



Characterization of volatile fractions in green mate and mate leaves (*Ilex paraguariensis* A. St. Hil.) by comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC × GC/TOFMS)



Allan dos S. Polidoro^a, Enelise Scapin^a, Milena Malmann^a, Jenniffer U. do Carmo^a, Maria E. Machado^a, Elina B. Caramão^{a,b}, Rosângela A. Jacques^{a,*}

^a Instituto de Química, UFRGS, Porto Alegre, RS, Brazil

^b Instituto Nacional de Ciência e Tecnologia em Energia e Ambiente, INCT-EA, Salvador, Bahia, Brazil

ARTICLE INFO

Article history:

Received 9 February 2016

Received in revised form 29 March 2016

Accepted 30 March 2016

Available online 9 April 2016

Keywords:

Ilex paraguariensis A. St. Hil.

Essential Oil

Comprehensive two-dimensional Gas chromatography

Time-of-flight mass spectrometry

ABSTRACT

Ilex paraguariensis A. St. Hil. is well known due to its great economic and social value. However, investigations detailing the differences between the volatile chemical profiles of fresh mate leaves and their processed product, green mate, remain largely neglected. Taking these considerations into account, this study aimed to obtain a deeper understanding of the constituents of fresh mate leaf and green mate essential oils (EOs). Toward this purpose, GC × GC/TOFMS and retention indices were used to improve the chemical characterization of these EOs. A total of 157 and 291 compounds were tentatively identified in fresh leaf and green mate EOs, respectively (168 of which are reported for the first time in green mate). The major constituents of the fresh leaf EOs were found to be 3-allylguaiacol (28.51%), linalool (17.61%), and methyl salicylate (5.36%), while those of green mate were 2,6-dimethyl-1,7-octadien-3-ol (9.77%), linalool (5.58%), and α-terpineol (4.67%). Furthermore, hazardous contaminants such as hydrocarbon isomers and PAHs (in particular, alkylated PAHs) were tentatively identified in green mate. The results represent the most detailed characterization of these EOs reported to date and suggest the need to use smokeless methods in mate processing.

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1. Introduction

Ilex paraguariensis A. St. Hil. (“mate”, “erva-mate” or “yerba mate”), from the family Aquifoliaceae, is a plant of great economic and social interest, widely consumed as a tea-like beverage called “chimarrão”, “tererê” or “mate”, which is prepared by an infusion of its leaves and twigs [1]. This plant is native to subtropical regions of South America and is cultivated mainly in Brazil, Argentina, Uruguay, and Paraguay [1]. In 2012, Brazil produced over 500,000 tons of mate [2], while in Argentina, 2011 mate production was over 700,000 tons [3]. Currently, this species has been exported to several parts of the world, mainly the USA and Europe, due to its nutritional and medicinal properties (tonic, choleric, diuretic, antioxidative, hypocholesterolemic, antiaging, anti-thrombotic, anti-inflammatory, antiobesity, etc.) [1,4–13].

Although volatile compounds greatly influence the properties of infused beverages made from parts of plants [14], there are only a few studies related to volatile mate components [14–18]. The first study, carried out in 1991 by Kawakami and Kobayashi, identified 172 compounds in green mate, including 2-butoxyethanol (9.5%) and linalool

(7.5%) as the main components [16]. More recently, in 2009, Purcaro and co-workers analyzed mate volatile fractions by using comprehensive two-dimensional gas chromatography with a rapid-scanning quadrupole MS (qMS) and identified 241 compounds [18].

Mate is not consumed as a raw product but is instead processed before it reaches the consumer. The overall processing consists of the following stages: blanching (the leaves and stems are flash heated (500 °C) from 10 s to 3 min), drying (the product is heated to 100 °C for 8–24 h to reduce the moisture from 10–12% to 4.5%), aging (the dried product is aged for 12 months), sieving, powdering, and packaging. The final product is called green mate [1,16].

During mate processing, changes occur in the chemical profile and in the concentration of bioactive compounds of *I. paraguariensis*. In 2006, Bastos and co-workers verified a significant variation in the caffeine and 5-caffeoylquinic acid content of mate infusions during the three stages of *I. paraguariensis* processing (fresh leaves, after blanching and dried leaves) [19]. Isolabella and co-workers have demonstrated that the mate samples obtained after the processing stages possess a higher content of bioactive compounds when compared with the fresh leaf samples [20]. However, there is a lack of information in the literature regarding the modifications of the volatile fraction of the mate leaves caused by their processing.

* Corresponding author.

E-mail address: rosangela.j@iq.ufrgs.br (R.A. Jacques).

Plant volatiles are usually complex samples containing hundreds of compounds, including alcohols, aldehydes, ketones, esters, lactones, phenols, phenylpropanoids and terpenoids [21,22]. GC/MS is the standard tool for characterization of the volatile fraction of *I. paraguariensis*. However, one-dimensional gas chromatographic analysis (1-D GC) often fails to provide a satisfactory analytical result, even on only moderately complex samples, due to the insufficient resolution power of a single column [18].

A higher peak capacity is required to achieve satisfactory separation of a complex sample, indicating in this case the use of comprehensive two-dimensional gas chromatography (GC × GC) [23–26], a technique developed by Liu and Phillips [27]. Furthermore, GC × GC has the advantage of increasing both resolution and sensitivity due to the re-concentration of the chromatographic band through the modulation process, allowing the detection of compounds at trace levels and separation of related compounds in the second dimension [28].

Taking these considerations into account, the goal of this study was to employ GC × GC/TOFMS in the investigation of the essential oil constituents of fresh mate leaves and green mate, aiming to evaluate the chemical changes caused by mate processing. Calculating linear temperature programmed retention indices (LTPRI) was one of the tools used to confirm peak assignments. To the best of the authors' knowledge, this is the first report detailing the differences between the volatile chemical profiles of fresh mate leaves and green mate using GC × GC.

2. Materials and methods

2.1. Materials

Fresh leaves and green mate were obtained from a local Rio Grande do Sul (Brazil) industry, during the harvesting period in August 2013. All solvents and reference standards (linear C₆–C₃₀ alkane mixture) used were purchased from Sigma–Aldrich (St. Louis, MO, USA) with high purity grade. Water was purified using a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Sample preparation

The mate samples (100 g) were hydrodistilled for 4 h using a Clevenger-type apparatus, following the method recommended by the Brazilian Official Pharmacopoeia V [29]. The essential oils were extracted with dichloromethane dried over anhydrous sodium sulfate p.a. grade from Merck (Darmstadt, Germany) and the organic layer was transferred into dark vials and stored at –4 °C for further analysis.

2.3. GC × GC/TOFMS analysis

GC × GC/TOFMS analyses were carried out using an Agilent 6890N gas chromatograph coupled to a LECO Pegasus IV time-of-flight mass spectrometer (TOFMS) (St. Joseph, MI, USA). The samples were injected in splitless mode using helium as the carrier gas (Linde Gases, Canoas, Brazil, 99.999% purity) supplied at a constant flow rate of 1 mL min⁻¹. The first-dimensional chromatographic separation was performed on a DB-5 (5% phenyl–95% dimethylpolysiloxane) column with 60 m length × 0.25 mm I.D. × 0.10 μm film thickness. A DB-17 (50% phenyl and dimethylpolysiloxane) column (2.15 m × 0.18 mm × 0.18 μm) was used in the second dimension. Both columns were acquired from Agilent Technologies–J&W Scientific (Palo Alto, CA, USA). The temperature program of the first column was set as follows: 45 °C for 5 min and then the temperature was increased to 295 °C at 3 °C min⁻¹. The second column temperature was maintained 10 °C above the temperature of the first column. The modulation period was 6 s, and the hot pulse was 40% of the modulation period. The injector, ion source and interface temperatures were held at 280 °C. The mass spectrometer had a detector voltage of 1.4 kV and a mass range of 45 to 450 m/z.

ChromaTOF software version 3.32 was employed for processing the total ion current chromatograms (TICs), including such tools as the peak finder and mass spectral deconvolution. For data processing, a signal to noise ratio (S/N) of 3 was used.

To calculate the linear-temperature-programmed retention indices (LTPRIs) [30], a linear C₆–C₃₀ *n*-alkane mixture was analyzed using identical GC × GC/TOFMS conditions.

Compounds were tentatively identified by comparing the similarity of their fragmentation with a commercial database (NIST) [31] and their LTPRI with those reported in the literature [31,32]. A similarity above 70% and a difference between the sample and literature LTPRI of no greater than ±20 units were determined to be acceptable for tentative compound identification. The relative amounts of individual compounds were calculated based on GC × GC/TOFMS peak area without using a correction factor.

3. Results and discussion

The weight percentage yields (w/w%) of essential oils (EOs) obtained from fresh leaves and green mate were 0.07% and 0.03%, respectively.

GC × GC/TOFMS analysis of both EOs presented a complex mixture of compounds with quite similar mass spectral data. Fig. 1 presents the GC × GC/TOFMS 2D color diagram expansion for the EOs from fresh leaves (A) and green mate (B). The compounds were tentatively identified using a combination of their mass spectral similarity and LTPRI. A total of 157 and 291 compounds were tentatively identified in fresh leaf and green mate EOs, respectively, with satisfactory library matches and good concordance between the LTPRI values obtained for the identified compounds and those reported in the literature (from Adams [32] and NIST [31] databases). Table 1 presents the tentatively identified compounds in both EOs, their relative percentages of the identified chromatographic area, the LTPRI values reported in the literature and the differences between the obtained LTPRI values and those reported in the literature.

In the fresh leaf EO, the following types of compounds were identified: 28 aliphatic and 3 aromatic hydrocarbons, 12 aliphatic alcohols, 11 aliphatic aldehydes, 5 aliphatic ketones, 8 aliphatic esters, 5 furan(one)s, 25 phenylpropanoids, 18 norisoprenoids, 11 monoterpenes, 23 oxygenated monoterpenes, 4 sesquiterpenes, 3 oxygenated sesquiterpenes and 1 oxygenated diterpene. The major constituents in the fresh leaf EOs were found to be 3-allylguaiacol (28.51%), the oxygenated monoterpene linalool (17.61%), and the phenylpropanoid methyl salicylate (5.36%). It is interesting to note that the fresh leaf and green mate EOs displayed significant differences between their chemical profiles. Indeed, 43 of the 157 compounds identified in the fresh leaf EOs were detected only there, among which 3-allylguaiacol and geraniol (1.59%) were the most representative.

In the green mate EO, the following compounds were identified: 38 aliphatic and 14 aromatic hydrocarbons, 11 aliphatic alcohols, 26 aliphatic aldehydes, 12 aliphatic ketones, 7 aliphatic esters, 8 furan(one)s, 6 acids, 2 ethers, 3 phenols, 28 phenylpropanoids, 31 norisoprenoids, 18 monoterpenes, 44 oxygenated monoterpenes, 20 sesquiterpenes, 14 oxygenated sesquiterpenes and 2 oxygenated diterpenes, 6 nitrogen compounds, and 1 sulfur compound. The main components were the oxygenated monoterpenes 2,6-dimethyl-1,7-octadien-3-ol (9.77%), linalool (5.58%), α-terpineol (4.67%), and geraniol (4.00%). A group of 178 compounds, including 2,6-dimethyl-1,7-octadien-3-ol, geraniol, 3,7-dimethyl-2,6-octadien-1-ol (3.99%), appeared only in the green mate EO.

Comparing the literature data with the results presented here, some similarities in the chemical composition of the green mate volatile fraction during qualitative analyses are observed. In 1991, Kawakami et al. [16] investigated the volatile components of green mate and mate tea obtained by steam distillation using GC/MS, tentatively identifying 172 components. Sixty-nine of those compounds were also identified in this present study, including linalool, α-terpineol, and geraniol. Bastos

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