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Correlation between maturity of tree and GC × GC–qMS chemical profiles of essential oil from leaves of *Aniba rosaeodora* Ducke

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ABSTRACT

The Amazonian tree *Aniba rosaeodora* Ducke, which provides a valued essential oil for perfume industry, is at risk of extinction. An alternative source of this product would be the oil obtained by steam distillation of the leaves of the same plant—which does not involve sacrifice of the tree. However, there is still not a technical criterion to ensure that determined oil is result of a sustainable production process. One step towards defining to know the differences in the oil compositions of trees of different stages of growth to establish the age from which the tree can be commercially explored and to differentiate products obtained from cultivated young plants and from native trees. In this paper, the characterization an differentiation of the essential oil extracted from the leaves collected from trees with different ages (4, 10 and 20 years old) was performed by Comprehensive Two-Dimensional Gas Chromatography coupled with Quadrupole Mass Spectrometric detection (GC × GC–qMS). GC × GC–qMS allowed the identification of ca. three times more chemical compounds on these samples, when compared to conventional gas chromatography. Depending on the age of the tree used to produce the oil, few differences in minor constituents of the oil samples were found; the amounts of the major compounds are similar all samples. Reliable differentiation of the essential oils according to the age of the source was only possible by GC × GC–qMS.

1. Introduction

Commercial rosewood essential oil has been intensively explored by steam distillation of chipped wood of the Amazonian tree *Aniba rosaeodora* Ducke, since the 1920s. The international perfume industry is the main consumer of this singular fragrance. As a consequence of the intense exploration of native forest to produce this oil, this species was recently included in the CITES-listed database (Convention on International Trade in Endangered Species of Wild Fauna and Flora) as a plant at risk of extinction.

The major volatile compound found on rosewood oil is linalool, ranging from 78 to 93% [1,2] although percentages of up to 99% have already been reported [3]. There is some discussion on the literature regarding sustainable sources of rosewood oil and its chemical composition [2]. It is feasible to explore cultivated rosewood trees, avoiding the collection of native specimens [4,5]; also, an alternative source of oil are the leaves of young plants [6]. However, there is scarce literature related to a chemical profile of the oil obtained from leaves of this tree, which does not demand the sacrifice of the plant, and therefore would be a sustainable alternative to obtain this valuable product. Therefore, it is fundamental to determine the chemical profile of rosewood leaf essential oil in more detail. Also, it is not known if material collected from older plants could provide oils chemically similar to those that extracted from younger rosewood trees. This could unveil other interesting approach from the economical and environmental points of view, ensuring a larger stock of feasible sources of rosewood oil.

Literature has a relatively low number of studies discussing the effect of the plant age and its growth stage on the composition of the corresponding essential oils. Dunfor et al. [7] reported the effect of age on the distribution of oil in red cedar tree segments, as a way to improve the extraction efficiency. The seasonal variation of monoterpene emission with for coniferous trees with different ages was studied by Kim et al. [8]: for one of species there was a significant dependence of the emissions of monoterpenes with tree age. The majority of volatile compounds identified in essential oil of *Cinnamomum cassia* by Geng et al. [9] presented high fluctuations in percentage of composition in different growth stages. Geng also investigated essential oil for the segment of the plant. The present study, however, had the leaves as the only target segment. Essential oil of *Cryptomeria japonica* has insecticidal activity and was studied at different ages [10]. The authors did not found significant correlation between age and yield; also, the composition of the oils did not change significantly.

As for the analytical technique employed on these studies, gas chromatography (GC) is universally adopted. However, for such complex

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matrices higher separation capacity would be desirable. During last decade, comprehensive two-dimensional gas chromatography (GC×GC) has become a possible alternative for these samples [11]. There are several reports in the literature concerning the application of GC×GC to the analysis of fragrances, aromas and essential oils [12–15], including rosewood essential oil [16]. As for the detection, quadrupole mass spectrometry (qMS) has been pointed as a viable alternative to time-of-flight mass spectrometry (ToF-MS). The former has much lower cost and ultimate generation rapid-scanning qMS instruments are quite suitable for GC×GC instrumental analysis requirements [11–18].

Linear temperature-programmed retention indices (LTPRI) are frequently used as an auxiliary tool for compound identification. The usual procedure to calculate LTPRI is the use of an 'equivalent' first dimension retention time (^1t_R) value, obtained by subtraction of the second dimension retention time (^2t_R) from the total retention time [19,20]. Von Mühlen et al. [21] compared LTPRI values calculated using the 'equivalent' ^1t_R and those obtained using total retention times. The authors observed that the values differ by less than 3 units of LTPRI. Thus, total retention time for LTPRI can be selected as a good estimative.

Since rosewood plantations in the Amazon could be the answer to conservation of this Aniba species, from an economic point of view, it is fundamental to know if young plants can produce essential oil with the quality required by the perfumery industry. Thus, the aim of this work was to carry out, by GC×GC–qMS, the chemical characterization of rosewood leaf essential oil extracted from trees at different growth stages, from four, ten and twenty year old plants to investigate its composition differences and the potential use as a sustainable source of rosewood essential oil.

2. Experimental

2.1. Plant material

Leaves and fine branches of four, ten and twenty years old *A. rosaeodora* trees were collected in December 2009 in the city Maués, in Amazon State, Brazil (S 03°32′44″, W 57°41′30″) where the characteristic climate is hot and humid. Specimens were identified by one of us (P. T. B. Sampaio). This rosewood material was steam distilled for 6 h in an industrial 1500 L iron reactor two thirds filled with leaves. The oil was separated from water after reaching roam temperature. Yield was, on average 0.75%. The oil was transferred to glass flasks filled to the top and kept at a temperature of −4 °C for further analysis.

2.2. Analysis of the essential oil

The analyses were performed on a GCMS-QP2010 Plus gas chromatograph from Shimadzu, adapted to work as GC×GC–qMS with technology developed in our laboratory [18,22–26]. The home-made four jet modulator was turned off to carry out conventional GC–qMS runs. The column set used was: HP-5 (5% phenyl-dimethylpolysiloxane), fused-silica column (30 m×0.25 mm, 0.25 μm film thickness) + DB-Wax (Polyethyleneglycol — PEG) column (1 m×0.1 mm, 0.1 μm film thickness). The chromatograph was temperature programmed as follows: 60°–250 °C at 3 °C/min. The carrier gas was He at a flow of 0.6 mL/min. The injection port was set at 250 °C. Samples were injected using a split ratio of 1:100. MS operating parameters: transfer line temperature: 240 °C; electron impact ionization at 70 eV with mass scan range of 40–284 *m/z* at a sampling rate of 0.03 scan/s; ion source temperature: 200 °C. Compounds were identified by computer search using digital libraries of mass spectral data [27] and by comparison of authentic mass spectra [28] and their retention indices, relative to C_8–C_{20} n-alkane series in a linear temperature-programmed run. GC×GC–qMS and GC–qMS analyses were performed using the same gas chromatograph and MS operating conditions and temperature program, described above. Data were acquired by GCMS Real Time Analysis (GCMS Solutions, Shimadzu Corp.) and processed using GC Image software, ver. 2.1 (GC Image, LLC, Lincoln, NE). Proper software for GC×GC data manipulation (GCImage 2.0, Zoex Corp.-Houston, TX) was used for data handling. A value of spectral similarity above 900 was fixed as an acceptable Identity Spectrum Match factor resulting from the NIST Identity Spectrum Search algorithm (NIST MS Search 2.0).

3. Results and discussion

In order to compare one-dimensional (1D) reference LTPRI values (commercial Libraries) with experimental LTPRI obtained in this work, the sample was spiked with a solution of n-alkanes. The retention indexes were calculated by GCMS solution software for the compounds, using the van den Dool and Kratz formula [29]. The compounds were tentatively identified with a combination of the mass spectral similarity and the LPTRI. A previous work reports the use of one-dimensional retention indexes to GC×GC data [16].

Conventional (GC–qMS) chromatographic runs identified 31 compounds in the essential oil extracted from the 4 year tree, 25 from the 10, and 25 from the 20 (Table 1), while in the first sample 93 compounds were tentatively identified based on spectral similarity and LTPRI by GC×GC–qMS, 90 in the second and 89 in the third sample (Table 2). Table 1 lists the identified compounds, their respective experimental linear retention indexes and literature LTPRI values (from Adams [28] and NIST [27]), for the three samples analyzed under similar conditions.

Mondello et al. [30], studied the composition of rosewood essential oil from leaves and wood by conventional gas chromatography.

Table 1
Identified compounds and the respective literature and calculated retention indexes obtained by GC–qMS.

No.	Compounds	$LTPRI^{I}_{calc}$	$LTPRI^{II}_{lit}$	% peak areas			Average spectral similarity/%
				4	10	20	
1	α-Pinene	932	932	0.35	0.37	0.46	97
2	Linalool 3,7-oxide	971	971[a]	0.18	0.06	0.12	93
3	β-Pinene	979	974	0.24	0.48	0.30	96
4	6-Methyl-5-hepten-2-one	988	981	0.05	–	–	93
5	β-Myrcene	992	988	0.06	0.10	0.07	95
6	Limonene	1030	1024	0.38	0.46	0.28	95
7	1,8-Cineole	1033	1026	0.34	0.16	0.13	96
8	β-Ocimene	1048	1044	0.07	0.06	0.05	93
9	cis-Linalool oxide (furanoid)	1074	1067	0.83	0.44	0.76	96
10	trans-Linalool oxide (furanoid)	1091	1084	0.79	0.43	0.75	94
11	Linalool	1107	1095	82.2	90.5	87.1	96
12	Hotrienol	1109	1104[a]	0.62	–	–	95
13	Myrcenol	1122	1119	0.04	–	–	89
14	Ocimenol	1155	1155[a]	0.09	–	–	93
15	Terpinen-4-ol	1180	1174	0.10	0.03	0.03	95
16	α-Terpineol	1195	1186	3.60	1.11	1.21	97
17	Nerol	1233	1227	0.39	0.10	0.15	96
18	Geraniol	1258	1249	1.33	0.28	0.58	98
19	Cycloisosativene	1373	1369	0.04	–	–	85
20	α-Copaene	1380	1374	0.48	0.38	0.23	94
21	β-Elemene	1397	1389	0.17	0.10	0.09	94
22	(E)-Caryophyllene	1426	1417	0.09	0.10	0.07	93
23	β-Selinene	1483	1489	0.17	–	–	93
24	α-Selinene	1495	1498	1.05	0.73	0.75	93
25	δ-Guaiene	1503	1502	0.79	–	–	91
26	γ-Cadinene	1522	1513	0.08	–	–	85
27	(E)-Nerolidol	1571	1561	0.11	0.07	0.07	97
28	Spathulenol	1587	1577	0.23	0.63	0.40	91
29	Caryophyllene oxide	1591	1582	0.14	0.15	0.19	92
30	α-Cadinol	1652	1652	0.07	–	–	89
31	Benzyl benzoate	1776	1759	0.75	0.17	1.61	96

[a] Obtained from literature ([28] or [27]).

Table 2
Comparison of the chemical composition of the essential oil samples extracted from leaves of 4, 10 and 20 years old trees. Identified compounds and the respective literature and calculated retention indexes obtained by GC × GC–qMS are show. N.I: not identified.

No.	Compounds	^1t_R/ min	^2t_R/ s	LTPRI$_{calc}$ [a]	LTPRI$_{lit}$ [b]	Identified in sample		
						4	10	20
1	(Z)-3-Hexen-1-ol	5.50	3.30	874	850	X	X	X
2	1-Hexanol	5.70	2.76	881	863	X	X	X
3	Octane, 3-methyl-	5.70	0.75	881	871*	X	X	X
4	Cumene	7.10	1.29	927	924	X	X	X
5	α-Pinene	7.40	0.90	937	932	X	X	X
6	Camphene	7.80	0.96	950	946	X	X	X
7	Benzaldehyde	8.30	4.68	967	952	X	X	X
8	Benzene, 1,3,5-trimethyl-	8.40	1.47	970	994	X	X	X
9	Linalool 3,7-oxide	8.50	1.02	973	971*	X	X	X
10	ß-Pinene	8.70	1.02	980	974	X	X	X
11	Cyclopentane, 1-methyl-3-(2-methylpropyl)-	8.90	0.84	987	–	X	X	X
12	5-Hepten-2-one, 6-methyl-	9.00	1.98	990	981	X	X	X
13	ß-Myrcene	9.10	1.11	993	988	X	X	X
14	Pseudocumene	9.20	1.62	997	~990*	X	X	X
15	6-Hepten-2-ol, 2,6-dimethyl-	9.80	2.64	1013	989	X	X	X
16	2-Carene	10.0	1.14	1018	1001	X	X	X
17	o-Cymene	10.30	1.44	1026	1022	X	X	X
18	D-Limonene	10.50	1.17	1032	1024	X	X	X
19	(Z)-ß-Ocimene	10.80	1.23	1039	1032	X	X	X
20	(E)-ß-Ocimene	11.20	1.26	1050	1044	X	X	X
21	γ-Terpinene	11.60	1.23	1060	1054	X	X	X
22	trans-Linalool oxide (furanoid)	12.10	5.91	1074	1084	X	X	X
23	Linalool	13.50	3.75	1109	1095	X	X	X
24	2H-pyran-3(4H)-one, 6-ethenyldihydro-2,2,6-trimethyl-	13.70	2.25	1114	1108*	X	X	X
25	Fenchol	14.00	3.33	1121	1118	X	X	N.I.
26	1-Terpinenol	14.70	3.00	1138	1130	X	N.I.	X
27	Dihydro-α-terpineol	15.10	2.76	1148	1160	X	X	X
28	ß-Terpineol	15.20	3.57	1150	1140	X	X	X
29	Camphene hydrate	15.40	3.06	1155	1145	X	X	X
30	Ocimenol	15.40	3.90	1155	1155*	X	X	X
31	Ocimene	15.50	1.02	1157	1152*	X	X	X
32	Nerol oxide	15.50	1.95	1157	1154	X	X	X
33	Borneol	16.10	4.32	1171	1165	X	X	X
34	cis-Linalool oxide (pyranoid)	16.20	5.04	1174	1170	X	X	X
35	trans-Linalool oxide (pyranoid)	16.40	5.55	1178	1173	X	X	X
36	Terpinen-4-ol	16.50	2.85	1181	1174	X	X	X
37	Myrcenol	17.10	2.67	1195	1119	X	X	X
38	α-Terpineol	17.20	3.90	1198	1186	X	X	X
39	trans-Dihydrocarvone	17.40	2.73	1202	1200	X	X	N.I.
40	1-p-Menthen-9-al	18.20	2.61	1221	1217*	X	X	X
41	ß-Citronellol	18.70	4.38	1232	1223	**X**	**X**	N. I.
42	(Z)-Citral	19.30	3.00	1246	1235	X	X	X
43	Nerol	19.80	5.40	1258	1227	X	X	X
44	(E)-Citral	20.60	3.18	1277	1264	X	X	X
45	α-Cubebene	23.90	1.20	1355	1345	X	X	N.I.
46	Nerol acetate	24.50	2.22	1369	1359	X	X	X
47	1-Hepten-6-one, 2-methyl-	24.50	2.88	1369	-	X	X	X
48	Cyclosativene	24.70	1.23	1374	1369	X	X	X
49	α-Copaene	25.10	1.26	1383	1374	X	X	X
50	β-Elemene	25.40	1.44	1390	1389	X	X	X
51	α-Gurjunene	26.50	1.26	1417	1409	X	X	X
52	Benzene, 1,3,5-trimethoxy-	26.60	1.77	1420	1405*	X	X	X
53	(Z)-ß-Farnesene	26.80	5.13	1425	1440	X	X	X
54	(Z)-Caryophyllene	26.90	1.44	1427	1408	X	X	X
55	Germacrene D	27.30	1.41	1437	1484	X	X	X
56	α-Guaiene	27.60	1.35	1445	1437	X	X	X
57	Isoamyl benzoate	27.60	3.18	1445	1433	X	X	X
58	3-Buten-1-ol, 3-methyl-, benzoate	28.00	3.96	1455	–	X	X	X
59	4-Hexen-2-one, 3-methyl-	28.20	2.49	1460	–	X	X	X
60	α-Caryophyllene	28.30	1.59	1462	1452	X	X	X
61	Aromadendrene	28.60	1.50	1470	1439	X	X	X
62	ß-Selinene	29.50	5.34	1492	1489	X	X	X
63	ß-Guaiene	29.90	5.34	1502	1502	X	N.I.	X
64	ß-Chamigrene	30.00	1.71	1505	1476	X	N.I.	X
65	2,4-Diisopropenyl-1-methylcyclohexane	30.00	2.73	1505	–	X	X	X
66	(Z)-α-trans Bergamotol	30.20	1.98	1510	1690	X	X	X
67	β-trans-Guaiene	30.40	1.59	1515	1502	X	X	X
68	α-Muurolene	30.70	1.74	1523	1500	X	X	X
69	δ-Cadinene	31.00	1.68	1531	1522	X	X	X
70	cis-Calamenene	31.10	2.01	1533	1528	X	X	X
71	α-Amorphene	31.60	1.77	1546	1483	X	X	X
72	α-Calacorene	31.80	2.37	1551	1544	X	X	X
73	Isocitronellol	32.60	1.77	1572	–	X	X	X
74	D-Nerolidol	32.60	3.12	1572	1531	X	X	X
75	Palustrol	32.80	2.31	1577	1567	X	X	X
76	Spathulenol	33.20	3.81	1587	1577	X	X	X
77	Caryophyllene oxide	33.40	2.58	1592	1582	X	X	X
78	Veridiflorol	33.40	3.21	1592	1592	X	X	X
79	α-Farnesene	33.50	2.13	1595	1509*	X	X	X
80	Ledane	33.80	2.64	1603	–	X	X	X
81	Guaiol	33.90	3.27	1605	1600	X	X	X
82	Ledol	34.20	2.70	1614	1602	X	X	X
83	α-Humulene epoxide	34.40	2.85	1619	1608	X	X	X
84	Globulol	34.50	3.60	1622	1590	X	X	X
85	Cubenol	35.10	2.82	1639	1645	X	X	X
86	(+)-3-Carene, 2-(acetylmethyl)-	35.10	4.89	1639	–	X	X	X
87	β-Eudesmol	35.20	3.78	1642	1649	**X**	**X**	**X**
88	α-Bisabolol	35.30	5.13	1644	1685	X	X	X
89	tau.-Muurolol	35.60	3.75	1653	1640	X	X	X
90	Limonene epoxide	36.30	3.45	1672	–	X	X	X
91	cis-Lanceol	36.60	3.54	1680	1760	X	X	X
92	α-Cadinol	36.70	2.10	1683	1652	X	X	X
93	Isoaromadendrene epoxide	38.50	0.51	1734	1612*	X	X	X
94	Benzyl benzoate	40.20	3.30	1783	1759	X	X	X

[a] Calculated values.
[b] Obtained from literature ([28] or [27]: *).

A comparison of the essential oil analysis from the old plants and from those used in the present work shows that the compositions are similar. Almost all compounds were found in two samples or a stereoisomer was identified. Both studies obtained quite similar relative percent peak areas for linalool, the major compound. Other major compounds are the same, although in different percentages. However, no direct comparison was performed and GC × GC–qMS was used in order to obtain a more complete sample chemical profile.

Fig. 1 presents the chromatograms obtained by GC–qMS and GC × GC–qMS. The small number of peaks in the GC chromatogram does not reveal the real complexity of the sample. Using GC × GC–qMS, it was possible to identify a much larger number of compounds (about 3 times more in each of the three sample), and several co-elutions were resolved as a result of the higher separation capacity and mass spectral quality. Moreover, GC × GC improves detectability and minor compounds are highlighted. After the modulation process, during GC × GC runs, peaks became narrower and more intense.

As high amounts of analyte can cause MS shutdown or damage, it was necessary to carry out a MS program during GC × GC runs, with detector voltage attenuation in the interval of elution of the major compound, linalool. The attenuation reduces MS sensitivity and avoids inconveniences. On the other hand, this strategy does not allow us to estimate the relative peak area percent once the relationship between peak areas are no more the original. Anyway it was possible to compare the peak area percent of the major compounds by GC–qMS.

The use of GC × GC–qMS enabled good improvement in separation and number of identified peaks of rosewood leaves essential oil. One can see that not all compounds identified in the essential oil extracted

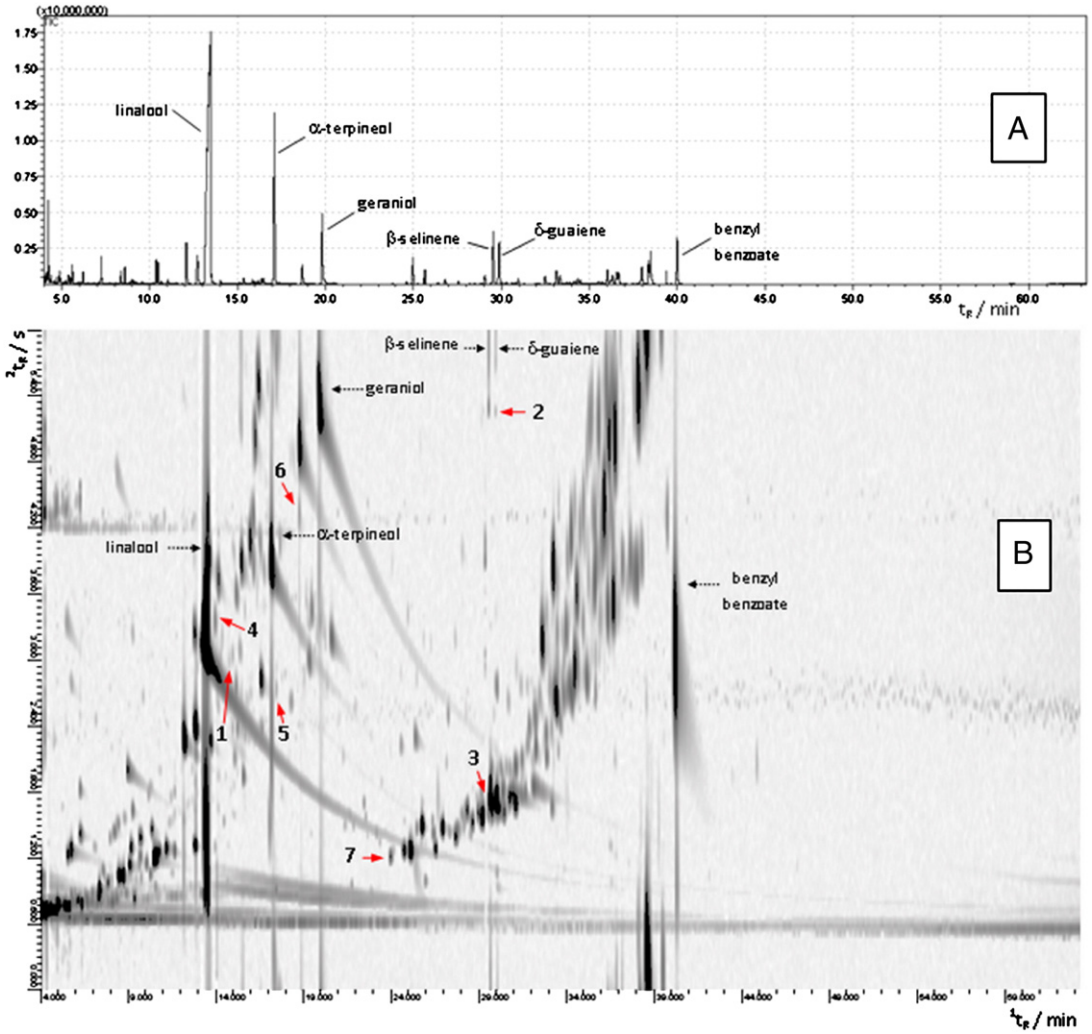

Fig. 1. GC–qMS (A) and GC × GC–qMS (B) chromatograms of a rosewood leaf essential oil sample extracted from leaves from a four year tree. ^1t_R: first dimension retention time. ^2t_R: second dimension retention time. Some of the major compounds are indicated so as those not identified in the samples obtained from ten and twenty years trees.

from leaves of the younger tree were found in the other samples. Three of these compounds were not identified in the sample from the ten years tree and four were not identified after the GC × GC–qMS analyses of the sample from the older tree. Among the first three unidentified compounds (respectively 1, 2 and 3 in Fig. 1) one is monoterpene (1-terpineol) and two are sesquiterpenes (β-guaiene and β-chamigrene). Among the four unidentified compounds cited (respectively 4, 5, 6 and 7 in Fig. 1), three of them are monoterpenoids (fenchol, trans-dihydrocarvone and β-citronellol) and one is sesquiterpenoid (α-cubebene). Fig. 1 shows the localization of these seven compounds.

Although some compounds show a relatively large window of LTPRI values when the retention indexes values for a single column are used with GC × GC separations, tentative identifications were carried out since the improved separation and detectability provided by the two-dimensional technique makes possible improved spectral quality. The results obtained show that rosewood leaf essential oil extracted from leaves of rosewood of different grown stages have a more complex composition than that obtained by conventional gas chromatography. Table 2 shows the identified components. However, when one considers that an essential oil is a complex sample, the chemical profiles from leaf oils analyzed are similar. Some of the more commonly encountered monoterpenoid hydrocarbons can be formed by dehydration of alcohols and so their presence in essential oils could be as artifacts arising from the extraction process. As sesquiterpenoids contain 15 carbon atoms they have lower volatilities and hence higher boiling points than monoterpenoids. Therefore, fewer of them (in percentage terms) contribute to the odor of essential oils but those that do often have low-odor thresholds and contribute significantly as end notes [31].

As can be seen in Table 1, some compounds were identified by conventional gas chromatography only in sample 4, but not in sample 10 and vice versa, the same occurring with sample 20, relative to the other samples, in respect to some compounds. The compounds 6-methyl-5-hepten-2-one, hotrienol, myrcenol, ocimenol, cycloisosativene, β-selinene, δ-guaiene, γ-cadinene and α-cadinol were identified in sample 4, but not in sample 10 (Table 1), while, γ-gurjunene (0.11/1481), germacrene A (0.58/1501), and δ-cadinene (0.06/1529), were found in sample 10, but not in sample 4 (the numbers in the parenthesis are the % peak area and calculated retention index, respectively). In sample 20, so as sample 10, gurjunene (0.11/1481), germacrene A (0.59/1501) and δ-cadinene (0.06/1529), were identified differently from sample 4, but some do not, as can be seen by Table 1. The library can mistake some components like isomers, but it is also possible that co-elutions results in difficult in the identification process. This can be the case of germacrene A (samples 10 and 20) and δ-guaiene, whose retention indexes are close. This is a consequence of the high number of sample components and the relative low separation capacity of conventional gas chromatography. Major compounds as cis and trans linalool oxides (furanoid), linalool, α-terpineol, geraniol, α-selinene and benzyl benzoate show similar % peak area tendencies in the three

essential oils. The number of components identified in the samples by conventional gas chromatography could points out to a higher similarity between the samples 10 and 20 towards sample 4. However, in spite of the differences and similarities found in the identification obtained by conventional gas chromatography, GC×GC–qMS analysis showed fewer differences in the identification (Table 2), mainly when one consider the higher number of identified components. This shows us that GC×GC–qMS can be more precise and hence more reliable to the chemical characterization of samples like rosewood essential oils obtained from leaves.

4. Conclusions

This project showed that compositions of the analyzed samples are very similar when one considers the complexity of essential oils. This could be concluded only by comprehensive two-dimensional gas chromatography coupled with quadrupolar mass spectroscopy, because the separation capacity of conventional gas chromatography is quite limited in the case of complex samples such as essential oils. Differences in the minor compounds content among the essential oils analyzed can be corrected relatively ease when one wishes to reach the better fragrance quality. Thus, from an economic point of view it seems that young plants from four years old can produce essential oil with quality similar to those from older trees. The economical interest in this raw material and the risk of extinction increases the importance of further investment in this research.

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References

[1] J.G.S. Maia, E.H.A. Andrade, H.A.R. Couto, A.C. da Silva, F. Marx, C. Henke, Plant sources of Amazon rosewood oil, Quim. Nova 30 (2007) 906–1910.
[2] F. Lupe, R. Souza, L.E.S. Barata, Seeking a sustainable alternative to Brazilian rosewood, Perfum. Flavor. 33 (2008) 40–43.
[3] J.-M. Chantraine, J.-M. Dhénin, C. Moretti, Chemical variability of rosewood (*Aniba rosaeodora* Ducke) essential oil in French Guiana, J. Ess. Oil Res. 21 (2009) 486–495.
[4] L.E.S. Barata, R.Q. de Carvalho, Amazon scents: replacing rosewood in perfumery? Part I. Perfum. Flav. 32 (2007). Available at http://www.perfumerflavorist.com/fragrance/rawmaterials/natural/6599747.html [Accessed on 17/04/2012].
[5] L.E.S. Barata, R.Q. de Carvalho, Amazon scents: replacing rosewood in perfumery? Part II. Perfum. Flavor. 32 (2007). Available at http://www.perfumerflavorist.com/fragrance/rawmaterials/natural/6845957.html [Accessed on 17/04/2012].
[6] L.E.S. Barata, P. May, Rosewood exploitation in the Brazilian Amazon: options for sustainable production, Econ. Bot. 58 (2004) 257–265.
[7] N.T. Dunford, S. Hiziroglu, R. Holcomb, Effect of age on the distribution of oil in Eastern redcedar tree segments, Bioresour. Technol. 98 (2007) 2636–2640.
[8] J.C. Kim, K.J. Kim, D.S. Kim, J.S. Han, Seasonal variations of monoterpene emissions from coniferous trees of different ages in Korea, Chemosphere 59 (2005) 1685–1696.
[9] S. Geng, Z. Cui, X. Huang, Y. Chen, D. Xu, P. Xiong, Variations in essential oil yield and composition during *Cinnamomum cassia* bark growth, Ind. Crops Prod. 33 (2011) 248–252.
[10] S.-S. Cheng, M.-T. Chua, E.-H. Chang, C.-G. Huang, W.-J. Chen, S.-T. Chang, Variations in insecticidal activity and chemical compositions of leaf essential oils from Cryptomeria japonica at different ages, Bioresour. Technol. 100 (2009) 465–470.
[11] L. Mondello, P.Q. Tranchida, P. Dugo, G. Dugo, Comprehenseive two-dimensional gas chromatography–mass spectrometry: a review, Mass Spectrom. Rev. 27 (2008) 101–124.
[12] P. Pripdeevech, S. Wongpornehai, A. Promsiri, Highly volatile constituents of *Vetiveria zizanioides* roots grown under different cultivation conditions, Molecules 11 (2006) 817–826.
[13] R.A. Shellie, P.D. Morrison, P.J. Marriott, S.D.H. Poynter, Selection of columns for GC×GC analysis of essential oils, LC GC Europe 23 (2010) 76–80.
[14] P.Q. Tranchida, R.A. Shellie, G. Purcaro, L.S. Conte, P. Dugo, G. Dugo, L. Mondello, Analysis of fresh and aged tea tree essential oils by using GC×GC–qMS, J. Chromatogr. Sci. 48 (2010) 262–266.
[15] O. Kutlular, M.Z. Ozel, Analysis of essential oils of origanum onites by superheated water extraction using GC×GC-TOF/MS, J. Essent. Oil Bear. Plants 12 (2009) 462–470.
[16] C.H.V. Fidelis, F. Augusto, P.T.B. Sampaio, P.M. Krainovic, L.E.S. Barata, Chemical characterization of rosewood (*Aniba rosaeodora Ducke*) leaf essential oil by comprehensive two-dimensional gas chromatography coupled with quadrupole mass spectrometry, J. Ess. Oil. Res. 24 (2012) 245–251.
[17] P.Q. Tranchida, R.C.Z. de Souza, L.E.S. Barata, M. Mondello, P. Dugo, G. Dugo, L. Mondello, Analysis of Macacaporanga (*Aniba parviflora*) leaf essential oil by using comprehensive two-dimensional gas chromatography combined with rapid-scanning quadrupole mass spectrometry, Chromatogr. Today (2008) 5–9.
[18] L.A.F. de Godoy, E.C. Ferreira, M.P. Pedroso, C.H.V. Fidelis, F. Augusto, R.J. Poppi, Quantification of kerosene in gasoline by comprehensive two-dimensional gas chromatography and N-way multivariate analysis, Anal. Lett. 41 (2008) 1603–1614.
[19] R.A. Shellie, P.J. Marriott, Comprehensive two-dimensional gas chromatography–mass spectrometry analysis of *Pelargonium graveolens* essential oil using rapid scanning quadrupolar mass spectrometry, Analyst 128 (2003) 879–883.
[20] S. Bieri, P.J. Marriott, Generating multiple independent retention index data in dual-secondary column comprehensive two-dimensional gas chromatography, Anal. Chem. 78 (2006) 8089–8097.
[21] C. von Mühlen, C.A. Zini, E.B. Caramão, P.J. Marriott, Comparative study of *Eucalyptus dunnii* volatile oil composition using retention indices and comprehensive two-dimensional gas chromatography coupled to time-of-flight and quadrupolar mass spectrometry, J. Chromatogr. A 1200 (2008) 34–42.
[22] M.P. Pedroso, L.A.F. De Godoy, E.C. Ferreira, R.J. Poppi, F. Augusto, Identification of gasoline adulteration using comprehensive two-dimensional gas chromatography combined to multivariate data processing, J. Chromatogr. A 1201 (2008) 176–182.
[23] L.A.F. de Godoy, L.W. Hantao, M.P. Pedroso, R.J. Poppi, F. Augusto, Quantitative analysis of essential oils in perfume using multivariate curve resolution combined with comprehensive two-dimensional gas chromatography, Anal. Chim. Acta 699 (2011) 120–125.
[24] L.A.F. de Godoy, M.P. Pedroso, L.W. Hantao, R.J. Poppi, F. Augusto, Quantitative analysis by comprehensive two-dimensional gas chromatography using interval Multi-way Partial Least Squares calibration, Talanta 83 (2011) 1302–1307.
[25] L.A.F. de Godoy, M.P. Pedroso, E.C. Ferreira, F. Augusto, R.J. Poppi, Prediction of the physicochemical properties of gasoline by comprehensive two-dimensional gas chromatography and multivariate data processing, J. Chromatogr. A 1218 (2011) 1663–1667.
[26] M.P. Pedroso, E.C. Ferreira, L.W. Hantao, S. Bogusz Jr., F. Augusto, Identification of volatiles from pineapple (*Ananas comosus* L.) pulp by comprehensive two-dimensional gas chromatography and gas chromatography/mass spectrometry, J. Sep. Sci. 34 (2011) 1547–1554.
[27] National Institute of Standards and Technology, NIST Chemistry WebBook, webbook.nist.gov/chemistry.
[28] R.P. Adams, Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy, Allured Publ, Corp, Carol Stream, IL, 2007.
[29] H. van den Dool, P.D. Kratz, A generalization of the retention index system including linear temperature programmed gas–liquid partition chromatography, J. Chromatogr. A 11 (1963) 463–471.
[30] B. d'A. Zellner, M. Lo Presti, L.E.S. Barata, P. Dugo, G. Dugo, L. Mondello, Evaluation of leaf-derived extracts as an environmentally sustainable source of essential oils by using gas chromatography–mass spectrometry and enantioselective gas chromatography–olfatometry, Anal. Chem. 78 (2006) 883–890.
[31] C. Sell, K. Hüsnü, Chemistry of Essential Oils, in: Can Baser and Gerhard Buchbauer (Eds.), Handbook of Essential Oils Science, Technology, and Applications, CRC Press, 2010, pp. 121–149. Print ISBN: 978-1-4200-6315-8; eBook ISBN: 978-1-4200-6316-5