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Ilex paraguariensis as chimarrão, terere and mate-tea forms: a comparative study of the volatile fractions of the plant materials and their respective aqueous extracts using Headspace Solid-phase Microextraction by CG-MS

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Research Article

Keywords: Ilex paraguariensis, volatile fraction, headspace solid-phase microextraction, CG-MS

Posted Date: September 22nd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2057262/v1

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Abstract

Popularly known as "Yerba mate", *Ilex paraguariensis* St Hil. (Aquifoliaceae) is a South American plant extensively used to prepare many beverages, with "chimarrão", "terere" and "mate-tea" as three of the most consumed, and the preparations methods, from the plant material process to extraction mode, determines the type of the final beverage. With many health benefits and also cultural and economic relevance, in this work the volatile compounds composition of these three main beverages were analyzed through headspace solid-phase microextraction (HS-SPME) used in combination with gas chromatography–mass spectrometry (GC-MS). Through this technique, 39 main volatile compounds were identified with spectral similarity above 90%. Using Principal Component Analysis (PCA) and Heatmap analysis, the volatile compounds composition was profiled for all the extracts and start material, and a good homogeneity among the replicates was observed, along with similarities between extracts and their start materials, as well as a notorious distinctive clustering of the different *Ilex paraguariensis* products, all good indications of the capability of the entire methodology used to analyze volatile compounds profiles. Limonene and n-hexanal were found as the main volatile compounds, and their abundances seem to be affected by the extraction modes, mainly due to water temperature and plant drying process. This work successfully compared the volatile profile of "chimarrão", "terere" and "mate-tea" beverages and their start materials using HS-SPME-GC-MS techniques, with the identification of the volatile compounds as well as the impact of the preparation modes in these ones.

1. Introduction

Ilex paraguariensis St. Hil. (Aquifoliaceae) is a native plant from South America, more specifically from Argentina, Uruguay, Paraguay and Brazil (Vieira et al. 2010), popularly known as “Yerba mate”. Several health benefits related to this plant and its rich chemical composition have been reported, such as hypocholesterolemic action (Stein et al. 2005), prevention of cancer development (Amigo Benevant et al. 2017; Mejia et al. 2010), hepatoprotective (Tamura et al. 2013) and neuroprotective potential (Branco et al. 2013) and excellent anti-inflammatory, antioxidant and anti-obesity effects (Arçari et al. 2013; Pimentel et al. 2013). Additionally, this herb represents important social and economic interests, being extensively consumed through three different beverages, called: “mate” or “chimarrão”, “terere” and “mate-tea”.

These three traditional drinks, all aqueous extracts, differ from each other mainly due to early treatment given to the plant material and the extraction temperature. The state of the yerba to prepare chimarrão and terere is called “cancheada”. This name is given after a process consisting of three main steps: 1. A quick exposition to high temperatures, called “Zapeca”; 2. drying until a 3% of moisture is reached, in a system called “barbacuá”, where the material is exposed to hot air in rotating drums heated by gas or wood; and; 3. a milling process with additional drying (Esmelindro et al. 2002). For mate-tea, on the other hand, the material used is the roasted product of the yerba (Bastos et al. 2007). Furthermore, while chimarrão and mate-tea are made with the addition of hot water, terere is obtained by using cold or iced water (Bastos and Torres 2003).

The growing geographic location and factors such as industrial processing steps, dry material conditions and preparation methods have been reported to have an influence in the variations of the final chemical composition of *Ilex paraguariensis* extracts (López et al. 2006; Isolabella et al. 2010). This diversity is also found in the volatile fraction with very significant differences (Kawakami and Kobayashi 1991; Dellacassa et al. 2007). Leprevost et al. (1987) reported the roasting step of mate-tea production as an important changing factor regarding volatiles, where the characteristic aroma is achieved. Additionally, Polidoro et al. (2016) showed a significative variation between fresh leaves of the plant and their processed product.

For containing a huge number of different compounds, including alcohols, ketones, phenols, esters, aldehydes, phenylpropanoids and terpenoids, plant volatiles are considered complex samples (Santos et al. 2013; Saucier et al. 2014). In this context headspace solid-phase microextraction (HS-SPME) used in combination with gas chromatography–mass spectrometry (GC–MS) is an excellent option for analysis. This method is less susceptible to interfering substances due to the fact that only volatile compounds in the sample are carried to the GC system by the fiber and low interference peaks can be obtained by selected ion monitoring (SIM) of GC–MS. Despite that, only a few studies were published using the headspace solid-phase microextraction (HS-SPME) GC–MS method for the characterization of the volatile fraction of *Ilex paraguariensis* and its related forms and products (Araújo et al. 2007; Purcaro et al. 2009).

Volatile compounds have a great influence on the properties of infused drinks made from plant parts (Bastos et al. 2006). Despite that, there is a lack of information in the literature regarding volatile components of *Ilex paraguariensis* concerning its different forms of consumption. In addition, regardless of the importance that volatile compounds have on the flavor quality, aromas characterization research of popularly consumed extracts has not been covered by the literature yet. Therefore, the purpose of this work was to compare the volatile fraction of the yerba mate samples of the three vegetal materials destined to make chimarrão, terere and mate-tea, as well as their respective aqueous extracts, by HS-SPME, aiming to trace a characteristic profile of each beverage.

2. Material And Methods

2.1. Material

The SPME device and fibers (65 µm poly(dimethylsiloxane)–divinylbenzene -PDMS–DVB) were obtained from Supelco (Bellefonte, PA, USA). All fibers were conditioned prior to use as recommended by manufacturer. The reference standards used, C8\\C20 n-alkane mixture, 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). The Yerba mate was obtained from different commercial companies: Tomelero (chimarrão and terere) and Organic (mate-tea).

2.2. Aqueous extracts

Aqueous extracts of *Ilex paraguariensis* were obtained (n = 5) mimicking the popular preparation modes for chimarrão, terere and mate-tea drinks. For chimarrão, the extraction process followed the protocol initially proposed by Meinhart et al. (2010) with modifications, as shown in Mesquita et al. (2021). For mate-tea, the extraction was made according to the protocol proposed by Tamura et al. (2013), immersing 5 grams of the preparation in 100 mL of boiled water for 5 minutes.

2.3. Sample preparation and SPME procedure

For better understanding, this part of the methodology is illustrated in Fig. 1. The volatile fractions of the different forms of yerba mate and their respective aqueous extracts were collected using solid phase micro-extraction (SPME) and analyzed by a gas chromatograph coupled to a mass spectrometer (GC-MS). Initially, 85 grams of chimarrão and terere and 5 grams for mate-tea, according to the quantities employed in popular preparations, were put into a Becker and the system was sealed using polyester bags on the opening. For the plant material intended for hot preparations, chimarrão and mate-tea, an acclimatation step was performed by exposition to a water bath at 50ºC for 10 minutes. Thereafter, bags were perforated with a pin and SPME syringe with a PDMS–DVB fibre was introduced into the head space of the system during 15 minutes. Finally, immediately after collection, SPME fibre samples were directly injected into a GC. An empty Becker was used as negative control. Regarding aqueous samples preparation, all the extracts obtained through the mimetic processes were used after reaching room temperature. Once temperature was stabilized and measured at ~ 25ºC, the steps of assembling and sealing the systems, acclimatization in water bath (for hot preparations) and exposure to fiber were conducted identically to applied in the dry material. Filtered water was used as negative control.

2.4. Gas chromatography–mass spectrometry conditions

GC-MS analyses were performed on a gas chromatograph (2010A, Shimadzu, Tokyo, Japan) coupled to a quadrupole mass spectrometer (QP2010, Shimadzu) and fitted with a Rtx-5MS capillary column (30 m × 0.32 mm i.d.; 0.25 µm film thickness, J&W Scientific, Folsom, CA, USA) The analyses were performed following the protocol initially proposed by Araújo et al. (2007). The oven temperature was kept at 40°C during 4 min, then increased to 200ºC at a rate of 2°C/min and held for more 2 min until the end of the run, totalizing 86 minutes. Helium was used as the carrier gas at a constant flow of 1.0 mL/min. Injection was performed in split-less mode, and the PDMS–DVB fiber was kept for 1 min in the injector at 260ºC. The quadrupole mass detector was operated at 150°C in the electron impact mode at 70 eV. The ion source temperature was 230°C and the transfer line was set at 280°C. MS were scanned from m/z 39 to 370 at a rate of 1.9 scans/s.

2.5. Identification and quantitative analysis

The chromatographic data were analysed by GC-MS Solution software (Shimadzu, Tokyo, Japan). To obtain the Kovats Retention Index (RI) of each compound peak, a C_8-C_{20} n-alkane mixture was injected using the same chromatographic conditions. The constituents' peaks were integrated by hand and identified based on a comparison of the mass spectra and RIs to those of reference compounds in the

data system of the Wiley 6th ed Mass Spectral and NIST05 and NIST online library Standard Reference Databases.

2.6. Statistical analysis

The volatile oil composition and its relative abundance of compounds (determined by GC-MS) were statistically evaluated through Principal Component Analysis (PCA), along with Heatmap, using RStudio version 3.6.2 and the packages "factoextra" (developed by A. Kassambara and F. Mundt) and "pheatmap" (by R. Kolde). For Heatmap analyses, the average of replicates was used, and the data was scaled by extraction method. The PCA was also used to compare the quality of the replicates.

3. Results And Discussion

3.1. Volatile compounds composition of *Ilex paraguariensis* preparations were successfully characterized through HS-SPME-GC-MS

The chromatograms obtained using HS-SPME in combination with GC–MS of the volatile fractions of the three different plant materials used to prepare the mostly consumed beverages of *Ilex paraguariensis* (Chimarrão, terere and mate-tea) and their aqueous extracts per se, are illustrated in Fig. 2. The volatile compounds were identified by comparison of their linear retention index (RI) determined in relation to a series of n-alkanes (C8//C20) with those from pure standards or reported in the literature.

Despite a complex chromatogram found in all samples, 39 compounds were identified with a spectral similarity > 90% (Table 1). It is relevant to say that these determinations of the volatile profile of commercial material and beverages mimetic extracts are important to construct an idea of the human exposition that happen daily, since the use is extensive.

Table 1
Main volatile constituents identified using GC-MS chromatograms referring to extractions (HP-SPME) performed in the headspace of different dry plant materials from *Ilex paraguariensis* and their respective extracts (chimarrão, terere and mate-tea).

Volatile substances (Area %)							
		Plant Material			**Extracts aqueous**		
Volatile constituent[a]	**RI[b]**	**Chimarrão**	**Terere**	**Mate-tea**	**Chimarrão**	**Terere**	**Mate-tea**
n-Hexanal	803	24.71	16.29	-	19.34	5.71	-
2-hexenal	866	0.54	-	-	-	-	3.52
Heptane, 2,2,4-trimethyl-	871	-	4.57	-	-	2.73	3.70
4-Heptenal	905	4.47	3.55	-	8.48	2.81	-
α-Pinene	926	6.18	7.88	2.25	1.62	-	-
Camphene	940	1.04	1.34	0.40	0.34	-	-
2,4-nonadiene	946	2.85	-	-	1.76	-	-
β-pinene	968	5.80	5.81	2.16	2.19	0.34	1.70
6-Methyl-5-hepten-2-one	992	13.20	7.43	-	8.28	2.68	-
Hexanoic acid	1004	-	7.34	-	-	0.60	-
2,4-Heptadienal	1006	7.82	-	-	9.68	-	-
Octanal	1006	-	-	1.21	-	-	0.81
p-Cymene	1021	5.73	-	-	3.59	-	-
Limoneno	1025	22.99	28.90	43.24	27.99	8.48	36.90
1-Hexanol, 2-ethyl-	1037	-	-	6.95	-	-	1.35
γ-Terpinene	1060	-	2.18	4.97	-	13.74	-
Linalool oxide	1071	-	-	2.03	-	-	0.38
(+)-4-Carene	1085	-	-	1.07	3.69	-	-
3,4-Dimethylcyclohexanol	1107	-	2.77	-	-	4.40	-
Nonanal	1108	-	-	4.15	1.48	0.46	-
Linalool	1109	1.27	-	-	3.02	-	-

[a]Tentative identification based on computer matching of the mass spectra of peaks with NIST 05 library and published data (Adams, 2007), besides comparison of retention indices on published data (Adams, 2007). [b]RI Retention index relative to n-alkanes on a DB-5 column. -:

Volatile substances (Area %)							
1,6-Octadiene, 2,7-dimethyl-	1118	2.43	-	-	0.23	-	-
L-camphor	1138	0.86	1.56	4.09	0.78	-	1.59
Menthone	1151	-	2.01	-	-	5.35	-
2,4-Dimethyl-1-heptene	1155	-	-	1.64	-	-	0.18
Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1α,2β,5α)-	1174	-	-	2.67	-	-	10.60
Allyl heptanoate	1192	-	0.98	-	-	-	4.60
Tetradecane	1200	0.05	0.04	1.05	3.96	-	-
Dodecane	1201	0.23	-	-	0.10	-	-
α-Ionone	1271	-	-	0.799	-	28.29	-
Decane, 3,7-dimethyl-	1375	-	-	0.50	-	-	6.78
Decane, 3,6-dimethyl-	1460	-	-	1.95	-	-	1.56
2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	1594	-	-	7.81	-	-	1.47

[a]Tentative identification based on computer matching of the mass spectra of peaks with NIST 05 library and published data (Adams, 2007), besides comparison of retention indices on published data (Adams, 2007). [b]RI Retention index relative to n-alkanes on a DB-5 column. -:

Regarding compositions, analyzing the profiles made possible to identify a rich composition, including several compounds with interesting properties shown by the literature, like limonene (Lima et al. 2013; Song et al. 2021), α-Pinene (Kasuya et al. 2015)d camphor (Hritcu et al. 2012). *Ilex paraguariensis* is a plant known for its properties and considered a medicinal herb for several researches (Santos et al. 2015), in addition to being popular related to reports of promoting well-being (Assmann N.S 2018). Interestingly, the most abundant substance in all analyzed samples, limonene, is associated with properties such as anti-anxiety activity. Thus, it is possible to suggest that the volatile fraction may be a factor that contributes to the feeling of well-being perceived by yerba mate consumers.

3.2. Volatile compound profiles where effectively distinguished using unsupervised machine learning

Regarding the analysis, the different extractions using the plant materials or their aqueous extracts were compared by PCA. A preliminary analysis using the Scree plot (Fig. 3) indicated that the variance of the data could be well explained by the principal components 1 and 2 (PC1 and PC2), which were used to plot the graph PCA-biplot (Fig. 4). In this representation, the replicates presented a good clustering, indicating

strong agreement in the volatile composition profile for each *Ilex paraguariensis* preparation, while the different types of beverages and plant materials presented a clear separation, indicating a notable difference in the composition of aroma for different infusion preparations and their plant materials base. It is also noteworthy that the pairs of “plant material” and each corresponding “aqueous extract” appeared close together in the same graphic region of the PCA analyses despite being clustered apart, which suggests a good ability of the methodology used to obtain the infusions, given that both, plant material and its aqueous extract, presented a similar profile, and also the ability of the entire methodology to evaluate volatile composition profiles.

3.3. Abundant compounds and their relation with the pre-preparation of dry materials and temperature of extraction

The observations made with PCA was also confirmed in by heatmap analysis (Fig. 5), where it is possible to clearly see the distinction in the composition of volatiles between the different starting materials and the extraction methods. These data illustrate the influences of “pre”-preparation in the dry material and its extracts. It is known that natural factors, as well as processing systems, interfere directly in the physicochemical components and organoleptic properties of yerba mate (Jacques et al. 2007). Despite these differences, it is noteworthy that two volatiles remain the most abundant: Limonene and n-Hexanal, as shown in Table 1. This can also be easily noticed in the Fig. 5B, where data is normalized per sample and not per compound.

Finally, another factor which deserves attention is the effects of the temperature in the different systems used. Although all the samples were extracted using water, the temperature - cold for terere and hot for chimarrão and mate tea – alter the effectiveness in relation to some compounds, like Limonene and n-Hexanal. Analyzing the plant material of chimarrão and terere preparations, in both cases, these substances are the predominant volatile elements registered in this work (n-Hexanal: 24,71% Chimarrão and 16,29% terere; Limonene: 22,99% Chimarrão and 28,90% terere). Interestingly, in the profile presented by their respective aqueous extracts, it is possible to observe a considerable difference in the rate of these substances: on one hand, chimarrão still presenting high concentrations of n-hexanal and Limonene (19,34% and 27,99%), and on the other hand, samples of terere show relatively lower content (5,71% and 8,48% respectively). This difference could be tied to the difference in the temperature during the extraction process, since it is important to keep in mind that the process for producing the dry material for both modes is quite similar and follows the same pattern. Furthermore, the fact that mate-tea, which is extracted with hot water, presents high concentrations of Limonene as well (43,24% for dry material and 36,9% in the extract), corroborates this observation.

4. Conclusion

In this work, the volatile composition of three main consumed *Ilex paraguariensis* derived beverages – “chimarrão”, “terere” and “mate-tea” – was compared through headspace solid-phase microextraction (HS-SPME) in combination with gas chromatography–mass spectrometry (GC-MS). The volatile

compounds were successfully identified, with 39 compounds presenting spectral similarity above 90%. Their profiles were consistently similar among replicates, and also notably distinctive for each extraction method. Limonene and n-hexanal are the main volatile compounds found and their abundances are strongly dependent on the plant material treatment and mainly the water temperature during extraction. This work profiled the volatile compounds of “chimarrão”, “terere” and “mate-tea” (beverages and start material), as well as demonstrated the capability of HS-SPME-GC-MS technique to evaluate volatile profiles of such materials.

Declarations

Funding declaration

The authors are thankful to CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Finance Code 001), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, #311166/2016-4; #427859/2018-2; #309411/2019-0), FAEPEX-UNICAMP and FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo, Process number 06/06079-4, 12/09101-1, 15/03726-8 and 17/03598-5) for the financial support.

Ethical Approval

This paper does not contain any studies involving a human participant or an animal being performed upon by any of the authors.

Informed Consent

Not applicable.

Conflict of Interest

Fernando Henrique Bosso declares that he has no conflict of interest. Marcos José Salvador declares that he has no conflict of interest.

Author's Contribution

Mariana M.: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - Original Draft; Fernando Henrique B.: Santos. E.: Methodology, Digital analysis, Figures; Marcos J.S.: Supervision, Funding acquisition, Project administration, Writing & Editing. All authors reviewed the manuscript.

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Figures

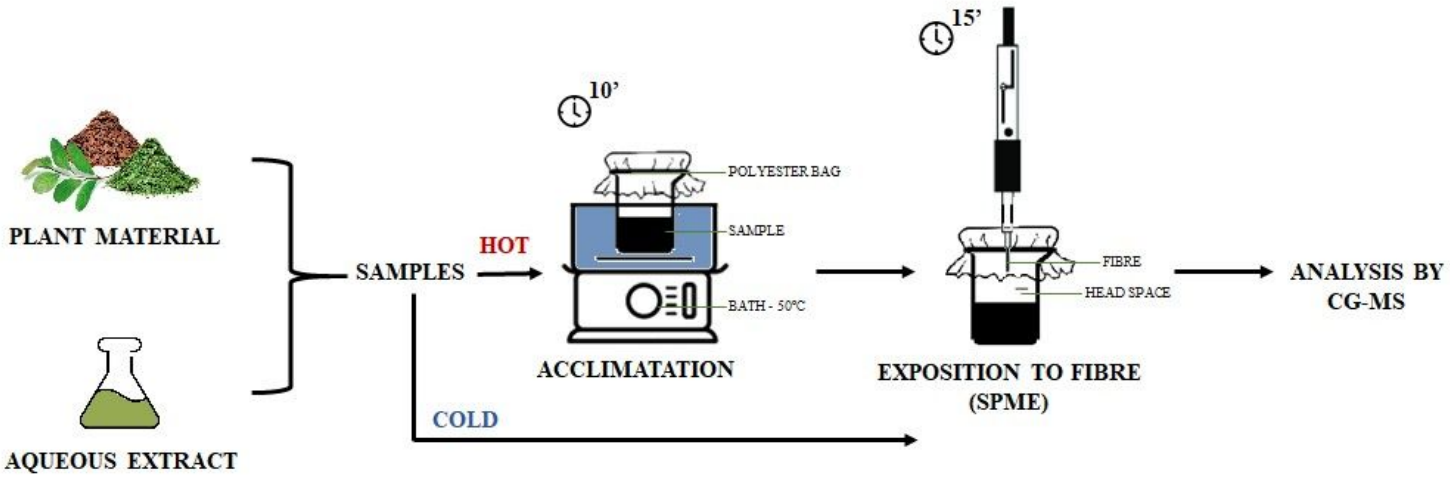


Figure 1

Sample preparation and SPME procedure

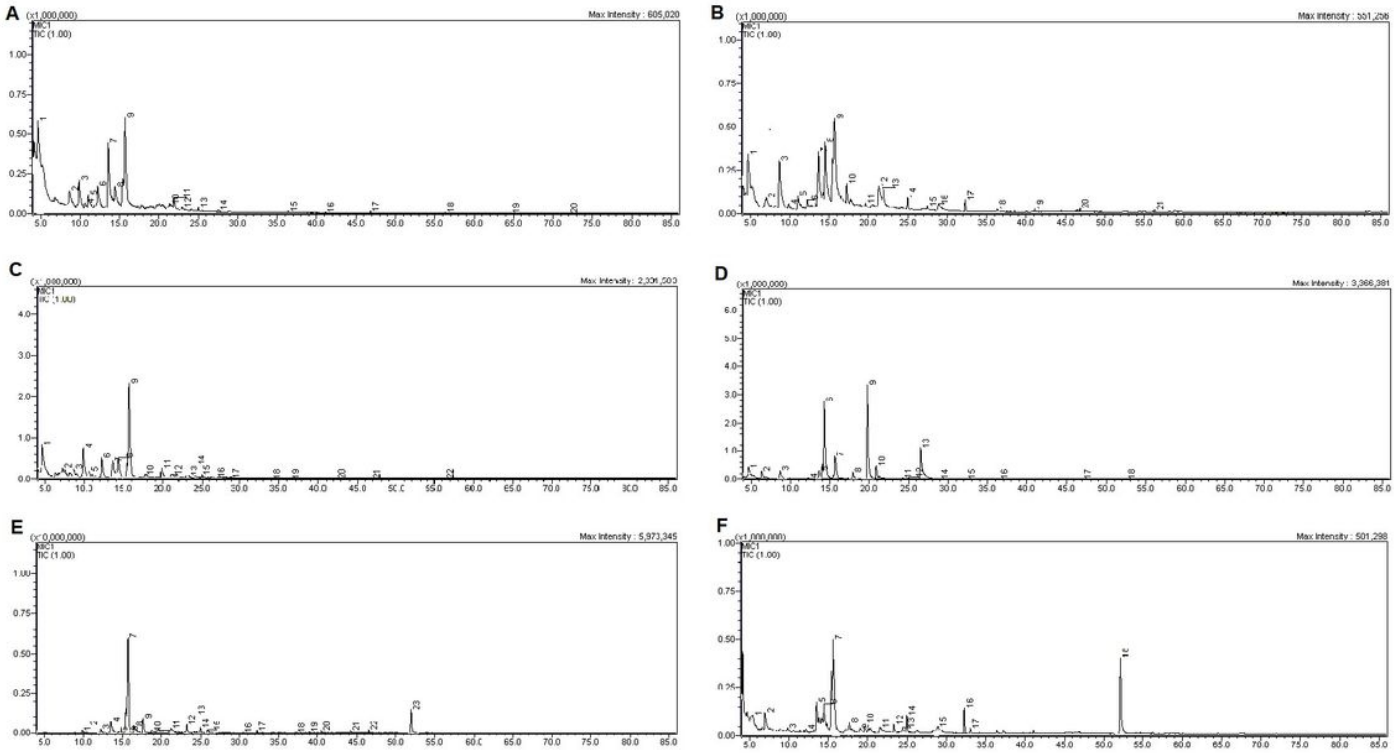


Figure 2

GC-MS chromatograms referring to extractions (HP-SPME) performed in the headspace of different dry plant materials from *Ilex paraguariensis* and their respective extracts chimarrão, terere and mate-tea.

Chromatograms (CG/EM) referring to extractions (HP-SPME) performed in the headspace of dry plant materials and their respective extracts: Chimarrão plant material (A); Chimarrão extract (B); Terere plant material (C); Terere extract (D); Mate-tea plant material (E); Mate-tea extract (F).

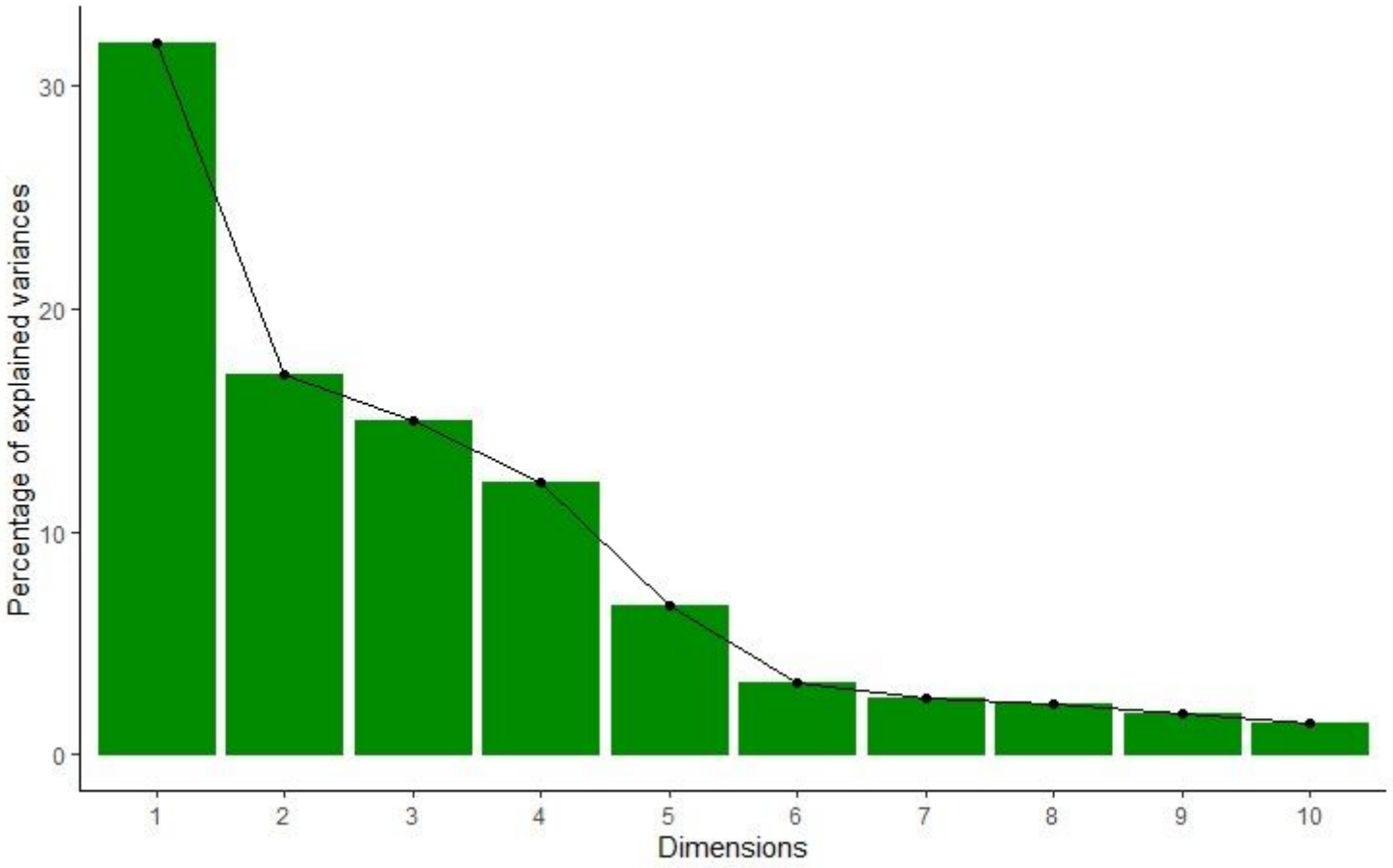


Figure 3

Scree-plot: Percentage of explained-variances for each principal components (PC) of the PCA analysis performed with the relative abundance values of volatile compounds characterized by GC-MS.

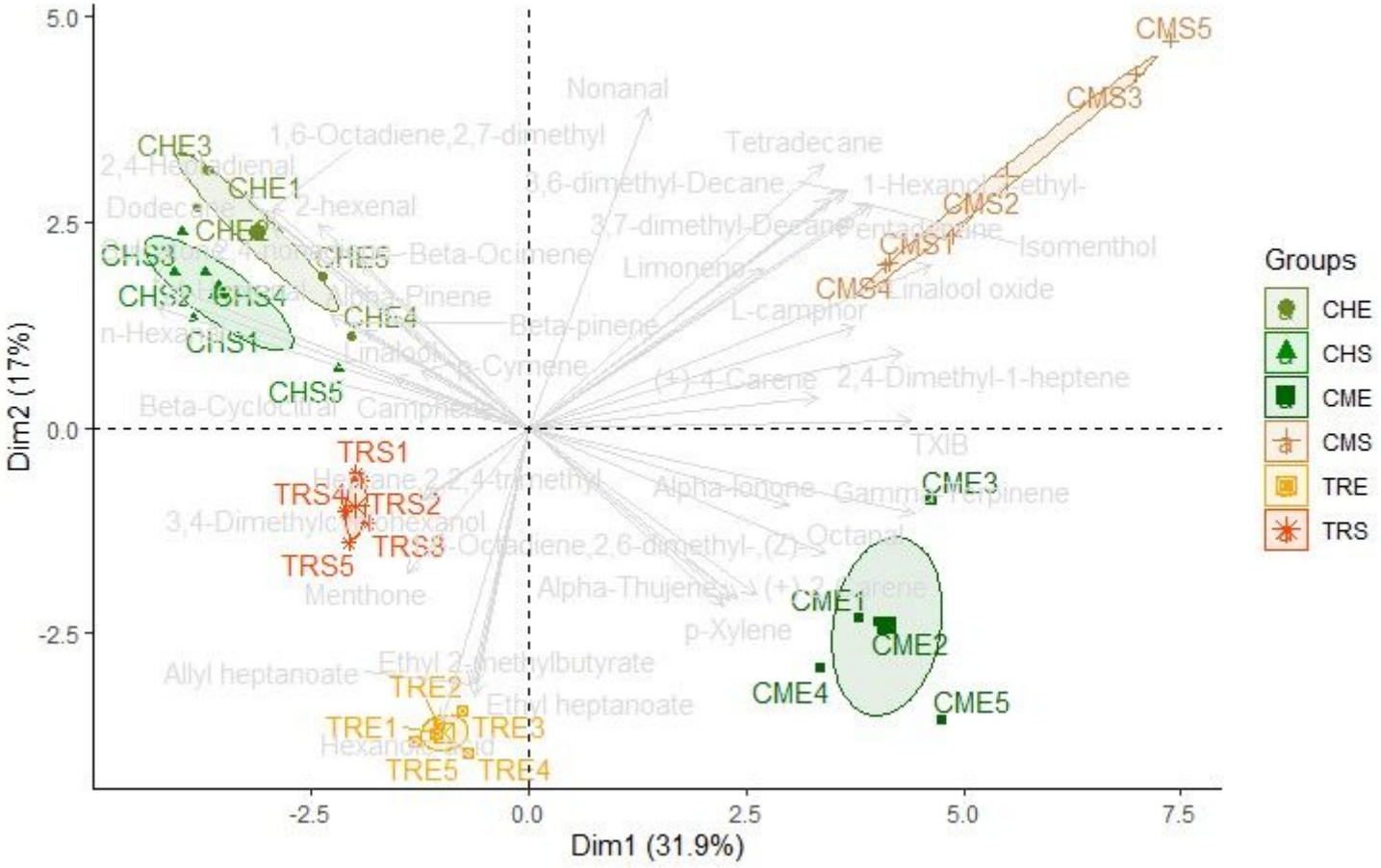


Figure 4

PCA analysis performed with the values of relative abundance of volatile compounds characterized by GC-MS, being PC1 and PC2 (respectively dimensions 1 and 2) followed by the explained-variances percentage.

The different materials analyzed were: Chimarrão extract (CHE); Chimarrão plant material (CHS); Mate-tea extract (CME); Mate-te plant material (CMS); Terere extract (TRE); Terere plant material (TRS). The vector-plot of the explanatory variables (volatile compounds) is shown in the background (light gray).

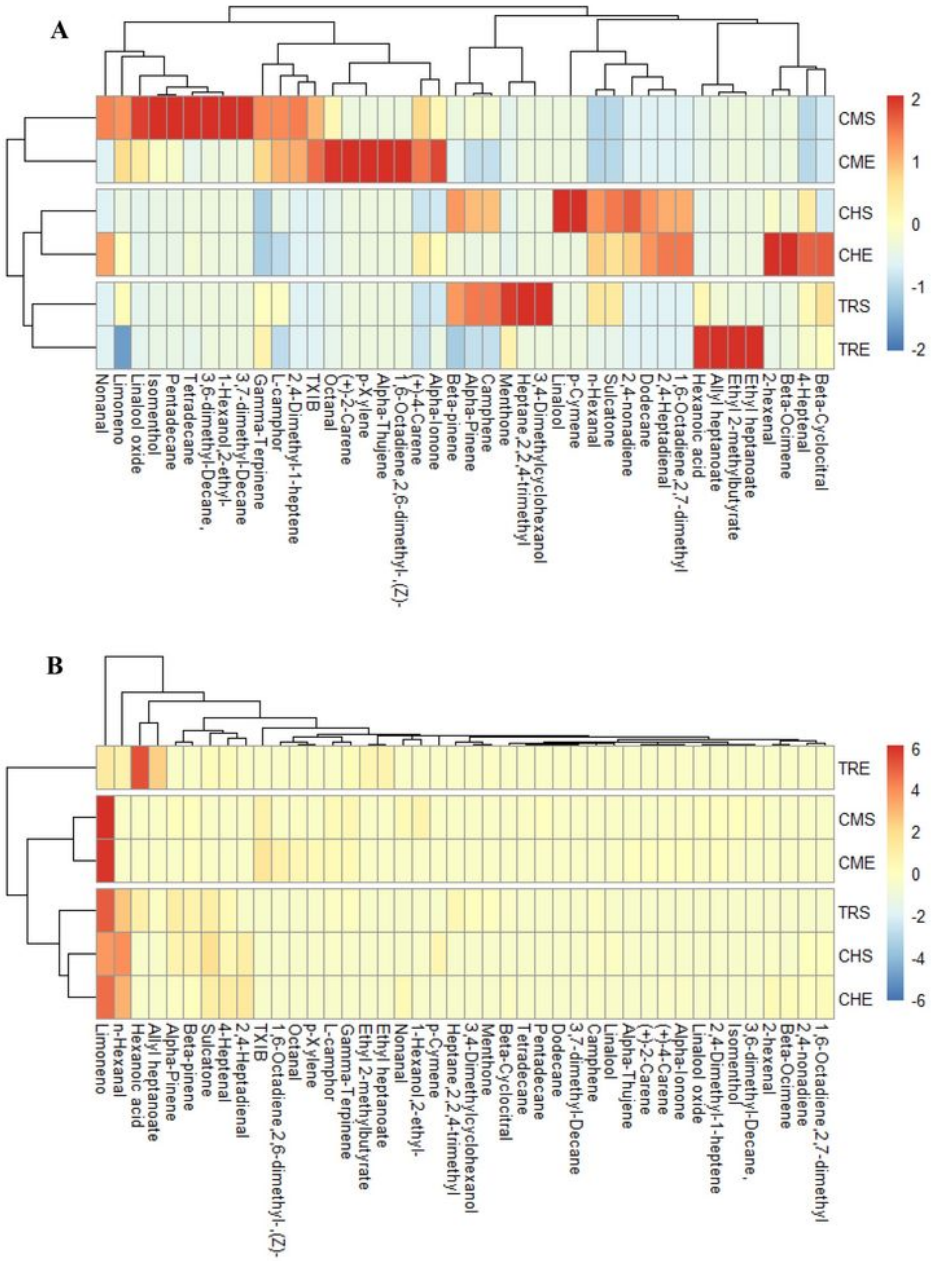


Figure 5

Heatmaps scaled by compound (A) and scaled by sample (B). Heatmap of the relative abundance values of volatile compounds characterized by compound-normalized GC-MS, with presentation of data cluster dendrograms.