



## Original Article

## Volatiles composition and extraction kinetics from *Schinus terebinthifolius* and *Schinus molle* leaves and fruit



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## ABSTRACT

Essential oils extracted from *Schinus molle* L. and *Schinus terebinthifolius* Raddi, Anacardiaceae, leaves and fruit hydrodistillation, as well as, their chemical composition and extraction kinetic were evaluated. For this proposal, 6 h extraction and aliquots collected at sequencing different times (0.5, 1, 2, 4 and 6 h) were carried out allowing calculating accumulated content (% w/w) and verifying essential oil chemical profile.  $\beta$ -caryophyllene (35.2%),  $\alpha$ -pinene (28.1%) and germacrene D (15.5%) represent *S. terebinthifolius* dried leaves essential oil major components, as well as,  $\alpha$ -pinene (44.9%), germacrene D (17.6%) and  $\beta$ -pinene (15.1%) in the fruit. Cubenol (27.1%), caryophyllene oxide (15.3%) and spathulenol (12.4%) represent *S. molle* dried leaves essential oil major components, and  $\beta$ -pinene (36.3%)  $\alpha$ -pinene (20.3%), germacrene D (12.1%) and spathulenol in the fruit. Essential oil extraction kinetics showed a hyperbolic distribution; monoterpene content presented exponential decay in time function and sesquiterpene showed exponential growth. Faster monoterpene extraction than the sesquiterpene extraction was observed, however, both presented increasing exponential distribution.

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## Introduction

Economic potential for aromatic plants and their essential oils employment is relevant, but just a little explored in Brazil, however, studies searching for natural products employed on human and animal health were intensified (Adorjan and Buchbauer, 2010; Silva and Fernandes Júnior, 2010; Lange et al., 2013).

Another factor stimulating essential oils studies has been the search for natural active substances presenting insecticidal, fungicidal and bactericidal activities, as well as, low environmental impact and human health damage, serving as an alternative to agriculture pesticides use (Santos et al., 2010; Regnault-Roger et al., 2012; El-Wakeil, 2013; Ootani et al., 2013; Regnault-roger, 2013).

Two Anacardiaceae family tree species (*Schinus molle* L. and *Schinus terebinthifolius* Raddi) are important in regarding to the natural resources exploitation possibility from their special metabolism

(Kramer, 1957; Morton, 1978; Mack, 1991; Goldstein and Coleman, 2004). The first one is native from northern South America arid regions, including Peru, Chile and Argentina, as well as, their fruit are not suitable for human feeding in the reason of toxic properties (Blood, 2001). The second one has widely been distributed in the Brazilian Atlantic forest from south to northeast Brazil and its fruit are traded as pepper (*poivre-rose*), greatly appreciated in European cooking (Gomes et al., 2013a,b).

Some studies have reported *S. terebinthifolius* and *S. molle* essential oils content and chemical composition based on equal or less time period than 90 min distillation (Santos et al., 2009; Dellacassa, 2010). However, it is supposed these time periods are not sufficient for essential oil compounds complete extraction.

*S. molle* and *S. terebinthifolius* leaves and fruit essential oils by hydrodistillation at different sequencing time periods (0.5, 1, 2, 4 and 6 h) were extracted, and results nonlinear analyses allowing observing extraction time modulating essential oil content and quality; decreasing monoterpenes content as increasing sesquiterpenes content on samples analyzed examined in time function.

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## Materials and methods

### Materials

The dichloromethane solvent, alkanes series (C8–C20 e C21–C40) and sodium sulfate anhydrous were purchased from Sigma–Aldrich (Brazil). Nitrogen gas (99.98%) of purity was purchased from White Martins SA (Brazil). Supplies were purchased from Axygen (Brazil) and glass vials for essential oils storage were purchased in Didática-SP (Brazil).

### Plant material

*Schinus molle* L. was collected at Volta Redonda (March 2013), and *S. terebinthifolius* Raddi at Seropédica (March 2014), both in the city of Rio de Janeiro, Brazil. Fruit and leaf samples were separated for drying at room temperature, protected from light, moisture and stored until the time of distillation. A voucher specimen has been deposited in the herbarium of Biology Institute (UFRRJ) with the following ID: RBR 36405 (*S. terebinthifolius*) and RBR 35791 (*S. molle*).

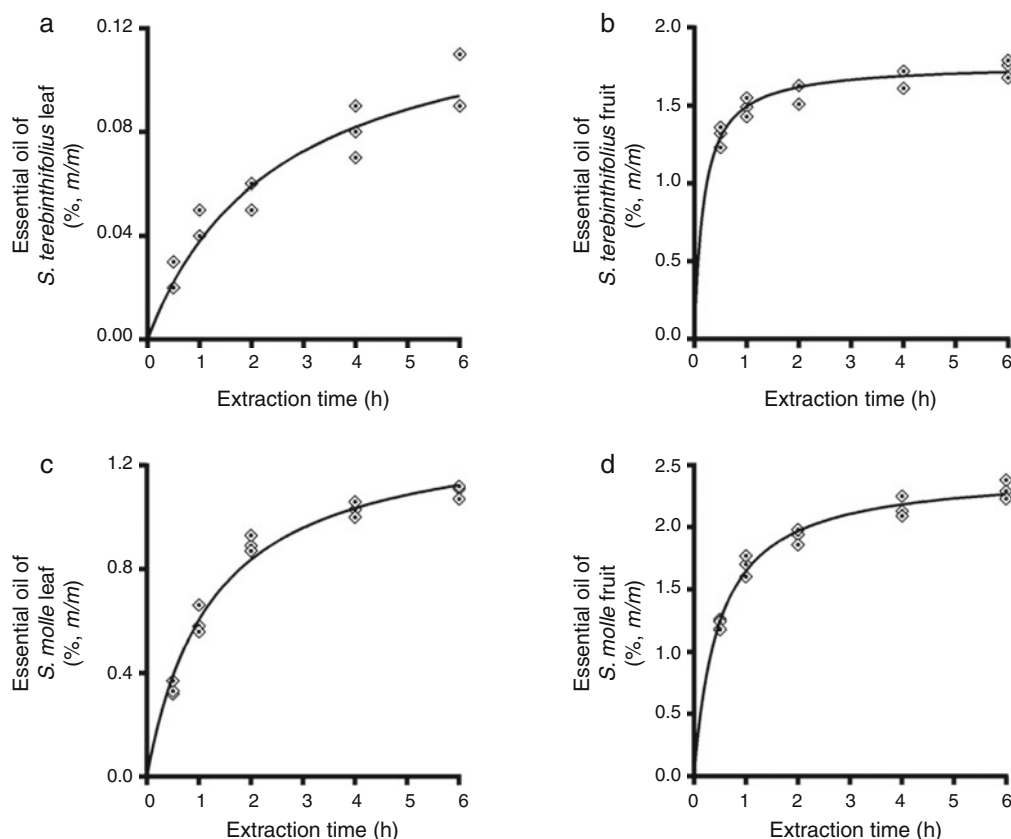
### Essential oil extraction

*S. terebinthifolius* and *S. molle* dried fruits and leaves, 80 g triplicate separate in organs and species, were homogenized for 3 min in a food processor and then subjected to hydrodistillation using the Clevenger apparatus for 6 h. About 15 ml of distilled water plus essential oil samples were collected in different sequence time periods (0.5, 1, 2, 4 and 6 h) and then partitioned with 3 × 5 ml of dichloromethane, then, the less polar phase was dried over anhydrous sodium sulfate, filtered and concentrated with nitrogen gas

at room temperature until constant weight (after three weighing equals). Gravimetric measurements were performed base on the dry weight of leaves and fruits and converted to essential oil percentage (w/w).

### Chromatographic analysis and identification

To separate, detect and quantify the constituents, 1  $\mu\text{l}$  of essential oils samples ( $10 \mu\text{l ml}^{-1}$ ), in the defined times, were injected into the gas chromatography (GC). A Hewlett-Packard 5890 Series II (Palo Alto, USA), equipped with flame ionization detection and a split/splitless injector, in a split ratio of 1:20 was used to separate and detect the constituents in the essential oil. The substances were separated with a fused silica capillary column, similar DB5 with  $30 \text{ m} \times 0.25 \text{ mm (i.d.)} \times 0.25 \mu\text{m}$  (film thickness). Helium was used as the carrier gas at a flow rate of  $1.0 \text{ ml min}^{-1}$ . The column temperature was programmed as follows:  $60^\circ\text{C}$  for 2 min followed by heating at  $5^\circ\text{C min}^{-1}$  to  $110^\circ\text{C}$ , followed by heating at  $3^\circ\text{C min}^{-1}$  to  $150^\circ\text{C}$  and finally followed by heating at  $15^\circ\text{C min}^{-1}$  until  $290^\circ\text{C}$  and holding constant for 15 min. The injector temperature was  $220^\circ\text{C}$  and the detector temperature was  $290^\circ\text{C}$ . To separate and identify the substances,  $1.0 \mu\text{l}$  of essential oils samples ( $10 \mu\text{l ml}^{-1}$ ), in the defined times, were injected in the gas chromatograph coupled to mass spectrometer (GC-MS) QP-2010 Plus (Shimadzu, Japan). The flow of the helium gas carrier, the capillary column and the temperature conditions for the GC-MS analysis were the same as described for the GC. The temperature of the injector was  $220^\circ\text{C}$  and the temperature of the interface was  $250^\circ\text{C}$ . Mass spectra were obtained with a quadrupole detector operating at 70 eV, with 40–400  $m/z$  mass range and scanning rate equal to  $0.5 \text{ scan s}^{-1}$ . The identification of volatile compounds in the essential oil has been based on Linear Retention Indices (LRI) and mass



**Fig. 1.** *Schinus terebinthifolius* and *Schinus molle* leaves and fruit essential oils extraction kinetics by hydrodistillation as function of different sequencing time periods (0.5, 1, 2, 4 and 6 h). Best fit obtained with hyperbolic nonlinear regression ( $\alpha = 0.05$ ,  $n = 3$ ),  $r^2 = 0.9183$  (a); 0.8865 (b); 0.9739 (c) and 0.9690 (d).

**Table 1***Schinus molle* and *S. terebinthifolius* leaves and fruit essential oils composition after 6 h extraction by hydrodistillation.

IN	Compounds	LRI	<i>S. terebinthifolius</i>		<i>S. molle</i>	
			Leaf	Fruit	Leaf	Fruit
			Percentage			
1	tricyclene	926	8.3	–	–	–
2	$\alpha$ -tujene	930	–	–	2.0	–
3	$\alpha$ -pinene	939	28.1	44.9	–	20.3
4	sabinene	975	–	2.6	–	2.9
5	$\beta$ -pinene	979	4.8	15.1	2.2	36.3
6	limonene	1029	–	1.4	–	1.8
7	eucalyptol	1038	8.5	–	–	–
8	linalool	1096	–	–	1.6	–
9	nopinone	1140	–	–	1.7	–
10	<i>trans</i> -pinocarveol	1139	–	0.6	4.5	1.9
11	<i>trans</i> -verbenol	1144	–	–	4.2	–
12	pinocarvone	1164	–	–	1.1	–
13	$\alpha$ -phellandren-8-ol	1170	–	0.8	1.0	–
14	terpinen-4-ol	1177	–	0.4	–	–
15	$\alpha$ -terpineol	1188	–	0.8	–	–
16	mirtenal	1195	–	–	5.3	–
17	verbenone	1205	–	–	6.2	–
18	bornyl acetate	1288	–	0.5	–	–
19	$\delta$ -elemene	1338	–	0.1	–	–
20	$\alpha$ -copaene	1376	–	0.4	–	–
21	$\beta$ -elemene	1390	–	–	–	0.9
22	$\beta$ -caryophyllene	1419	35.2	1.9	0.7	8.9
23	$\alpha$ - <i>trans</i> -bergamotene	1434	–	1.8	–	–
24	$\alpha$ -humulene	1454	–	0.3	–	–
25	$\gamma$ -muurolene	1479	–	0.3	–	–
27	germacrene D	1485	15.1	17.6	–	12.1
29	$\alpha$ -selinene	1498	–	–	–	2.7
30	$\alpha$ -muurolene	1500	–	0.2	–	–
32	$\gamma$ -cadinene	1513	–	–	3.8	–
33	$\delta$ -cadinene	1523	–	0.9	–	0.9
34	germacrene B	1561	–	1.1	–	–
35	spathulenol	1578	–	0.5	12.4	11.4
36	caryophyllene oxide	1583	–	–	15.3	–
37	globulol	1590	–	–	1.2	–
38	ledol	1602	–	–	1.5	–
39	1, 10-di- <i>epi</i> -cubenol	1619	–	–	3.4	–
40	<i>cis</i> -cadinen-4-en-7-ol	1636	–	1.6	–	–
43	cubenol	1646	–	–	27.1	–
44	$\alpha$ -cadinol	1654	–	1.9	1.7	–
45	( <i>E</i> )-bisabol-ol-11	1668	–	1.9	–	–
46	khusilol	1678	–	–	1.0	–
Monoterpene hydrocarbons			41.2	64.0	4.2	61.3
Oxygenated monoterpenes			8.5	3.1	25.6	1.9
Total monoterpenes			49.7	67.1	29.8	63.2
Sesquiterpene hydrocarbons			50.3	24.6	4.5	25.5
Oxygenated sesquiterpenes			–	5.9	63.6	11.4
Total sesquiterpenes			50.3	30.5	68.1	36.9
Total identified			100	97.6	97.9	100

IN, number of identified compound; compounds, identified based on LRI and mass spectrum reported in literature and NIST database (2008); LRI, linear retention indices relative to n-alkanes C8–C40 on the DB5 capillary column; percentage, relative to the total peak area.

spectra of the samples, compared with authentic standards injected under the same conditions, with the NIST database (2008) and the index Kovats, IK (Adams, 2007). The LRI was calculated based on co-injection of alkanes series (Van Den Dool and Kratz, 1963).

#### Statistical analysis

The mean, standard error of the mean, test-*t* and graphics were calculated and produced by GraphPad Prism 6.0 (GraphPad Software, EUA).

## Results and discussion

#### Essential oil content and extraction kinetics

Differences in essential oils contents between the *Schinus* species and organs were observed. *S. terebinthifolius* leaves essential

oil (0.10%) compared to same species fruit (1.74%) and *S. molle* leaves (1.10%) presented lower content. *S. molle* fruit essential oil content (2.30%) was higher than that on from the same species leaves and from *S. terebinthifolius* fruit.

*S. terebinthifolius* leaves essential oil low content does not correspond to those ones presented in the literature (Barbosa et al., 2007; Santos et al., 2009). Moreover, *S. terebinthifolius* fruit essential oil content (Gomes et al., 2013a) and *S. molle* leaves and fruit essential oils were similar to those ones presented in the literature (Dikshit et al., 1986; Zahed et al., 2010; Torres et al., 2012; Scopel et al., 2013) and higher to those ones presented by Santos et al. (2009).

It was observed that essential oils chemical profile obtained from this survey was not overall similar to those ones presented by other authors (Santos et al., 2009; Dellacassa, 2010; Gomes et al., 2013b) as ISO (IRAM-18608-1: 2006) cited by Dellacassa (2010).

**Table 2***Schinus terebinthifolius* dried leaves essential oils composition after 6 h extraction by hydrodistillation at different sequencing time periods (0.5, 1, 2, 4 and 6 h).

IN	Compounds	LRI	Extraction time (hours)				
			0.5	1	2	4	6
			Percentage				
1	tricyclene	926	4.0	16.6	21.8	24.1	20.6
3	$\alpha$ -pinene	935	41.2	14.5	10.2	7.3	6.1
5	$\beta$ -pinene	982	9.3	–	–	–	–
7	eucalyptol	1038	12.9	–	10.5	–	–
22	$\beta$ -caryophyllene	1422	16.3	68.9	57.6	68.6	73.3
27	germacrene D	1485	16.4	–	–	–	–
Monoterpene hydrocarbons			54.5	31.1	32	31.4	26.7
Oxygenated monoterpenes			12.9	–	10.5	–	–
Total monoterpenes			67.4	31.1	42.5	31.4	26.7
Oxygenated sesquiterpenes			32.7	68.9	57.6	68.6	73.3
Total sesquiterpenes			32.7	68.9	57.6	68.6	73.3
Total identified			100	100	100	100	100

IN, number of identified compound; compounds, identified based on LRI and mass spectrum reported in literature and NIST database (2008); LRI, linear retention indices relative to n-alkanes C8–C40 on the DB5 capillary column; percentage, relative to the total peak area.

Variations in essential oils contents from plant tissues can be related to different factors, some of them intrinsic and controlled by the plant genetic traits (Souza, 2012; Gomes et al., 2013b). On the other hand, quantitative traits are susceptible to the edapho-climatic effects, such as seasonality, water availability and soil nutrients (Sangwan et al., 2001; Lima et al., 2003).

Nonlinear hyperbolic distribution was the model which presented the best fit to essential oils extraction kinetics (Fig. 1),

allowing estimating maximum essential oils contents greater than 40, 10, 25 and 15 h as uneconomical extraction time period, however, in the experimental proposed time (6 h), the maximum practically achieved corresponded to 75, 98, 81 and 93% total estimated, Fig. 1a–d, respectively.

Faster fruit essential oil extraction speed than the leaves extraction speed was observed, thus, to obtaining half of estimated maximum content of essential oils were needed 150 and 75 min

**Table 3***Schinus terebinthifolius* dried fruit essential oils composition after 6 h extraction by hydrodistillation at different sequencing time periods (0.5, 1, 2, 4 and 6 h).

IN	Compounds	LRI	Extraction time (hours)				
			0.5	1	2	4	6
			Percentage				
3	$\alpha$ -pinene	934	52.5	29.5	27.7	14.8	8.4
4	sabinene	976	7.0	–	–	–	–
5	$\beta$ -pinene	982	18.2	8.3	8.8	5.2	2.7
6	limonene	1032	2.3	0.7	1.1	0.4	0.6
10	<i>trans</i> -pinocarveol	1154	–	2.4	–	–	–
13	$\alpha$ -phellandre-8-ol	1189	1.6	5.8	0.6	0.7	0.5
14	terpinen-4-ol	1193	–	–	0.9	1.5	2.2
15	$\alpha$ -terpineol	1211	–	2.7	1.3	2.3	4.1
18	bornyl acetate	1294	–	1.2	–	0.6	0.9
19	$\delta$ -elemene	1338	–	–	–	0.6	0.5
20	$\alpha$ -copaene	1377	–	–	0.9	1.4	1.7
22	$\beta$ -caryophyllene	1421	1.9	3.3	2.4	2.9	3.0
23	$\alpha$ - <i>trans</i> -bergamotene	1437	–	–	3.0	4.3	5.0
24	$\alpha$ -humulene	1459	–	–	–	1.0	1.1
25	$\gamma$ -muurolene	1480	–	–	1.8	3.4	4.2
27	germacrene D	1484	14.9	10.4	19.7	14.7	12.3
30	$\alpha$ -muurolene	1504	–	–	1.3	2.2	2.4
31	$\beta$ -bisabolene	1512	–	–	0.5	0.6	0.6
32	$\gamma$ -cadinene	1518	–	–	1.1	2.2	2.4
33	$\delta$ -cadinene	1524	–	–	4.6	7.3	9.9
34	germacrene B	1565	–	3.7	1.3	1.4	2.0
35	spathulenol	1592	–	1.9	2.6	2.2	2.4
40	<i>cis</i> -cadinen-4-en-7-ol	1655	–	7.7	3.9	4.5	6.3
41	<i>tau</i> -cadinol	1666	–	–	–	1.2	0.9
42	<i>tau</i> -muurolol	1669	–	–	2.1	2.4	1.9
44	$\alpha$ -cadinol	1680	–	5.2	6.5	8.5	10.2
45	( <i>E</i> )-bisabol-ol-11	1686	1.5	8.9	2.7	2.0	2.8
Monoterpene hydrocarbons			80.0	38.5	37.6	20.4	11.7
Oxygenated monoterpenes			1.6	12.1	2.8	5.1	7.7
Total monoterpenes			81.6	50.6	40.4	25.5	19.4
Sesquiterpene hydrocarbons			16.8	17.4	36.6	42.0	45.1
Oxygenated sesquiterpenes			1.5	23.7	17.8	20.8	24.5
Total sesquiterpenes			18.3	41.1	54.4	62.8	69.6
Total identified			99.9	91.7	94.8	87.7	88.5

IN, number of identified compound; compounds, identified based on LRI and mass spectrum reported in literature and NIST database (2008); LRI, linear retention indices relative to n-alkanes C8–C40 on the DB5 capillary column; percentage, relative to the total peak area.

**Table 4**Chemical profile of *Schinus molle* dried leaves essential oils composition after 6 h extraction by hydrodistillation at different sequencing time periods (0.5, 1, 2, 4 and 6 h).

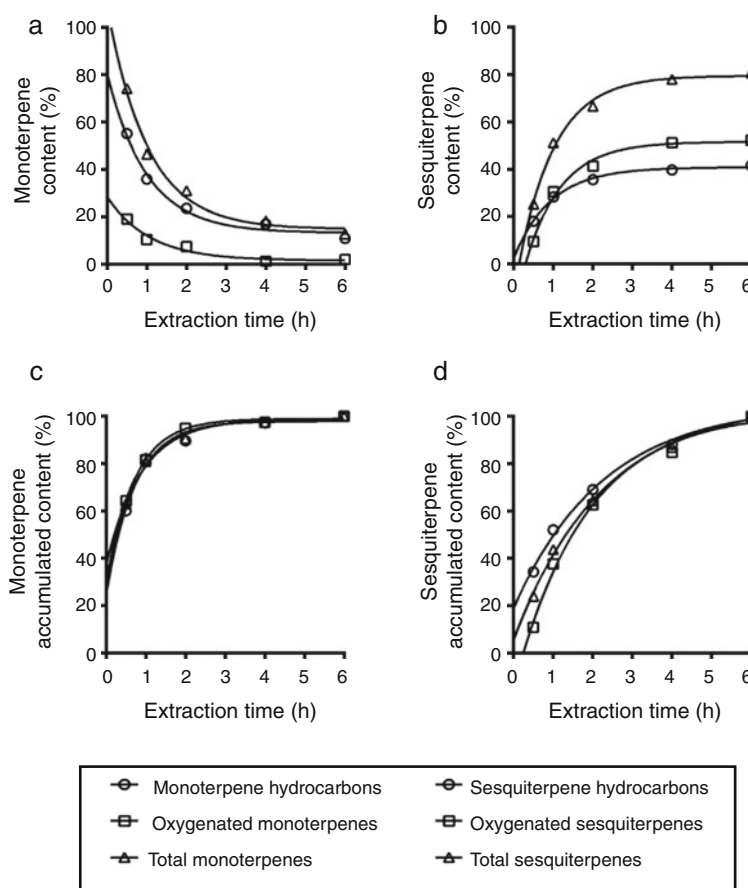
IN	Compounds	LRI	Extraction time (hours)				
			0.5	1	2	4	6
			Percentage				
2	$\alpha$ -tujene	931	3.6	5.0	0.8	–	–
4	sabinene	971	0.8	0.6	–	–	–
5	$\beta$ -pinene	974	4.3	4.9	1.0	–	–
8	linalool	1100	–	–	9.7	–	–
9	nopinone	1133	4.8	–	–	–	–
10	<i>trans</i> -pinocarveol	1135	13.3	7.5	0.6	–	–
11	<i>trans</i> -verbenol	1141	12.3	3.6	0.4	–	–
12	pinocarvone	1159	3.5	–	–	–	–
13	$\alpha$ -felandren-8-ol	1164	1.9	2.3	0.6	–	–
16	mirtenal	1193	13.0	5.5	1.0	–	–
17	verbenone	1206	10.4	11.1	4.2	–	–
22	$\beta$ -caryophyllene	1415	0.7	–	0.5	–	1.3
32	$\gamma$ -cadinene	1511	3.3	3.5	2.5	3.0	4.0
35	spathulenol	1577	4.9	9.8	14.3	17.4	13.6
36	caryophyllene oxide	1582	9.2	19.6	19.9	16.8	9.4
37	globulol	1590	–	–	1.6	2.2	1.9
38	ledol	1599	–	–	1.9	2.2	2.1
39	1, 10-di- <i>epi</i> -cubenol	1616	0.8	2.3	4.0	4.6	4.5
43	cubenol	1647	10.4	23.8	31.0	45.8	50.3
44	$\alpha$ -cadinol	1661	–	–	1.8	3.4	6.3
46	khusilol	1676	–	–	1.0	2.3	4.5
Monoterpene hydrocarbons			8.7	10.5	1.8	–	–
Oxygenated monoterpenes			59.2	30.0	16.5	–	–
Total monoterpenes			67.9	40.5	18.3	–	–
Sesquiterpene hydrocarbons			4.0	3.5	3.0	3.0	5.3
Oxygenated sesquiterpenes			25.3	55.5	75.5	94.7	92.6
Total sesquiterpenes			29.3	59.0	78.5	97.7	97.9
Total identified			97.2	99.5	96.8	97.7	97.9

IN, number of identified compound; Compounds, identified based on LRI and mass spectrum reported in literature and NIST database (2008); LRI, linear retention indices relative to n-alkanes C8–C40 on the DB5 capillary column; Percentage, relative to the total peak area.

**Table 5**Chemical profile of *Schinus molle* dried fruits essential oils composition after 6 h extraction by hydrodistillation at different sequencing time periods (0.5, 1, 2, 4 and 6 h).

IN	Compounds	LRI	Extraction time (hours)				
			0.5	1	2	4	6
			Percentage				
3	$\alpha$ -pinene	935	24.3	21.8	7.5	6.6	2.2
4	sabinene	977	4.3	1.7	–	–	–
5	$\beta$ -pinene	983	46.6	39.3	15.1	9.7	3.1
6	limonene	1033	1.6	1.3	0.8	–	–
10	<i>trans</i> -pinocarveol	1154	2.8	–	–	–	–
21	$\beta$ -elemene	1396	–	–	1.8	1.9	2.2
22	$\beta$ -caryophyllene	1425	6.2	7.9	14.1	15.9	13.3
24	$\alpha$ -humulene	1463	–	–	0.8	0.7	0.9
26	$\gamma$ -himachalene	1484	–	–	0.7	0.9	1.5
27	germacrene D	1489	10.8	13.0	17.7	15.6	11.1
28	$\beta$ -selinene	1495	–	–	0.8	0.8	1.4
29	$\alpha$ -selinene	1505	1.5	2.6	5.8	4.8	4.7
30	$\alpha$ -muurolene	1509	–	–	–	–	0.6
32	$\gamma$ -cadinene	1523	–	–	1.0	1.1	2.0
33	$\delta$ -cadinene	1530	–	–	2.6	3.4	4.7
35	spathulenol	1597	1.9	12.4	27.5	32.3	28.2
41	<i>tau</i> -cadinol	1674	–	–	2.2	3.4	5.4
42	<i>tau</i> -muurolol	1676	–	–	–	–	0.9
44	$\alpha$ -cadinol	1686	–	–	–	0.9	2.7
46	khusinol	1690	–	–	0.9	1.4	2.4
Monoterpene hydrocarbons			76.8	64.1	23.4	16.3	5.3
Oxygenated monoterpenes			2.8	–	–	–	–
Total monoterpenes			79.6	64.1	23.4	16.3	5.3
Sesquiterpene hydrocarbons			18.5	23.5	45.3	45.1	42.4
Oxygenated sesquiterpenes			1.9	12.4	30.6	38.0	39.6
Total sesquiterpenes			20.4	35.9	75.9	83.1	82.0
Total identified			100	100	99.3	99.4	87.3

IN, number of identified compound; compounds, identified based on LRI and mass spectrum reported in the literature and NIST database (2008); LRI, linear retention indices relative to n-alkanes C8–C40 on the DB5 capillary column; percentage, relative to the total peak area.



**Fig. 2.** Monoterpenes and sesquiterpenes content and accumulated content from *Schinus terebinthifolius* and *Schinus molle* leaves and fruit essential oils by hydrodistillation as function of different sequencing time periods (0.5, 1, 2, 4 and 6 h). Best fit obtained with exponential nonlinear regression ( $\alpha = 0.05$ ;  $n = 4$ ),  $r^2 = 0.9843$ ;  $0.9670$ ;  $0.9867$  (a);  $0.9947$ ;  $0.9885$ ;  $0.9923$  (b);  $0.9749$ ;  $0.9968$ ;  $0.9883$  (c) e  $0.9949$ ;  $0.9924$ ;  $0.9968$  (d), respectively to  $\bigcirc$ ,  $\square$  and  $\triangle$ .

for leaves extraction and just 11 and 29 min for fruits from *S. terebinthifolius* and *S. molle*, respectively.

#### Chemical profile of essential oil

In Table 1,  $\alpha$ -pinene (28.1%),  $\beta$ -caryophyllene (35.2%) and germacrene D (15.5%) were *S. terebinthifolius* dried leaves major compounds, as well as,  $\alpha$ -pinene (44.9%),  $\beta$ -pinene (15.1%) and germacrene D (17.6%) were in the fruits *S. terebinthifolius* were the; in the dried leaves of *S. molle* were the sesquiterpenes cubenol (27.1%), caryophyllene oxide (15.3%) and spathulenol (12.4%); in the dried leaves of *S. molle* were the monoterpenes  $\alpha$ -pinene (20.3%),  $\beta$ -pinene (36.3%) and the sesquiterpenes germacrene D (12.1%) and spathulenol (11.4%).

It can be observed some similarity in the essential oil profile of *S. terebinthifolius* with regarding the majority compounds from fruits and leaves ( $\alpha$ -pinene,  $\beta$ -caryophyllene and germacrene D) and also compared with essential oils from fruits of *S. molle* ( $\alpha$ -pinene,  $\beta$ -pinene, germacrene D and spathulenol), whose difference is due to the presence of spathulenol. On the other hand, the same similarity was observed between the leaves and fruits of *S. molle*, which showed only the spathulenol in common (Table 1).

In Tables 2–5 can be noted the chemical variation of essential oils as a function of extraction time. The essential oil obtained from the leaves of *S. terebinthifolius* (Table 2) was what had the lowest number of different compounds, only six substances. Despite the sesquiterpene content to increase with the time of distillation, it was observed that the sesquiterpene was completely extracted in the first half-hour of distillation.

The essential oil obtained from fruits of *S. terebinthifolius* exhibited a more complex profile comparing to the leaves, totaling 26 identified substances (Table 3). Some substances, especially sesquiterpenes were not observed during the first distillation time. For example,  $\gamma$ -murolene and  $\delta$ -cadinene, which to be start extracted from the second hour of distillation.

The chemical analysis of the essential oil of *S. molle* leaves allowed the identification of 21 different substances (Table 4). Also the analysis of chemical composition as a function of extraction time allowed to evaluate that the monoterpenes were extracted in their totality until the second hour of distillation, while that some sesquiterpenes, for example,  $\alpha$ -cadinol and globulol have been extracted after the second hour distillation.

In Table 5 can be observed twenty different substances that have been identified. It was found with the time, decrease of monoterpene content extracted, for example, the content of monoterpenes  $\alpha$ -pinene and  $\beta$ -pinene decreased respectively from 24.3 and 46.6 to 2.2% and 3.1%. On the other hand, the sesquiterpene spathulenol increased from 1.9 to 28.2%, in function of time.

In the extractions involving the fruits and leaves of *S. terebinthifolius* and *S. molle* were revealed similar behavior in the extraction process of monoterpenes and sesquiterpenes in function of time (Fig. 2), for which was noted decrease of monoterpenes content and increase of sesquiterpenes content in the samples analyzed, which are in agreement with those reported by Marongiu et al. (2004) and Barbosa et al. (2007).

On the other hand, the accumulated contents of all terpenoids have been increasing in the function of time elapsed extraction. Based on this result can be inferred about extraction speed of



terpenes, since it took around 1 and 4 h for 80% extraction of monoterpenes and sesquiterpenes, respectively.

This fact is related to ability of diffusion of volatile molecules from the site of synthesis and storage, crossing the plant tissue, until reaching the atmosphere after to be carried by steam. Thus, how much greater is the impediment, smaller is diffusion and greater will be time required for extraction these volatiles (Reverchon et al., 1995).

In this way, volatile with lower molecular weight and lower polarity tend to have a better diffusibility, while the volatiles with higher molecular weight and having polarity tend to have a lower diffusivity.

Therefore, the extraction time suitable to extract a real chemical profile of essential oil, as quality and content of volatiles, depends of an extraction time that is not too small. On the other hand, long extraction times besides the necessary, can increase the costs of the operation, without thereby significantly increase the content of essential oil extracted.

### Conflict of interest

The authors declare no conflicts of interest.

### Authors' contributions

ASC and DSP (undergraduate student) contributed for the procedures regarding to extraction of *S. terebinthifolius* essential oils and its quantification. MSA (PhD student) was responsible for chemical analysis (GC-FID and MS) of *S. terebinthifolius* essential oils. LCPS (undergraduate student) contributed for chemical analysis (GC-FID and MS) of *S. molle* essential oil. MNS (PhD student) contributed on the advisor of undergraduate student and isolation of compounds from *Schinus* genus. DSAC contributed for the collecting plant sample and identification, designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. MAAS Responsible for the review of the data, tabulation and statistical analysis and manuscript editing. All the authors have read the final manuscript and approved the submission.

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