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Screening of Volatile Compounds in Mate (*Ilex paraguariensis*) Tea—Brazilian *Chimarrão* Type—By HS-SPDE and Hydrodistillation Coupled to GC-MS

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Abstract: The volatile fraction of mate (*Ilex paraguariensis*) tea—specifically Brazilian *chimarrão* type, which has an odor profile comprising distinctive fresh, green, grass, and herbal notes—was investigated. Hydrodistillation in a Clevenger apparatus was employed in order to extract volatiles from the tea matrix. Headspace–solid-phase dynamic extraction (HS-SPDE) was employed to extract the volatiles from two types of infusions of this tea—a simple single infusion and a traditional preparation of consecutive infusions. Volatiles were analyzed by gas chromatography–flame ionization detection/mass spectrometry (GC-FID/MS). In total, 85 compounds were either identified or tentatively identified and semi-quantified. Semi-quantification comprised peak area integration of all the peaks (including the unidentified ones) in the chromatogram. Results obtained by hydrodistillation and by HS-SPDE were distinct, covering mostly different ranges of volatility and showing only 15 compounds in common. The identified compounds had their respective average and minimum odor thresholds and odor characteristics compiled from the literature. Several major compounds considered as key odorants in other mate tea products were not detected or only present at low levels in the samples of this research. Approximately half of the odorants identified in these samples were commonly reported in different mate tea types; the remaining 41 molecules—predominantly terpenoids (isoprenoids)—could be listed as specific to the Brazilian *chimarrão* type and are suggested to underlie its typical freshness.

Keywords: *chimarrão*; mate cocido; infusion; erva-mate; yerba mate

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1. Introduction

1.1. Volatiles in Different Mate Tea Types

The volatile compounds present in different mate (*Ilex paraguariensis*) teas have been identified and/or quantified (or semi-quantified) in several studies [1–7]. In a recent review, Lasekan and Lasekan [4] mentioned 10 odorants, namely linalool, α-ionone, β-ionone, α-terpineol, octanoic acid, geraniol, 1-octanol, nerolidol, geranylacetone, and eugenol, as described in the work of Kubo et al. [6], that are present in mate teas. They also described several biochemical pathways influencing a much longer list of compounds, depending on factors such as raw materials, the production process, and aging. Most of these compounds were identified either in roasted or aged tea types [1,3]. A few studies investigated the key odorants in the Brazilian *chimarrão* type [2,5,7,8]. Usually, its sensory description focuses on 'green', 'grass', and 'bitter aroma' [8] and contrasts with 'mature', 'smoky', 'tobacco', and 'floral' in the case of aged or roasted mate teas [3]. Therefore, 'fresh' or 'freshness' will be deliberately used in this research, in opposition to 'aged'.

1.2. Analytical Approaches and Instrumentation

When assessing the contribution of volatiles to the flavor perception, some factors need to be taken into account. Different extraction techniques applied for the evaluation of mate teas have generated different results [3]. Some studies performed analyses of mate tea distillates [1,2,7,8]. Techniques based on headspace sampling and lower temperatures than those used for distillation were successfully applied for mate and other teas with good sensitivity and reproducibility [3,5,9,10]. Other studies about mate tea volatiles employed gas chromatography (GC)—for separation—and its common hyphenated techniques for detection, identification, quantification, and semi-quantification [1–7]. Semi-quantification has already been performed in other studies involving numerous mate tea volatiles by integrating all the peak areas registered in the chromatogram and calculating their individual relative peak area [1,2,5,8].

Finally, for the interpretation of analytical results it is necessary to consider that the potential sensory impacts of the different volatiles tend to be not just directly proportional to their concentrations but also inversely proportional to their odor thresholds—among several other factors [11].

1.3. Mate Tea Infusions: Traditional Consecutive Infusions and Single Infusions

An important feature of mate tea that must be taken into account is its most common way of consumption, in traditional consecutive infusions (TCI) in a gourd, popularly known as '*mate*' (or also '*chimarrão*' in Brazil). In this case, mate tea is poured into a gourd, accommodated on one side of it, and water is added to fill up the remaining empty space within the gourd. A metallic straw with a filter coupled to its lower end is then placed inside the gourd. The straw is slowly sucked and the infusion drunk until the gourd is empty. Once empty, more water is added to the gourd. This process is repeated several times. A gourd is commonly shared by several people in a communal way [12,13]. All these features differentiate these mate-tea-specific preparations from other herbal teas.

Even though mate tea is mostly consumed in the form of the traditional consecutive infusions described above, it is also used to prepare a simple single infusion (SI), as other common herbal teas, by infusing a small portion of tea under hot water during a certain extraction time [14,15]. Mate tea single water infusions were studied in other research, which employed or covered various tea-to-water ratios and water temperatures [13–19]. In some Spanish-speaking countries, this single infusion is called '*mate cocido*'. It is prepared with boiling water and drunk very hot, hot, or warm. Nevertheless, consumption at an excessively high water temperature is correlated with a higher occurrence of esophageal cancer and thus is not advisable [18]. Nowadays, the preparation of a mate tea infusion 'in tea bag form is also common' [19].

1.4. Mate Tea Types

Mate beverages are widely consumed in southern Brazil, Argentina, Paraguay, and Uruguay [12]. As could be expected, consumer preferences vary notably among these countries. Therefore, as a result of specific methods of processing and aging employed in the different countries and companies, various mate teas of different types or qualities exist (Figure 1) [14].

1.5. Aims of This Research

The aim of this work was to extend our knowledge of the potential key odorants present in Brazilian *chimarrão* mate teas and in their most common infusions by combining different approaches. These approaches were: identification and semi-quantification of the volatiles found in the essential oil and in the different water infusions (TCI and SI) of these teas; the definition of volatiles that are specific to this type of tea; and the appreciation of the odor thresholds of these molecules.

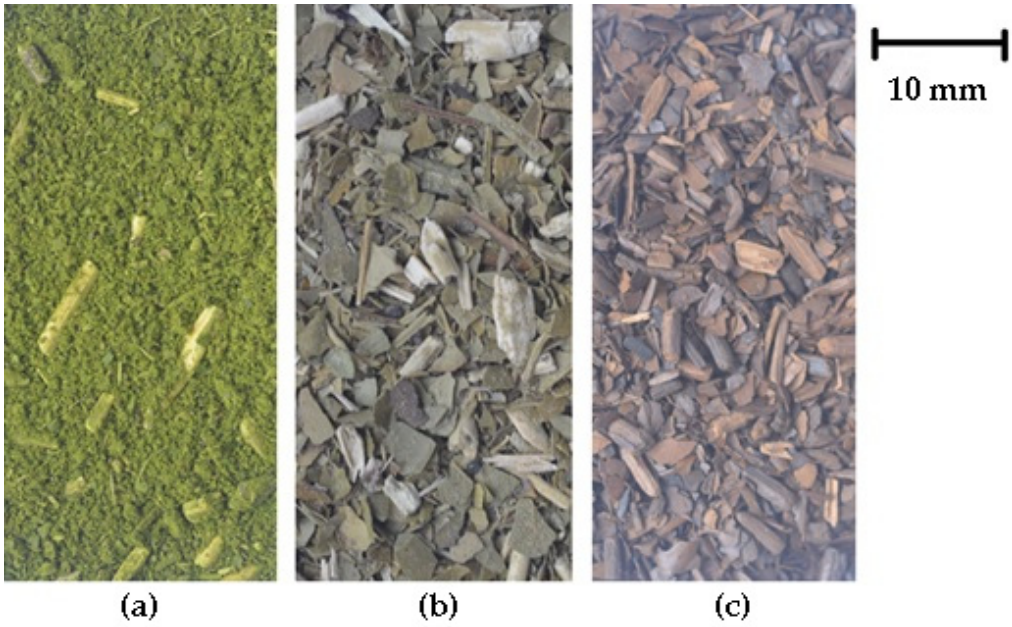

Figure 1. Some different mate tea types: (**a**) Brazilian typical standard (Brazilian *chimarrão*); (**b**) Argentinean typical standard; (**c**) Roasted mate (known as '*chá-mate*', in Brazil).

2. Materials and Methods

2.1. Samples

Samples of Brazilian mate tea—*chimarrão*—were produced following the traditional industrial process for this type of product. After blanching, drying, and grinding, mate teas were immediately packed in 1 kg vacuum packs and, without any aging period, transported to the laboratory in Germany and stored frozen (−20 °C) until analysis. The two samples were (as specified in the labels): one of Brazilian '*chimarrão tradicional premium*' type produced in August (A) and another of Brazilian '*chimarrão tradicional*' type produced in November (B). These samples were produced by Barão Comércio e Indústria de Erva Mate LTDA (Barão de Cotegipe, RS, Brazil) and analyzed as conventionally commercialized and consumed. No grinding processes were applied.

2.2. Hydrodistillation: Extraction of Volatiles in the Mate Tea Samples

Both mate tea samples (A and B) were subject to hydrodistillation. Hydrodistillation is based on the European Pharmacopeia 9.0 [20] and other studies [2,8]. A large sample of tea and a small volume of water had to be used in order to produce an appreciable sample of extracted essential oil. A total of 100 g of tea sample, 700 mL of distilled water, and ten boiling chips (IDL GmbH & Co KG, Nidderau, Germany) were added to a 2000 mL round bottom flask and the Clevenger apparatus was fitted on top of it. During the onset of boiling, an extra 100 mL of room temperature distilled water had to be slowly added from the central orifice of the Clevenger apparatus to control the initial foam formation in the neck of the round bottom flask. After two hours of distillation, 3 × 83.3 µL of n-hexane (Merck KGaA, Darmstadt, Germany) were used to flush the glass surfaces around the few droplets of essential oil extracted. This mixture was collected with 8 mL of the hydrosol (the aqueous phase obtained from the hydrodistillation) in a 10 mL test tube. The supernatant (non-polar phase) was collected with a pipette, transferred into a 1.5 mL vial, and immediately analyzed. Distillations were performed in triplicate for each tea sample and the mixture of essential oil and n-hexane was analyzed without further dilution prior to liquid injection.

2.3. Preparation of Popular Mate Tea Infusions: Single Infusion and Traditional Consecutive Infusions

Both mate tea samples (A and B) were also employed for a lab simulation of two different popular mate tea infusions: one to be representative of the preparation of a single infusion, such as for a conventional tea, and another to be representative of the preparation of traditional consecutive infusions in a gourd.

The conventional single infusion (SI) was accomplished by simply adding 3 g of tea and 200 mL of distilled water at 70 °C to a 250 mL beaker, simulating a domestic preparation of tea, similar to the approaches of other studies [13,16,17]. After one minute, the infusion was collected with a traditional 'bomba' or 'bombilla', a stainless-steel straw with a filter at its lower end (Bortonaggio, Garibaldi, RS, Brazil). The filter at the lower end had a diameter of 34 mm, with 160 holes (60 on each side) of 1 mm each. Over this stainless-steel filter, another finer filter (J.M. Filtros, José Luís Pereira & CIA. LTDA, São Leopoldo, RS, Brazil) with a pore size of around 200 µm was fitted. A 100 mL syringe was coupled with a silicon hose to the upper end of this metallic straw. After suction of the first 100 mL of infusion, 5 mL aliquots were added to the 20 mL SPDE vials. SI infusions were performed in triplicate for each tea type, followed by single headspace–solid-phase dynamic extraction (HS-SPDE) for each replicate.

Traditional consecutive infusions (TCI) (Figure 2) were performed based on the procedures of Meinhardt et al. [13]. A homogeneous sample of 48 g of mate tea was added to a 223 mL glass gourd (Meta Mate, Berlin, Brazil) and agitated manually back and forth, in horizontal position. After shaking, the recipient was positioned at a 45° angle and received the water for hydration (145 mL at 20 °C). After the hydration step of 5 min, the first cold infusion was sucked, and 9 consecutive infusions were performed. The consecutive infusions consisted of adding water up to the edge of the gourd, allowing 30 s of infusion time, and sucking the infusion with the syringe coupled to the upper tip of the metallic straw. Only the 10th infusion was then transferred to a 200 mL beaker and 5 mL aliquots were added to a 20 mL SPDE vial by pipetting. TCI infusions were performed in triplicate for each tea type, followed by single extraction by HS-SPDE for each replicate.

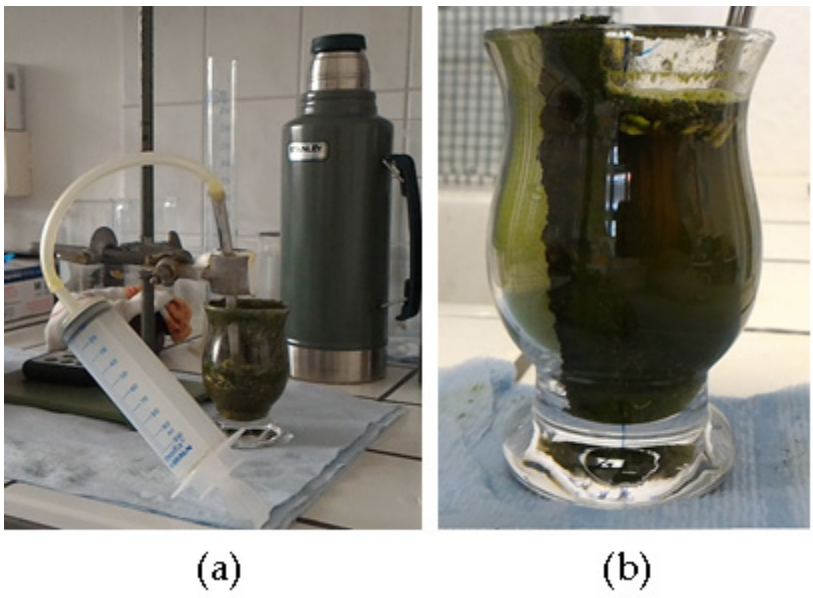

Figure 2. Apparatus for traditional consecutive infusions: (**a**)—whole laboratory setting; (**b**)—consecutive infusion happening.

2.4. HS-SPDE: Extraction of Volatiles in Infusions

The parameters for HS-SPDE were based on previous studies [21,22]. Within a 20 mL SPDE glass vial, 5 mL of the infusions and 100 µL of internal standard—0.154 mmol of 1-octanol in 10% ethanol (Merck KGaA, Darmstadt, Germany)—were carefully added. Just

during the extraction time (approximately 1 h), the vial was heated up to 70 °C and stirred with a magnetic stirrer at 750 RPM, while the syringe was also kept at 70 °C. The extraction was accomplished by 15 strokes, with an aspired volume of 2000 µL per stroke, and flow rates of 10 µL/s up and 100 µL/s down. The 74 mm SPDE needle (Chromtech GmbH, Bad Camberg, Germany), coated with 50 µm of polydimethylsiloxane, activated carbon, and divinylbenzene (PDMS/AC/DVB, respectively) was coupled to a 2.5 mL syringe. After desorption in the GC port, the needle was flushed in the flush station with nitrogen gas at 270 °C for 15 min.

2.5. HS-SPDE: Extraction of Volatiles in the Mate Tea Samples

For identification, in order to maximize the extraction of volatiles present at low concentrations and generate enough of a MS signal, another approach had to be developed. To the best of our knowledge, no similar simple procedure is described in the literature to accomplish this task by HS-SPDE. A vial of 20 mL was filled with 1 mL of water at room temperature, closed just provisionally by pressing the cap against its top, and shaken for a few seconds to spread the water onto the internal walls. Then, 2 g of tea were added to the vial, the vial was sealed, and the tea was spread onto the internal walls while they were still humid by gently rotating and shaking the vial. The tea particles spread and adhered to the humid wall, creating a large surface area for the volatilization of compounds. All the other extraction parameters (i.e., regarding the strokes, the syringe, the needle, and the temperatures) were the same as listed above. These experiments were meant only for identification.

2.6. GC, FID, and MS Parameters

The gas chromatograph (GC) and flame ionization detector (FID) used for both analyses was a TRACE GC (Thermo Fisher Scientific GmbH, Dreieich, Germany) equipped with a Combi PAL autosampler (CTC Analytics AG, Zwingen, Switzerland) and operated under the parameters shown in Tables 1–3. The mass spectrometer (MS)—a TRACE DSQ (Thermo Fisher Scientific)—was coupled to the gas chromatograph described above and operated under the parameters shown in Table 4.

Table 1. GC parameters for analyses of essential oils (from hydrodistillation).

Parameter	Specification
Injected volume	1 µL
Carrier gas	Helium
Carrier gas flow	1 mL/min (constant)
Injection	PTV, splitless
Injection temperature	250 °C
Temperature program	60 °C 2 °C/min ➡ 230 °C 3 °C /min ➡ 300 °C

Table 2. GC parameters for analyses of infusions (by HS-SPDE).

Parameter	Specification
Desorption volume	1000 µL of Helium
Pre-desorption time	45 s
Pre-desorption temperature	250 °C
Desorption speed	10 µL/s
Desorption temperature	250 °C
Carrier gas	Helium
Carrier gas flow	1 mL/min (constant)
Injection	PTV, splitless
Injection temperature	250 °C
Temperature program	40 °C (5 min hold time) 5 °C/min ➡ 70 °C 95 °C ➡ 0.5 °C/min 95 0.7 °C/min ➡ 105 °C 1 °C/min ➡ 140 °C/min 5 °C ➡ 160 °C at 5 °C, 160–250 at 7 °C/min, and 250 °C (2 min hold time)

Table 3. FID parameters for all the analyses.

Parameter	Specification
Base temperature	300 °C
Ignition threshold	0.5 pA
Flow (air)	350 mL/min
Flow (H_2)	35 mL/min
Flow (Makeup):	30 mL/min

Table 4. MS parameters for all the analyses.

Parameter	Specification
Scan mode	Full scan
Detector gain	1×10^5 (Multiplier voltage 1340 V)
Ionization	Positive
Mass range	1–650 Da
Start of the scan	0 min ('on' during the whole GC program)
Rates	Scans/s: 2.0833 Scan rate (amu/s): 1411.6

2.7. Identification and Semi-Quantification of Compounds

Analyses were carried out using Xcalibur and Chrom Perfect software (Thermo Fisher Scientific/Axel Semrau, Sprockhoevel, Germany). The identification of the compounds was performed by a combination of a MS NIST library search and retention indices. The retention indices (RIs) were calculated by linear interpolation of the retention times (RTs) obtained for a sequence of n-alkanes (C8-C40 Alkanes Calibration Standard; Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) analyzed under the same chromatographic conditions used for analyzing the essential oil, the HS-SPDE samples, and the analytical standards. The retention indices were mostly obtained from the compilation of Adams [23]. A minority of values not listed in this reference were obtained from the NIST database [24] for retention indices. Afterwards, the analytical standards were compared with their respective tentatively identified compounds in terms of their experimental retention indices and MS spectra.

The quantification was accomplished by peak area integration. The baselines were established as straight lines in between valleys where no specific masses but just background noise were detected. For chromatograms of essential oil, only the unidentified peaks with an average height (measured from the baseline) under 1000 mV were not considered for the calculation of the relative areas. For chromatograms of HS-SPDE, only peaks with a signal-to-noise ratio lower than 10 were not integrated but listed as 'trace'. Unidentifiable peaks had their peak area measured and included in the calculations of relative peak areas. Peaks identified as column or needle bleeding were completely disregarded.

Standard solutions containing 20 mg of standards and 10 mL of EtOH:H_2O (1:9, *v*/*v*) were prepared and stored at 2 °C until analysis. The standards/chemicals are described in Table S1.

2.8. Replicates

For the essential oil samples, three chromatograms (three replicates) for each tea type were evaluated. Mean peak areas (n = 3) of the analytes were calculated. The calculation of relative areas comprised the unidentified compounds as well. For the HS-SPDE samples, the three chromatograms obtained from both tea types (A and B) in both infusion procedures (SI and TCI) were evaluated. For these samples, prior to the calculation of the relative areas, the areas of the analytes were normalized by dividing them by the area of the internal standard.

2.9. Compilation of Odor Thresholds of the Identified Volatiles

In order to achieve a better comprehension of the analytical data and assist in the identification of the potential odor active compounds, the odor thresholds of the identified compounds were compiled. The vast majority of the odor threshold values in water were obtained from the extensive compilations of van Gemert [25], and a few values from other separate references specified below the appropriate table of results. First, the minimum threshold values found in the literature were compiled separately, in order to compose the list of the 'minimum odor threshold values' ever reported in the literature. When several threshold values were available (which was the case for most of the compounds), minimum and maximum values were excluded and the final selection was based on the following criteria (when applicable): mode—values found repeatedly in the different references were preferred; and year of the reference—the most recent studies were preferred. No distinction was made (when mentioned) between detection thresholds and recognition thresholds. The aroma descriptors were mostly obtained from The Pherobase [26], when available, or from other references specified below the appropriate table of results.

3. Results

3.1. Compounds Obtained by Hydrodistillation and SPDE

The compounds obtained by hydrodistillation (in the essential oils) and HS-SPDE (in the infusions) have their identification data shown in Table 5. Most of the peaks could be tentatively identified by a combination of a MS library search and retention indices. All these tentatively identified compounds matched the reference standards (which were tested) when comparing their mass spectra and retention time, being considered correctly identified. Some compounds that were identified with low MS library search matches and/or an imprecise RI match (in some cases, no RI data were found in the literature) are indicated with a question mark '(?)'. Regarding the unidentified compounds, the following is mentioned, instead of their names: either the most abundant masses (m/z) found in their spectra; or 'unknown', for peaks that did not display clearly distinguishable predominant masses. These peaks might be composed of mixtures of compounds, as reported by Purcaro et al. [5]. All the available data about each unidentified peak (m/z, RT, and RI) that may eventually be useful for future investigations are included.

Table 5. Identified compounds.

Compound	Retention Index			Retention Time (min)		CAS-Number	Identification Confirmed by Standard
	Literature [a]	Essential Oils	Infusions	Essential Oils	Infusions		
Hexanal	801	-	803	-	19.39	66-25-1	x
Oxime–metoxy–phenyl	-	-	891	-	25.31	-	
Pinene <α–>	932	-	931	-	29.19	80-56-8	x
Camphene	946	-	948	-	30.93	79-92-5	x
Benzaldehyde	952	954	959	12.91	32.06	100-52-7	x
Pinene <β–>	974	-	977	-	33.97	127-91-3	x
5–Hepten–2–one <6–methyl–5>	981	975	979	13.94	34.15	110-93-0	x
Myrcene <β–>	988	-	985	-	34.84	123-35-3	x
Pentyl furan <2–>	988	-	986	-	34.84	3777-69-3	
Heptadienal <(2E,4Z)–>	990 n	992	-	14.63	-	4313-02-4	
Octanal	998	-	1001	-	36.52	124-13-0	x
Heptadienal <(2E,4E)–>	1005	1006	1008	15.47	37.64	4313-03-5	
Cymene <p–>	1020	1020	1021	16.31	39.62	99-87-6	x
Limonene	1024	1024	1025	16.58	40.25	5989-27-5	x
Eucalyptol	1026	1028	1029	16.81	40.76	470-82-6	x
Ocimene <(E)–β–>	1044	1039	1039	17.47	42.35	3779-61-1	x
2–octenal <(E)–>	1049	1052	-	18.21	-	2548-87-0	
Terpinene <γ–>	1054	-	1052	-	44.3	99-85-4	x

Table 5. *Cont.*

Compound	Retention Index			Retention Time (min)		CAS-Number	Identification Confirmed by Standard
	Literature [a]	Essential Oils	Infusions	Essential Oils	Infusions		
1–octanol (internal standard)	1063	-	1063	-	45.86	11-87-5	x
Octadien–2–one <(3E,5E)–>	1066 n	1063	-	18.89	-	30086-02-3	
Linalooloxide <(Z)–>	1067	1065	-	19.02	-	5989-33-3	
Linalooloxide <(E)–>	1084	1082	-	19.98	-	34995-77-2	
Fenchone	1083	-	1085	-	49.27	1195-79-5	x
Linalool	1095	1100	1095	21.09	50.71	78-70-6	x
Unknown	-	-	1101	-	51.58	-	
Perillene (?)	1102	-	1109	-	52.99	539-52-6	
Pinocarveol <(E)–>	1135	1138	-	23.57	-	547-61-5	
Verbenol <(E)–>	1140	1142	-	23.82	-	1820-09-3	
Camphor	1141	1145	-	24.00	-	76-212	x
Nonadienal <(2E,6Z)–>	1150	1148	-	24.20	-	557-48-2	
Menthone	1148	1153	-	24.53	-	89-80-5	x
Isoborneol	1155	-	1159	-	61.91	124-76-5	x
Menthol	1167	-	1173	-	64.26	15356-60-2	x
Menthol <iso–>	1179	1175	-	25.93	-	3623-52-7	x
Terpinen–4–ol	1174	1178	-	26.14	-	562-74-3	x
Naphtalene	1178	1182	-	26.40	-	91-20-3	
MethylSalicylate	1190	1188	-	26.83	-	119-36-8	
Estragole	1195	-	1193	-	67.75	140-67-0	x
Terpineol <α–>	1186	1194	-	27.18	-	98-55-5	x
Safranal	1197	1196	-	27.35	-	116-26-7	
Decanal <n>	1201	1203	1203	27.75	69.5	112-31-2	x
Cyclocitral <β–>	1217	1217	1214	28.67	71.19	432-25-7	
Nerol	1227	1222	-	29.00	-	106-25-2	x
166;136;120;108;93;86;79;69	-	1227	-	29.32	-	-	
Carvone	1239	1242	-	30.27	-	99-49-0	x
Geraniol	1249	1249	-	30.73	-	106-24-1	
Ionene, <α–>	1266 n	1253	-	30.95	-	475-03-6	
2–Decenal <(E)– >	1260	1261	-	31.46	-	3913-81-3	
1H–2–Indenone,2,4,5,6,7,7a–hexahydro–3–(1–methylethyl)–7a–methyl	-	1279	1276	32.67	80.63	-	
Anethole <(E)–>	1282	1285	1282	33.05	81.65	4180-23-8	x
Safrole	1285	1289	-	33.28	-	94-59-7	
Carvacrol	1298	-	1293		83.36	499-75-2	x
Edulan I <dihydro–> (?)	1273 n	1294	-	33.59	-	63335-66-0	
172;157;142;128;115;91;77;69;57	-	1356	-	37.42	-	-	
Undecenal <(2E)–> (?)	1357	1367	-	38.10	-	53448-07-0	
Copaene <α–>	1374	1379	-	38.84	-	3856-25-5	
Damascenone <(Z)–β–>	1383	1383	1376	39.10	94.41	59739-63-8	x
192;147;144;131;119;105;93;91;79;69;55	-	1389	-	39.41	-	-	
Elemene <β–>	1389	1394	-	39.72	-	515-13-9	
Damascone <(E)–β–>	1413	1412	-	40.88	-	23726-91-2	
192;174;159;144;131;119;105;91;82;77;71	-	1414	-	40.98	-	-	
Caryophyllene <(E)–β–>	1417	1425	-	41.65	-	87-44-5	x
Ionone <(E)–α–>	1428	1426	-	41.75	-	127-41-3	
Merged peaks	-	1434	-	42.20	-	-	
Aromadendrene	1439	1443	-	42.77	-	489-39-4	
Geranylacetone <(E)–>	1453	1452	1451	43.33	102.9	3796-70-1	
204;178;163;161;150;135;121;107;91;79;71	-	1465	-	44.13	-	-	
Muurolene <γ–>	1478	1479	-	45.01	-	30021-74-0	
Ionone <(E)–β–>	1487	1483	1487	45.23	105.3	79-77-6	x
Muurola–4(14),5–diene <trans–>	1493	1486	-	45.41	-	54324-03-7	
Unknown	-	1494	-	45.92	-	-	
Bicyclogermacrene (?)	1500	1499	-	46.24	-	24703-35-3	

Table 5. *Cont.*

Compound	Retention Index			Retention Time (min)		CAS-Number	Identification Confirmed by Standard
	Literature [a]	Essential Oils	Infusions	Essential Oils	Infusions		
Farnesene <α–>	1505	1509	-	46.85	-	502-61-4	x
Cadinene <γ–>	1513	1522	-	47.62	-	39029-41-9	
Unknown	-	1529	-	48.04	-	-	
Nerolidol <(E)–>	1561	1565	-	50.22	-	40716-66-3	
Dendrolasin	1570	1577	-	50.89	-	23262-34-2	
Spathulenol	1577	1582	-	51.20	-	6750-60-3	
Caryophyllene oxide	1582	1586	-	51.43	-	1139-30-6	
Merged peaks	-	1587	-	51.61	-	-	
Guaiol	1600	1597	-	52.15	-	489-86-1	
Hexadecane <n–>	-	1602	-	52.42	-	544-76-3	
Merged peaks	-	1615	-	53.10	-	-	
Merged peaks	-	1631	-	53.99	-	-	
Cadinol <α–>	1652	1659	-	55.50	-	481-34-5	
6,9–Heptadecadiene (?)	1668 n *	1674	-	56.29	-	-	
Unknown		1677	-	56.45	-	-	
3–Heptadecene <(Z)–> (?)	1687 n *	1684	-	56.84	-	-	
236;258;189;161;145;133;123; 119;109;95;81;69;67;57	-	1690	-	57.16	-	-	
Pentadecanone <2–>	1697	1702	-	57.81	-	2345-28-0	
Merged peaks	-	1720	-	58.78	-	-	
Tetradecanoic acid	1770	1768	-	61.30	-	544-63-8	
122;196;166;138;123;109;96; 82;69;57	-	1785	-	62.23	-	-	
278;263;249;236;222;208;193; 179;165;151;137;123;109;95;82; 71;68;57	-	1844	-	65.26	-	-	
Hexahydrofarnesylacetone	1847 n	1849	-	65.51	-	502-69-2	
278;263;249;236;222;208;193; 179;165;151;137;123;109;95;82; 71;68;57	-	1886	-	67.40	-	-	
Farnesylacetone <(5E,9E)–>	1913	1915	-	68.83	-	1117-52-8	
Methyl hexadecanoate	1927 b	1933	-	69.69	-	112-39-0	
Isophytol(?)		1952	-	70.62	-	-	
Palmitic acid	1970 n	1985	-	72.19	-	57-10-3	x
272;257;229;215;203;189;175;161; 147;136;121;107;93;81;69	-	2029	-	74.28	-	-	
Methyl linolenate	2108 n	2105	-	77.78	-	301-00-8	
296;264;236;222;180;166;152;137; 123;110;96;83;74	-	-	-	77.85	-	-	
Phytol	2128n	2122	-	78.57	-	150-86-7	
Merged peaks	-	2146	-	79.64	-	-	
Merged peaks	-	2151	-	79.85	-	-	
9–Tricosene <(Z)–>	2271 n	2281	-	85.46	-	27519-02-4	
Tricosane	2300	2310	-	86.64	-	638-67-5	
242;299;273;257;231;217;203;191; 185;161;149;136;121;1007;95;81;69	-	2366	-	88.70	-	-	
Squalene	2847 n *	2832	-	102.47	-	111-02-4	x

[a]—Retention index reference values found in the literature [23], when just the numbers are mentioned; n—values from the NIST database [24]; b—values from the literature [2]; '(?)'—low MS library search matches and/or imprecise retention index correspondence; * values found for a similar column, other than DB-5MS; x—identification confirmed by comparison with the retention time and mass spectrum of the authentic standard; '-'—not detected or not available.

The relative peak areas of the volatiles detected by the different methods are represented in Figure 3, where only the major peaks are labeled. Complete information about all the semi-quantified compounds is provided in Table S2 (Supplementary Material).

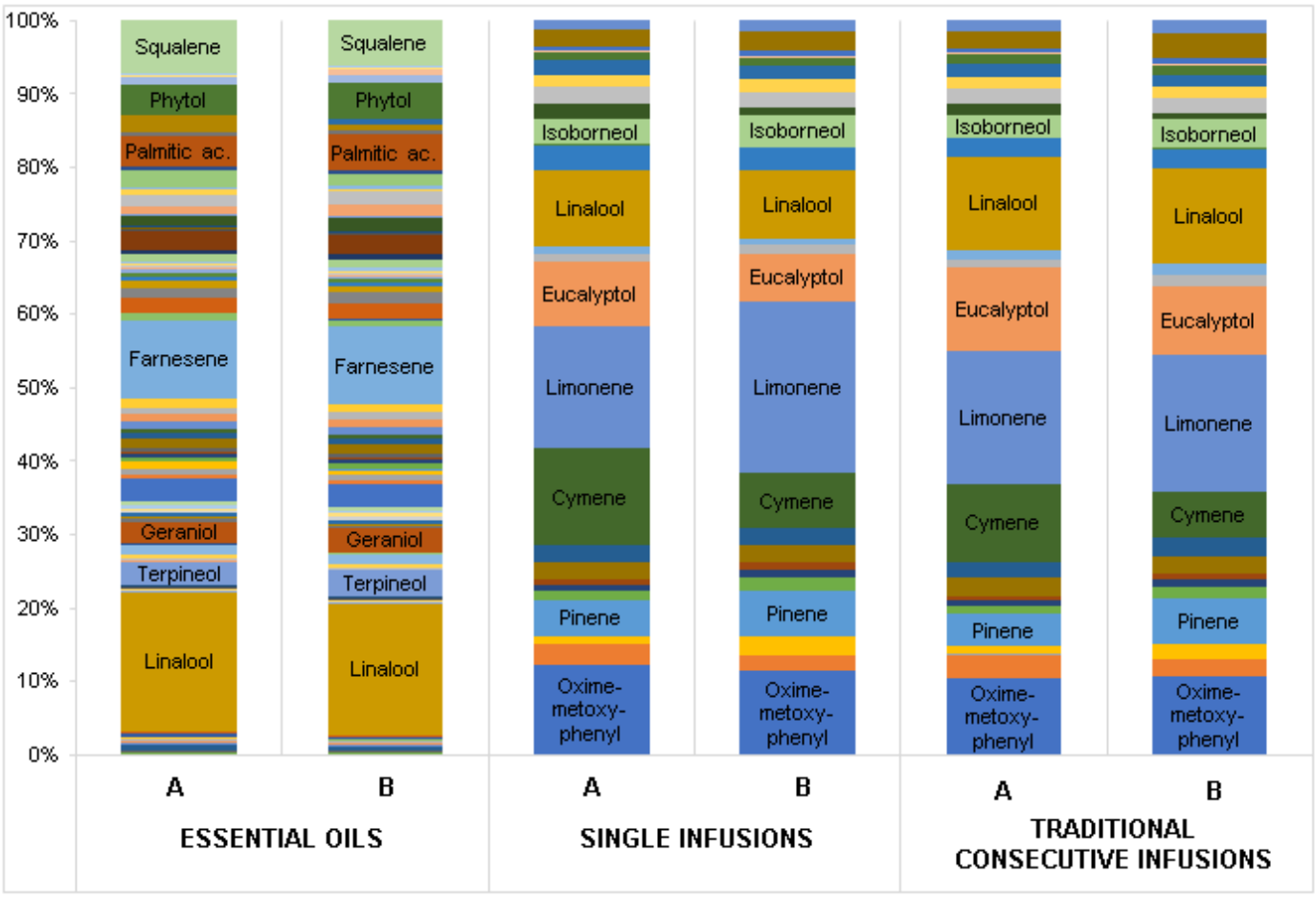

Figure 3. Relative peak areas of the volatiles (only major compounds are labeled). A—sample A; B—sample B.

3.2. Odor Thresholds

A rank of the average odor thresholds of the identified compounds is presented in Table 6, organized from the lowest to the highest average value, i.e., from the compounds that tend to be perceptible at lower concentrations to the ones that tend to be perceived just at higher concentrations, respectively. For some identified compounds, no information about threshold values nor odor characteristics was found. In this case, they were not included in this list.

Table 6. Odor thresholds and characteristics of the identified compounds.

Compound	Average Odor Threshold (ppm) [a]	Minimum Odor Threshold (ppm) [a]	Odor Characteristics [b]
Damascenone, <(Z)–β–>	0.000002	0.00000075	Honey, sweet, fruity, apple, tobacco, canned peach
Damascone, <(E)–β–>	0.000002	0.000002	Fruity, floral, berry, honey, rose, tobacco
Ionone <(E)–β–>	0.000007	0.000007	Violets, floral, raspberry, woody
2–Decenal, <(E)–>	0.0004	0.0003	Green, fatty, tallowy, orange
Octanal	0.0008	0.00032	Lemon, stewed, boiled meat, rancid, soapy, orange
Ionene, <α–>	0.002	0.002	-
Hexanal	0.0024	0.00032	Green, fruity, tallowy, fishy, grassy, herbal, leafy
Decanal <n>	0.003	0.00008	Stewed, burnt, green, waxy, floral, lemon, herbal
Ionone <(E)–α–>	0.00378	0.0004	Floral, violet, woody, fruity
2–octenal, <(E)–>	0.004	0.00034	Fatty, nutty, sweet, waxy, green, burnt, mushroom
Cyclocitral <β–>	0.005	0.003	Sweet, mild, green, grassy, floral, hay
Linalool	0.006	0.00001	Lavender, muscat, sweet, green, floral, lemon
Naphtalene	0.006	0.0068	Medicinal

Table 6. *Cont.*

Compound	Average Odor Threshold (ppm) [a]	Minimum Odor Threshold (ppm) [a]	Odor Characteristics [b]
Geraniol	0.0066	0.001	Rose, geranium, floral, sweet, fruity, citrus
Cymene <p–>	0.0114	0.0062	Lemon, fruity, fuel-like, sweet, herbal, spicy
Pinene <α–>	0.014	0.0025	Terpeny, fruity, sweet, green, woody, pine, citrus
Pentyl furan <2–>	0.0145	0.0058	Buttery, green bean
(β)–Myrcene	0.015	0.0012	Metallic, musty, geranium, sweet, fruity
Estragole	0.016	0.006	Liquorice, sweet, herbal, anise, spicy
Eucalyptol	0.023	0.0011	Camphor, minty, sweet, liquorice, pine
Safrole	0.033	0.01	Sweet, warm, spicy, woody, floral
Ocimene <(E)–β–>	0.034	0.034	Herbal, mild, citrus, sweet, orange, lemon
MethylSalicylate	0.04	0.0349	Wine, berry, warm, sweet, wintergreen
Heptadienal <(2E,4E)–>	0.056	0.0154	Orange oil, oily, fatty, rancid
2.4–Heptadienal, <(E,Z)–>	0.056	0.0154	Orange oil, oily, fatty, rancid
Anethole <(E)–>	0.086	0.0015	Herbal, anise, sweet, spicy
Farnesene <α–>	0.087	0.087	Woody
Linalooloxide <(Z)–>	0.1	0.1	Sweet, woody, floral, creamy, slightly earthy
3,5–Octadien–2–one <(E,E)–>	0.125	0.1	Fresh, sweet, woody, mushroom
Pinene <β–>	0.14	0.006	Musty, green, sweet, pine, resin, turpentine
Caryophyllene <(E)–>	0.15	0.064	Oily, fruity, woody
5–Hepten–2–one <6–methyl–5>	0.16	0.05	Mushroom, earthy, vinyl, rubbery, blackcurrant
Carvone	0.16	0.0067	Caraway, herbal minty
Geranylacetone, <(E)–>	0.186	0.06	Fresh, floral, rose, green, fruity
Limonene	0.2	0.034	Licorice, green, citrus, ethereal, fruity
Nerolidol <(E)–>	0.25	0.25	Waxy, floral
Terpinene <γ–>	0.26	0.065	Citrus, terpeny, herbal, fruity, sweet
Linalooloxide <(E)–>	0.32	0.19	Sweet, floral creamy, leafy, earthy, green
Terpineol <α–>	0.35	0.0046	Peach, anise, oily, minty, toothpaste
Caryophyllene oxide	0.41	0.2	Sweet, fruity, sawdust, fruity, herbal
Fenchone	0.44	0.44	Camphor
Phytol	0.64	0.64	Herbal, delicate, floral, balsamic
Nerol	0.68	0.29	Floral, rose, citrus, marine
Benzaldehyde	0.75	0.024	Burnt sugar, almond, woody
Carvacrol	0.8	0.07	Yuzu, caraway
Camphor	0.83	0.25	Camphor, green, dry, leafy
Isoborneol	0.9	0.001	Musty, dusty
Menthol, <iso–>	0.95	0.1	Fresh, green, cool, herbal
Terpinen–4–ol	1.2	0.34	Terpeny, woody, sweet, herbal, pine, musty
Camphene	1.98	1.86	Sweet, fruity, camphor, pine, oily, herbal
Methyl hexadecanoate	2	2	Oily, faint, waxy, sweet
Menthol	2.1	0.9	Fresh, green, cool, herbal
Menthone	2.4	0.17	Herbal, minty, sweet, earthy
Pinocarveol <(E)–>	-	-	Floral, herbal, camphor, woody, pine
Verbenol <(E)–>	-	-	Balsamic, pine
Safranal	-	-	Powerful saffron aroma, tobacco, camphor
Spathulenol	-	-	Fruity, herbal
Palmitic acid	-	-	Oily
Perillene (?)	-	-	Woody

[a]—values from the literature [25]; [b]—descriptors from The Pherobase [26].

4. Discussion

4.1. Compounds Obtained by Hydrodistillation and SPDE

A remarkable difference between the extraction techniques employed for the present work can be observed at a first glance: HS-SPDE was more sensitive to the more volatile compounds and hydrodistillation to the less volatile. For instance, cymene is the 11th identified compound in the HS-SPDE samples while it is the fifth in the essential oils (Table 5). These results are logical, considering that HS-SPDE occurs at a lower temperature, and during a shorter extraction time, but within a hermetic vial, which prevents any losses

of analytes. Some of the most volatile compounds in the essential oils could not be detected or identified either due to their low concentration or due to the saturated peak of the solvent (n-hexane) that covered them. Considering that mate tea traditional consecutive infusions are prepared while they are being drunk— therefore with warm but not boiling water [13]—hydrodistillation at 100 °C would be less representative than HS-SPDE at 70 °C, which was performed closer to the temperature of consumption. Independently of the temperature, HS-SPDE applied to the analysis of the infusions themselves presents the advantage of analyzing the final product that is ingested (the infusion) instead of the ingredient (mate tea) used for preparing the beverage.

In the essential oils, the 71 identified compounds showed generally similar mean relative areas in both samples. The exceptions, showing a difference larger than twofold (%) between samples A and B, were cymene <o->, 3,5-octadien-2-one <(E,E)->, linalooloxide <(Z)->, safranal, damascenone, farnesylacetone, and methyl linolenate. Therefore, both products can be considered similar and the average peak areas between both essential oils (A and B) were considered suitable for evaluating the highest means (above 1%) for: linalool (18.1%); farnasene (10.5%); squalene (6.6%); palmitic acid (4.5%); phytol (4.5%); terpineol <α-> (3.4%); damascenone, <(Z)-β-> (3.2%); geraniol (3.2%); 3-heptadecene <(Z)-> (2.6%); nerolidol <(E)-> (2.1%); farnesylacetone <(5E,9E)-> (1.97%); hexahydrofarnesylacetone (1.6%); methyl linolenate (1.5%); dendrolasin (1.4%); nerol (1.3%); geranylacetone <(E)-> (1.2%); ionone <(E)-β-> (1.1%); and 6,9-heptadecadiene (1.1%).

The HS-SPDE samples presented a total of 30 identified compounds, which showed always similar patterns of relative peak area, independently of tea sample (A or B) and infusion technique (SI or TCI). Therefore, the overall average relative area for each compound was considered representative for further evaluation of the relatively most abundant (>2% of the total area) compounds: limonene (17.9%); linalool (10.5%); oxime-methoxy-phenyl (10.4%); cymene <p-> (8.7%); eucalyptol (8.4%); hexanal (7.0%); pinene <β-> (5.0%); isoborneol (3.4%); unknown (2.7%); geranyl acetone (2.5%); pinene <α>(2.4%); octanal (2.2%); 2,4-heptadienal <(2E,4E)> (2.1%); and decanal <n> (2.1%).

Some researchers reported several volatiles of relatively high molecular mass by using different extraction techniques [27,28]. Corroborating with the results of the latest, in this research, essential oils also presented almost 40% of the total relative area situated above an AI of 1565. This upper range comprises the following major identified compounds (above 1% on average): squalene (6.6%); palmitic acid (4.5%); phytol (4.5%), 3-heptadecene <(Z)-> (2.6%); nerolidol <(E)-> (2.1%); methyl hexadecanoate (2.0%); hexahydrofarnesylacetone (1.6%); methyl linolenate (1.5%); tetradecanoic (1.5%); dendrolasin (1.4%); and 6,9-heptadecadiene (1.1%).

In combination, the results from hydrodistillation and HS-SPDE are comparable with the findings of Bastos et al. [2]. This study presented 32 identified and semi-quantified volatiles, which creates a certain intersection between the results from the two different extraction techniques. It is important to emphasize that in this other study, the addition of a non-polar solvent to the distillation procedure, namely dichloromethane, might have assisted in preventing the loss of the most volatile components. These could be found at appreciable levels in the present study just in the HS-SPDE samples but not in the essential oils. Examples are: limonene, cymene, and eucalyptol, which comprised more than 30% of the area in the chromatograms from HS-SPDE, while in case of the essential oils this sum was lower than 0.5%.

Only a few compounds (15) could be obtained both by hydrodistillation and HS-SPDE, namely: benzaldehyde; 5-hepten-2-one <6-methyl-5>; heptadienal <(2E,4E)->; cymene <p->; limonene; eucalyptol; ocimene <(E)-β->; linalool; decanal <n->; cyclocitral <β->; anethole <(E)->; damascenone <(Z)-β->; geranylacetone <(E)->; ionone <(E)-β->; and 1H-2-indenone,2,4,5,6,7,7a-hexahydro-3-(1-methylethyl)-7a-methyl. This reduced number of compounds suggests that the combination of different methods is necessary for more complete screenings of the volatiles in the mate tea samples and that by using other methods other compounds should be found. Other researchers already used combinations of

different extraction and analytical methods—e.g., dynamic headspace analysis (DHA), solvent-assisted flavor evaporation–solvent extraction (SAFE-SE), column adsorption extraction coupled to gas chromatography–olfactometry (GC-O), and gas chromatography–mass spectrometry (GC-MS). Using these different methods, unlike results were obtained for the same samples [3].

4.2. Odor Thresholds

First of all, it must be noted that the odor threshold values compiled from the literature (Table 6) are from different studies, which employed different methods and present variations of many folds. Furthermore, they were determined at room temperature using water as a matrix. In the case of mate tea *chimarrão*-type infusions, prepared with warm/hot water, these values change greatly once the vapor pressure of a substance increases exponentially with the temperature [11]. Therefore, caution is necessary when considering these data.

In between the values at the extremes of Table 6, a difference of 3.2 million fold can be observed. This indicates that the perception of the different volatiles is greatly driven by these values. Some of them possess such low odor thresholds that their occurrence above the threshold and consequent odor contribution already become likely upon their detection by a gas chromatograph, which is frequently less sensitive than the human olfactory system [11]. Examples of these compounds with extremely low values are: damascenone <(Z)-β->, damascone <(E)-β->, ionone <(E)-β->, 2-decenal <(E)->, octanal, ionene, <α->, hexanal, decanal <n>, ionone <(E)-α->, 2-octenal <(E)->, cyclocitral <β->, and linalool. On the other hand, compounds at the bottom of the table are more unlikely to impart their specific individual notes, e.g., camphene, methyl hexadecanoate, menthol, and menthone.

4.3. Potential Key Odorants in the Brazilian Chimarrão Type

The majority of the compounds found in both tea samples by both extraction techniques were previously identified in different types of mate tea samples in various relative concentrations [1–3,8]. Nevertheless, some compounds reported to be within the 10 main compounds of mate teas [6] were not detected in these *chimarrão* samples: octanoic acid, 1-octanol, and eugenol. Some odorants mentioned by Lozano et al. [3] within the major aroma contributors in different Argentinean mate samples were also not found in *chimarrão* samples: vinylguaiacol <p->; guaiacol; 3-hexenal <Z>; 1-octen-3-ol; geranial; and eugenol. Other compounds present at considerable levels in these aged products showed low levels in the *chimarrão* samples, e.g., hexanal; benzaldehyde; and 5-Hepten-2-one <6-methyl-5>.

Important compounds, commonly found in different mate tea types, were also detected in the *chimarrão* samples. Those that possess a low odor threshold and/or showed a large relative peak area in these samples are highly likely to integrate the odor profile of this product as well, e.g., linalool; terpineol <α->; damascenone <(Z)-β->; nerol; geraniol; damascone <(E)-β->; ionone <(E)-β->; ionone <(E)-α->; ionene <α->; 2-decenal <(E)->; octanal; hexanal; decanal <n>; 2-octenal <(E)->; cyclocitral <β->; 5-Hepten-2-one <6-methyl-5>; and geranylacetone <(E)->.

On the other hand, numerous compounds not even reported, present at low levels, or not regarded as potential key odorants in studies involving other types of mate teas were found in the present research. Remarkable odorants among them, showing a considerably large relative peak area and/or a low odor threshold, were: pinene <α->; pinene <β->; cymene <p->; limonene; eucalyptol; ocimene <(E)-β->; isoborneol; damascone <(E)-β->; farnesene <α->; nerolidol; dendrolasin; phytol; and squalene. Approximately 50% (41 out of 85 compounds) of all the compounds identified in this research were detected only in *chimarrão* but not in other types of mate teas: oxime-metoxy-phenyl; camphene; ocimene <(E)-β->; fenchone; isoborneol; pinocarveol <trans->; verbenol <trans>; camphor; menthone; menthol; estragole; terpinen-4-ol; carvone; ionene, <α->; 1H-2-indenone,2,4,5,6,7,7a-hexahydro-3-(1-methylethyl)-7a-methyl; anethole; carvacrol; safrole (just in one sample of this research); copaene <α->; elemene <β->; damascone <(E)-β->; caryophyllene <(E)-β->; aromadendrene; muurolene <γ->; muurola-4(14),5-diene <trans->; farnesene <α->;

cadinene <γ->; dendrolasin; spathulenol; caryophyllene oxide; guaiol; hexadecane <n->; cadinol <α->; tetradecanoic acid; hexahydrofarnesylacetone; farnesylacetone <(5E,9E)->; methyl hexadecanoate; palmitic acid; methyl linolenate; 9-tricosene <(Z)->; tricosane; and squalene. The vast majority of these compounds are terpenoids (isoprenoids). These odorants are, potentially, keys to differentiating and characterizing the volatiles specific to this product, which consumers recognize by and appreciate for its fresh and non-mature (non-aged) flavor [8].

Bastos et al. [2] analyzed samples of Brazilian 'green mate' (supposedly also of the Brazilian *chimarrão* type) and 'chá-mate' (roasted), both from the same batch of raw materials and reported about volatile compounds in both samples. Some of the major compounds in the non-roasted samples are in accordance with those found in the present research and were lower or absent in the roasted samples, namely: pinene <α->; myrcene; limonene; linalool; terpineol <α>; geraniol; 2-decenal <(E)- >; damascone <(E)-β->; and methyl hexadecanoate. These findings reinforce their presence in the list of typical major volatile compounds in this product.

In sum, a long list of compounds might be associated with the unique freshness of the Brazilian *chimarrão* mate tea. Remarkably, many terpenoids must be involved, even though several compounds from other classes of compounds are certainly inherent to its overall sensory profile. It is also important to consider that the freshness of *chimarrão* must be dependent not just on the presence of compounds imparting the typical fresher ('non-aged') notes at or above noticeable levels but also dependent on the concurrence of low levels or the absence of volatiles imparting the mature, aged, or roasted character. Many of these compounds (imparting aged notes) were described in other studies with other mate tea types [1,3,4,12]. Even though the results presented in this research are still inconclusive, they constitute a database to serve as a starting point for determining active and key odorants within the volatile fraction of this product in further future research, which should employ sensory analysis and other tools such as gas chromatography–olfactometry (GC-O).

5. Conclusions

In total, 85 compounds were identified (or tentatively identified) and semi-quantified in Brazilian *chimarrão* mate tea. Some compounds (mostly smaller peaks) remained unidentified. Approximately 50% of the identified compounds were commonly reported in studies with different mate tea types. Potential key odorants are supposed to be comprised within a list of numerous molecules (41) that seem to be specific to this product and are mostly composed of terpenoids (isoprenoids). The odor profile of this product (Brazilian *chimarrão* mate tea) must be characterized by: the presence of compounds imparting the typical freshness; and the absence or low levels of some compounds typically reported in other mate tea types, which derive from specific processes such as aging and roasting. Further investigations based on other tools such as GC-O and sensory analysis are necessary to define the key odorants in this product.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/separations8090131/s1, Table S1: Standards and chemicals used for GC-MS, Table S2: Relative peak areas of the compounds obtained by hydrodistillation and SPDE.

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References

1. Kawakami, M.; Kobayashi, A. Volatile Constituents of Green Mate and Roasted Mate. *J. Agric. Food Chem.* **1991**, *39*, 1275–1279. [CrossRef]
2. Bastos, D.H.M.; Ishimoto, E.Y.; Ortiz MMarques, M.; Fernando Ferri, A.; Torres, E.A.F.S. Essential oil and antioxidant activity of green mate and mate tea (Ilex paraguariensis) infusions. *J. Food Compos. Anal.* **2006**, *19*, 538–543. [CrossRef]
3. Lozano, P.R.; Cadwallader, K.R.; González de Mejia, E. Identification of Characteristic Aroma Components of Mate (*Ilex paraguariensis*) Tea. *ACS Symp. Ser.* **2007**, *946*, 143–152.
4. Lasekan, O.; Lasekan, A. Flavour chemistry of mate and some common herbal teas. *Trends Food Sci. Technol.* **2012**, *27*, 37–46. [CrossRef]
5. Purcaro, G.; Tranchida, P.Q.; Jacques, R.; Caramão, E.B.; Moret, S.; Conte, L.; Dugo, P.; Dugo, G.; Mondello, L. Characterization of the yerba mate (Ilex paraguariensis) volatile fraction using solid-phase microextraction-comprehensive 2-D GC-MS. *J. Sep. Sci.* **2009**, *32*, 3755–3763. [CrossRef] [PubMed]
6. Kubo, I.; Muroi, H.; Himejima, M. Antibacterial Activity against Streptococcus mutans of Mate Tea Flavor Components. *J. Agric. Food Chem.* **1993**, *41*, 107–111. [CrossRef]
7. Márquez, V.; Martínez, N.; Guerra, M.; Fariña, L.; Boido, E.; Dellacassa, E. Characterization of aroma-impact compounds in yerba mate (Ilex paraguariensis) using GC-olfactometry and GC-MS. *Food Res. Int.* **2013**, *53*, 808–815. [CrossRef]
8. Machado, C.C.B.; Bastos, D.H.M.; Janzantti, N.S.; Facanali, R.; Marques, M.O.M.; Franco, M.R.B. Determinação do perfil de compostos voláteis e avaliação do sabor e aroma de bebidas produzidas a partir da erva-mate (*Ilex paraguariensis*). *Quim Nova* **2007**, *30*, 513–518. [CrossRef]
9. Kim, Y.; Lee, K.G.; Kim, M.K. Volatile and non-volatile compounds in green tea affected in harvesting time and their correlation to consumer preference. *J. Food Sci. Technol.* **2016**, *53*, 3735–3743. [CrossRef]
10. Du, L.; Li, J.; Li, W.; Li, Y.; Li, T.; Xiao, D. Characterization of volatile compounds of pu-erh tea using solid-phase microextraction and simultaneous distillation-extraction coupled with gas chromatography-mass spectrometry. *Food Res. Int.* **2014**, *57*, 61–70. [CrossRef]
11. Meilgaard, M.C.; Civille, G.V.; Carr, B.T. *Sensory Evaluation Techniques*, 3rd ed.; CRC Press: New York, NY, USA, 2010.
12. Heck, C.I.; De Mejia, E.G. Yerba mate tea (*Ilex paraguariensis*): A comprehensive review on chemistry, health implications, and technological considerations. *J. Food Sci.* **2007**, *72*, 138–151. [CrossRef] [PubMed]
13. Meinhart, A.D.; Bizzotto, C.S.; Ballus, C.A.; Poloni Rybka, A.C.; Sobrinho, M.R.; Cerro-Quintana, R.S.; Teixeira-Filho, J.; Godoy, H.T. Methylxanthines and phenolics content extracted during the consumption of mate (*IIex paraguariensis* St. HiI) beverages. *J. Agric. Food Chem.* **2010**, *58*, 2188–2193. [CrossRef]
14. Loria, D.; Barrios, E.; Zanetti, R. Cancer and yerba mate consumption: A review of possible associations. *Rev. Panam Salud Pública* **2009**, *25*, 530–539. [CrossRef]
15. Gómez-Juaristi, M.; Martínez-López, S.; Sarria, B.; Bravo, L.; Mateos, R. Absorption and metabolism of yerba mate phenolic compounds in humans. *Food Chem.* **2018**, *240*, 1028–1038. [CrossRef]
16. Butiuk, A.P.; Martos, M.A.; Adachi, O.; Hours, R.A. Study of the chlorogenic acid content in yerba mate (Ilex paraguariensis St. Hil.): Effect of plant fraction, processing step and harvesting season. *J. Appl. Res. Med. Aromat Plants* **2016**, *3*, 27–33. [CrossRef]
17. Murakami, A.N.N.; Amboni, R.D.D.M.C.; Prudencio, E.S.; Amante, E.R.; Fritzen-Freire, C.B.; Boaventura, B.C.B.; Muñoz, I.; Branco, C.D.S.; Salvador, M.; Maraschin, M. Concentration of biologically active compounds extracted from *Ilex paraguariensis* St. Hil. by nanofiltration. *Food Chem.* **2013**, *141*, 60–65. [CrossRef]
18. Rolón, P.A.; Castellsagué, X.; Benz, M.; Muñoz, N. Hot and Cold Mate Drinking and Esophageal Cancer in Paraguay. *Cancer Epidemiol. Biomarkers Prev.* **1995**, *4*, 595–605. [PubMed]
19. Bates, M.N.; Hopenhayn, C.; Rey, O.A.; Moore, L.E. Bladder cancer and mate consumption in Argentina: A case-control study. *Cancer Lett.* **2007**, *246*, 268–273. [CrossRef]
20. Council of Europe. *European Pharmacopoeia 9.0*; European Directorate for the Quality of Medicines and Health Care of the Council of Europe (EDQM): Strasbourg, France, 2017.
21. Bicchi, C.; Cordero, C.; Liberto, E.; Rubiolo, P.; Sgorbini, B. Automated headspace solid-phase dynamic extraction to analyse the volatile fraction of food matrices. *J. Chromatogr. A* **2004**, *1024*, 217–226. [CrossRef] [PubMed]
22. Lv, S.; Wu, Y.; Li, C.; Xu, Y.; Liu, L.; Meng, Q. Comparative analysis of Pu-erh and Fuzhuan teas by fully automatic headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry and chemometric methods. *J. Agric. Food Chem.* **2014**, *62*, 1810–1818. [CrossRef]

23. Adams, R.P. *Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy*, 4th ed.; Allured Publishing: Carol Stream, IL, USA, 2007.
24. Linstrom, P.J.; Mallard, W.G. (Eds.) *NIST Chemistry webBook, NIST Standard Reference Database Number 69*; National Institute of Standards and Technology: Gaithersburg, MD, USA, 2018.
25. Van Gemert, L.J. *Odour Thresholds—Compilations of Odour Thresholds in Air, Water and Other Media*, 2nd ed.; Oliemans Punter and Partners: Utrecht, The Netherlands, 2011.
26. Pherobase. The Pherobase: Database of Pheromones and Semiochemicals. 2019. Available online: http://www.pherobase.com/ (accessed on 25 July 2019).
27. Smelindro, A.Q.E.; Os, J.O.D.; Irardi, S.A.G.; Ossi, A.L.M.; Osa, R. Influence of Agronomic Variables on the Composition of Mate Tea Leaves (*Ilex paraguariensis*) Extracts Obtained from CO_2 Extraction at 30 °C and 175 bar. *Agric. Food Chem.* **2004**, *52*, 1990–1995. [CrossRef] [PubMed]
28. Jacques, R.A.; Freitas, L.S.; Pérez, V.F.; Dariva, C.; Oliveira, A.P.; Oliveira, J.V.; Caramão, E.B. The use of ultrasound in the extraction of Ilex paraguariensis leaves: A comparison with maceration. *Ultrason. Sonochem.* **2007**, *14*, 6–12. [CrossRef] [PubMed]