusions (*Ilex paraguariensis*) prepared using plants from different regions of Southern Brazil (Rio Grande do Sul and Santa Catarina states) and different cultural practices (native and reforested). Results of the chemical and sensory analysis were correlated in order to identify the main compounds possibly related to the bitter taste, and explain the differences of taste between native and reforested plants.

2. Materials and methods

2.1. Samples

The erva-mate (*Ilex paraguariensis*) samples harvested in June 2004 were obtained from two regions of South Brazil: Ilópolis, in the state of Rio Grande do Sul, and Xaxim, in the state of Santa Catarina. Samples from both regions were divided into native or reforested according to the cultural practice used. Samples were grounded, dried packed in aluminum packages and polyethylene film, and stored at −20 °C to protect against humidity and keep their physical, chemical, and microbiological characteristics.

Infusion of erva-mate was prepared according to Purdon and McCamey (1987), Matsubara (2001) with some modification. A 5 g sample was mixed with 80 ml of distilled tri-deionized water at 80°C for 5 min. After cooling, the volume was adjusted to 100 ml and filtered. For all the analysis samples were run in triplicate.

2.2. Reagents

Caffeic acid, (±)-catechin hydrate, chlorogenic acid, caffeine, and gallic acid standards were purchased from Sigma–Aldrich (St. Louis, MO, USA). HPLC-grade methanol, acetic acid and acetonitrile were from Tedia (Fairfield, OH, USA).

2.3. Analytical determination

Analyses were performed using an HP 1100 series (Agylent®) liquid chromatograph system, equiped with quaternary pump, auto sampler, and thermostatted column compartment (35 °C). Compounds were separated using a C18 reverse phase column (5 μm, 150 × 4.6 mm ID) LUNA®, protected by a Phenomenex® C18 guard column (4 L × 3.0 mm ID). Detection was carried out at 274 nm.

The gradient elution consisted of 0.5% aqueous acetic acid (v/v) (solvent A), methanol (solvent B), and acetonitrile (solvent C). The gradient conditions were: 0–11 min, 70% A, 30% B; 11–14 min, 100% C; 14–20 min, returning to 70% A, 30% B. The flow rate was 0.5 ml/min and the injection volume was 2 μl.

Calibration curves were constructed for the compounds evaluated (caffeic acid, catechin, chlorogenic acid, caffeine, and gallic acid) at five concentrations ranging from 2 to 5000 μg ml^{-1}. Curves obtained had r^2 greater than 0.999. Quantification limit to the method for caffeic acid, catechin, chlorogenic acid, caffeine, and gallic acid were 0.8, 1.0, 1.0, 0.8 and 0.6 μg ml^{-1}, respectively. Recoveries from erva-mate samples spiked with known amounts of these compounds ranged between 93% and 110%.

3. Sensory analysis

Sensory tasters were recruited using questionnaires about their alimentary habits and healthy state. They were

Table 1
Phenolic compounds and caffeine in erva-mate beverage from samples harvested in the south of Brazil[A]

	Santa Catarina state		Rio Grande do Sul state		CV%
	Native	Reforested	Native	Reforested	
Catechin (g %)	4.51 ± 0.11^{a}	3.39 ± 0.09^{c}	3.83 ± 0.03^{b}	3.05 ± 0.06^{d}	0.85–2.78
Caffeic acid (mg %)	28.7 ± 0.30^{c}	4.0 ± 0.8^{b}	23.6 ± 0.6^{d}	37.0 ± 0.5^{a}	1.06–2.56
Chlorogenic acid (g %)	1.00 ± 0.00^{b}	0.90 ± 0.00^{c}	1.27 ± 0.07^{a}	0.73 ± 0.00^{d}	0.75–4.95
Caffeine (g %)	1.61 ± 0.04^{a}	0.97 ± 0.00^{c}	1.18 ± 0.01^{b}	0.86 ± 0.00^{d}	0.53–2.51
Gallic acid (mg %)	0.89 ± 0.00^{a}	0.69 ± 0.00^{c}	0.75 ± 0.00^{b}	0.64 ± 0.00^{d}	0.13–2.20

CV = coefficient of variation. Different letters in the same line indicate significant differences ($p \leqslant 0.05$).
[A] Results are expressed on a dry matter basis and are the average concentration ± standard deviation of four samples analyzed in June 2004.

trained using ordering tests for basic tastes and triangular discriminative tests. Triangular test was used in the training for bitterness perception. Training was done using the extract of mate beverage fortified with 0.0025%, 0.005% and 0.01% caffeine. The extract was prepared by mixing erva-mate sample with 70 °C water (1:50, w/v) for 3 min (Duarte, 2000). The beverage was put into thermo flasks to keep temperature at 60 °C. Thirty-two candidates were recruited, but due to personal schedule limits, only 20 people participated in the sensory training program, and 15 were selected to take part in the sensory evaluation of erva-mate samples.

Erva-mate extract for sensory evaluation was prepared as described above, except that it was not fortified with caffeine. Sensory evaluation was carried out employing trained tasters (15). For Triangular discriminative test samples of native (N) and reforested (R) erva-mate from Rio Grande do Sul (RS) and Santa Catarina (SC) were presented into six combinations (RS–N versus RS–R, SC–N versus SC–R, RS–N versus SC–N, RS–N versus SC–R, RS–R versus SC–R, and RS–R versus SC–N). In each combination, samples were displayed in two different sequences, for example: (a) RS–N, RS–N, and RS–R, or (b) RS–N, RS–R, and RS–R; so that 50% of the tasters could analyze sequence "a" and the other 50% sequence "b". Each combination was analyzed in one session, in adequate schedule, totaling three days.

The ordering test was carried out to determine the bitterest sample. Analyses followed the methodology described by Meilgaard, Cirille, and Carr (1991). Tasters received the four samples of erva-mate infusion (RS–N, RS–R, SC–N, and SC–R) and were asked to order samples according to the increase of the bitter taste, so that the bitterest sample received the higher score.

3.1. Statistical analyses

Results of chemical assays were analysed by two-way analysis of variance: Two regions (RS and SC) × two cultural practices (native and reforested), followed by Duncan's test. Analyses were performed using 'STATISTICA' 6.0. Results of the triangular test were analysed using the chi-square test in accordance to ASTM (1968). Results of the ordering test analysis were evaluated based on the Newell and Mc Farlane's table (Dutcosky, 1996).

4. Results and discussion

Table 1 shows the content of caffeic acid, catechin, chlorogenic acid, caffeine, and gallic acid in erva-mate samples from the south of Brazil. ANOVA revealed significant effects of the regions and the cultural practices on the levels of chemical compounds in erva-mate extracts. Catechin was the compound found at the highest level in the infusion of *Ilex paraguariensis* (Table 1). Caffeic acid and caffeine concentrations of erva-mate samples from the south of Brazil were in the range previously reported in samples from Argentina (0.023% and 1.92 g%, respectively) (Filip, López, Coussio, & Ferraro, 1998; Filip, López, Giberti, Coussio, & Ferraro, 2001). Beverage from reforested plants had significantly higher caffeic acid and lower catechin, chlorogenic acid, caffeine, and gallic acid content as compared to the beverage from native plants. Beverage from plants of Santa Catarina state had significantly higher catechin, caffeine, and gallic acid content than from Rio Grande do Sul state does.

Triangular sensory testing (Table 2) revealed differences in the taste of beverages brewed from native and reforested plants as well as brewed from plants from the state of Rio Grande Sul and state of Santa Catarina. Table 3 shows the results of the ordering sensory test. Ordering test revealed that samples from reforested areas of Rio Grande do Sul state were considered the bitterest comparing to the other samples.

The results of sensory analysis may be explained by the levels of catechin, caffeic acid, and chlorogenic acid found in the extracts indicating the role of these substances on the taste properties of erva-mate.

Table 2
Sensory score of triangular test for the taste of erva-mate beverages[a]

Sample comparison	Correct replies	Incorrect replies	Significance
N–RS versus R–RS	12	3	$p \leqslant 0.001$
N–SC versus R–SC	13	2	$p \leqslant 0.001$
N–RS versus N–SC	9	6	$p \leqslant 0.05$
N–RS versus R–SC	9	6	$p \leqslant 0.05$
R–RS versus R–SC	9	6	$p \leqslant 0.05$
N–SC versus R–RS	9	6	$p \leqslant 0.05$

[a] Sample codes: N: native; R: reforested; RS: Rio Grande do Sul; SC: Santa Catarina. Test evaluate by 15 sensory tasters.

Table 3
Sensory score of the ordering test according to the bitterness of erva-mate extracts[A]

Sample	R–RS	R–SC	N–RS	N–SC
Score	60[a]	41[b]	26[b]	23[b]

The critical value for the 15 tasters and 4 samples, at a level of significance of 5%, according to Newell and Mc Farlane's Table (Dutcosky, 1996) is 19. Different letters indicate significant differences ($p \leqslant 0.05$).
[A] Sample codes: N: native; R: reforested; RS: Rio Grande do Sul; SC: Santa Catarina.

Catechin is a monomeric flavonoid characterized by astringency and bitterness (Robichaud & Noble, 1990). Astringency is usually considered to be a tactile sensation resulting from the precipitation of salivary proteins and leading to a loss of mouth lubrication (Vidal et al., 2004), while bitterness is a taste mediated by sensory receptors (Vidal et al., 2004). Catechin is not chemically defined as astringent, since poli-flavonoids with molecular weight below 500 usually do not precipitate proteins (Bate-Smith, 1973). Nevertheless, the astringency of this small flavonoid molecule has been attributed to the precipitation or a strong link with proteins, due to the presence of dihydroxy-1,2 or trihydroxy-1,2,3 groups, which can explain the astringency property in flavan-3-ols monomers (McManus, Davis, Lilley, & Haslam, 1981). Few studies evaluated the interaction between bitter and astringent tastes. Recently Scharbert and Hofmann (2005) observed that the omission of catechin from a biomimetic reconstitution of tea taste decreases both astringency and bitterness. In contrast, we found higher catechin levels in beverages from native erva-mate samples which were rated as less bitter than the reforested ones. Therefore, we can propose that catechin may not be a key compound in the bitterness of erva-mate. Besides, the higher catechin content of native erva-mate extract could have masked the perception of bitterness of other substances, due to their astringency.

Caffeic acid is a bitter taste compound that is normally found in trace quantities in arabic coffee, and it may increase when chlorogenic acid is hydrolyzed in the bitter monsooned coffee (Variyar et al., 2003). Accordingly, samples from reforested plants which had higher concentrations of caffeic acid were considered bitterer by tasters when compared to native ones.

Chlorogenic acid is the major phenolic compound responsible for astringency of green coffee (Variyar et al., 2003). Chlorogenic acid, a caffeic acid ester, is known as caffeoylquinic acid with an agliconic portion of caffeic acid. As mentioned before, the astringent chlorogenic acid decreases as a consequence of hydrolysis releasing the bitter caffeic acid in samples of monsooned coffee (Variyar et al., 2003). Besides, there are indications that small molecules of phenolic compounds can obtain astringency gradually such as 5-CQA (5-caffeoylquinic) that has only one caffeic acid residue and has been considered astringent (Naish, Clifford, & Birch, 1993). Beverage from native plants had the highest chlorogenic acid concentrations, which probably helped to diminish the bitter taste of these samples when compared to the reforested ones.

Native plants are found within woods and therefore are submitted to a higher degree of shade. According to Hoft et al. (1996, 1998), Coelho (2000) plants under lower light intensity and stress conditions, increase the production of defence substances, such as caffeine. The increase of these protective substances against defoliating insects and fungi can be a way to guarantee longevity to leaves. Accordingly, native plants had higher caffeine content than reforested ones.

Since caffeine is a bitter substance, its higher concentration in native samples, which were less bitter, was unexpected. All erva-mate samples had significant levels of glucose (data not shown). Hence, a possible explanation for this unexpected result is that caffeine bitter taste may have been masked by a sweet taste. Taste may be influenced by the molecular packing of taste molecules close to the sensory receptor proteins that are inserted in the cell membrane (an hydrophobic phase) (Aroulmoji, Aguié-Béghin, Mathlouthi, & Douillard, 2004). Bitter taste was found to be suppressed by sweeteners such as sucrose (Aroulmoji, Hutteau, Mathlouthi, & Rutledge, 2001). As a general rule, sweeteners are rather hydrophilic and bitter molecules have predominantly a hydrophobic character. The inhibition of the bitter taste by sweet substances has been attributed to hydration and surface properties (Aroulmoji et al., 2001). A study of the molecular organization of bitter (caffeine) and sweet (sucrose) substances in an aqueous solvent near a modeled hydrophobic phase revealed that caffeine molecules form an adsorption layer, whereas sucrose may induce the dissociation of some caffeine aggregates and slow down their adsorption (Aroulmoji et al., 2004). Although no specific study was found on the taste interaction between caffeine and glucose, it is possible that caffeine–glucose interaction could mask the bitter taste of caffeine in erva-mate beverages.

Polymeric phenolic compounds, like gallic acid are more astringent than bitter (Robichaud & Noble, 1990). In beverages from native plants gallic acid concentration was higher when compared to reforested ones. According to tasters, beverage from native plants had lower bitter taste. Astringent compounds, like gallic acid could help to decrease the perception of bitterness. However, as this compound was found at very low concentration, its influence on the erva-mate beverage astringent taste may not be significant.

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