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Headspace solid-phase microextraction-gas chromatography–mass spectrometry analysis of the volatile compounds of *Evodia* species fruits

Federica Pellati [a,*], Stefania Benvenuti [a], Fumihiko Yoshizaki [b], Davide Bertelli [a], Maria Cecilia Rossi [c]

[a] *Department of Pharmaceutical Sciences, University of Modena and Reggio Emilia, Via G. Campi 183, 41100 Modena, Italy*
[b] *Tohoku Pharmaceutical University, 4-1 Komatsushima 4-chome, Aoba-ku, Senday 981-8558, Japan*
[c] *Centro Interdipartimentale Grandi Strumenti (C.I.G.S.), University of Modena and Reggio Emilia, Via G. Campi 213/A, 41100 Modena, Italy*

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Abstract

In this study the investigation of the aroma compounds of dried fruits of *Evodia rutaecarpa* (Juss.) Benth. and *E. rutaecarpa* (Juss.) Benth. var. *officinalis* (Dode) Huang (i.e. *E. officinalis* Dode) (Rutaceae family) was carried out to identify the odorous target components responsible for the characteristic aroma of these valuable natural products. To avoid the traditional and more time-consuming hydrodistillation, the analyses were carried out by means of headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography–mass spectrometry (GC–MS). The SPME headspace volatiles were collected using a divinylbenzene–carboxen–polydimethylsiloxane (DVB–CAR–PDMS) fiber. The extraction conditions were optimized using a response surface experimental design to analyze the effect of three factors: extraction temperature, equilibrium time and extraction time. The best response was obtained when the extraction temperature was around 80 °C, equilibrium time near 25 min and extraction time close to 18 min. Analyses were performed by GC–MS with a 5% diphenyl–95% dimethyl polysiloxane (30 m × 0.25 mm I.D., film thickness 0.25 μm) capillary column using He as the carrier gas and a programmed temperature run. The main components of the HS-SPME samples of *E. rutaecarpa* (concentration >3.0%) were limonene (33.79%), β-elemene (10.78%), linalool (8.15%), myrcene (5.83%), valencene (4.73%), β-caryophyllene (4.62%), linalyl acetate (4.13%) and α-terpineol (3.99%). As for *E. officinalis*, the major compounds were myrcene (32.79%), limonene (18.36%), β-caryophyllene (9.92%), *trans*-β-ocimene (6.04%), linalool (5.88%), β-elemene (7.85%) and valencene (4.62%).

Keywords: *Evodia*; HS-SPME; GC–MS; Volatile compounds; Plant materials; Optimization; Experimental design; Central composite design

1. Introduction

The unripe fruit of *Evodia rutaecarpa* (Juss.) Benth. or *E. rutaecarpa* (Juss.) Benth. var. *officinalis* (Dode) Huang (i.e. *E. officinalis* Dode) (Rutaceae family) is a traditional Chinese medicine that contains alkaloids, essential oil, carboxylic acids, limonoids and flavonoids. The constituents of *Evodia* species fruits have been found to exert several pharmacological effects against parasitic diseases, namely, anti-malarial [1], anthelmintic [2] and anti-microbial activities [3,4], and in particular against *Helicobacter pylori* [5,6]. Furthermore, uterotonic [7], anti-inflammatory [8,9], anti-nociceptive [8,10], vasodilative [11,12], anti-diarrheal [13], bronchoconstrictive [14], anti-thrombotic [15], cardiotonic [16], anti-obesity [17] and central stimulative [18] activities have been described. Extracts of *E. rutaecarpa* can also modulate drug-metabolizing enzymes [19,20]. Some constituents of *Evodia* fruits have been reported to exhibit inhibitory effects on the nuclear factor of activated T cells, suggesting a possible role in the development of new therapies for the treatment of autoimmune disease and transplant rejection [21]. In addition, some active compounds of *E. rutacecarpa* have been claimed to inhibit cell proliferation and migration in several types of cancer cells [22–24]. More recently, some constituents of *Evodia* spp. have been reported to inhibit ultraviolet A-induced increased generation of reactive oxygen species, suggesting a possible applica-

* Corresponding author. Tel.: +39 059 205 5144; fax: +39 059 205 5131.
E-mail address: pellati.federica@unimore.it (F. Pellati).

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tion in the prevention of ultraviolet A-induced photo-aging [25].

High-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) have been applied for the determination of *Evodia* indolequinazoline alkaloids and quinolone alkaloids [26–28]. More recently, synephrine, a phenethylamine alkaloid previously detected by HPLC in the peel and the edible part of *Citrus* species fruit [29,30], has been quantified in *Evodia* fruits by CE [31].

However, despite the many studies that have shown the alkaloid composition of *Evodia* species, there is little research on the volatile compounds responsible for the intense aroma of these fruits. The characterization of the aroma compounds of these plants could represent a useful pointer to the identity and the quality of *Evodia* species. Furthermore, the volatile organic constituents of *Evodia* fruits may contribute to some of the pharmacological effects reported above: in particular, the claimed pharmacological activities of *Evodia* extracts may suggest that the volatile compounds could possess antimicrobial and anti-inflammatory properties. A method able rapidly to identify the volatile constituents of the plants of this genus could prove a useful tool for the purpose of a complete phytochemical analysis.

Hydrodistillation is the most common extraction technique employed to obtain essential oil from aromatic plants. However, hydrodistillation is a time-consuming and laborious process and needs large amounts of sample. Solid-phase microextraction (SPME) is a unique sample preparation technique, which eliminates most drawbacks to extracting organics, including high cost and excessive preparation time; in particular, SPME is a simple and fast modern tool used to characterize the volatile fraction of aromatic and medicinal plants [32,33] and offers a valid alternative to hydrodistillation for gas chromatographic analysis of essential oil from different sources. In SPME, analytes are adsorbed from a solid sample by headspace extraction, using a polymer-coated fused silica fiber. The compounds are then desorbed by exposing the fiber in the injection port of a gas chromatographic apparatus.

In this study, headspace solid-phase microextraction (HS-SPME), combined with gas chromatography–mass spectrometry (GC–MS), was developed and applied to detect the volatile organic compounds of *E. rutaecarpa* and *E. officinalis*. No references have been found on the use of HS-SPME to describe the aroma of *Evodia* fruits objectively. A preliminary screening of fiber of various polarity was carried out in order to select the best type for the analysis of the volatile compounds of *Evodia* fruits. To optimize the extraction conditions, a response surface experimental design was set up to analyze the effect of three factors: extraction temperature, equilibrium time and extraction time.

Furthermore, comparative studies on the characteristic GC–MS profiles of the HS-SPME sampling from *E. rutaecarpa* and *E. officinalis* were performed with the aim of confirming the applicability of the method developed for both qualitative and semi-quantitative analyses of volatile compounds from *Evodia* fruits.

2. Experimental

2.1. Plant material

Authentic fruits of *Evodia rutaecarpa* (Juss.) Benth. and *E. rutaecarpa* (Juss.) Benth. var. *officinalis* (Dode) Huang (i.e. *E. officinalis* Dode) (Rutaceae family) were harvested from trees at Tohoku Pharmaceutical University, Sendai, Japan and were kindly donated by Professor Fumihiko Yoshizaki, Tohoku Pharmaceutical University. The dried samples were protected from light and humidity until required for chemical analysis. Voucher specimens were deposited at the Herbarium of the Botanical Garden of the University of Modena and Reggio Emilia (Italy).

2.2. Chemicals

Compounds used as references were purchased from Sigma–Aldrich–Fluka (Milan, Italy) (both from "General" and "Flavors and Fragrances" catalogues), Carlo Erba (Milan, Italy), Lancaster (Milan, Italy), Extrasynthese (Genay, France) and Roth (Karlsruhe, Germany).

2.3. Hydrodistillation

Evodia spp. essential oil was extracted by hydrodistillation from the plant fruits with a commercial Clavenger apparatus. Forty grams of dried plant material and 300 mL of distilled water were used; hydrodistillation was carried on for 5 h after the mixture had reached boiling point (100 °C).

2.4. SPME fiber screening

Before carrying out the optimization of the SPME conditions for the analysis of volatile compounds of *Evodia* fruits, fiber screening was carried out. The silica fibers and the manual SPME holder were purchased from Supelco (Bellefonte, PA, USA). Four fibers were tested and compared: polydimethylsiloxane (PDMS, 100 μm), polydimethylsiloxane–divinylbenzene (PDMS–DVB, 65 μm), Stableflex divinylbenzene–carboxen–polydimethylsiloxane (DVB–CAR–PDMS, 50/30 mm) and Stableflex Carbowax–divinylbenzene (CW–DVB, 65 μm). The coating of all fibers was 1 cm long, with the exception of that of the DVB–CAR–PDMS fiber, which was 2 cm long. Before GC–MS analysis, each fiber was conditioned in the injector of the GC system, according to the instructions provided by the manufacturer.

2.5. HS-SPME

A 0.5 g amount of *Evodia* fruits was hermetically sealed in a 15 mL screw top amber vial with a polypropylene hole

cap and PTFE/silicone septa (Supelco, Bellefonte, PA, USA) and equilibrated during the equilibrium time (depending on the experimental design) in a thermostatic bath at the desired temperature (depending on the experimental design). Then, the SPME device was inserted into the sealed vial by manually penetrating the septum and the fiber was exposed to the plant material headspace during the extraction time (depending on the experimental design). For the preliminary fiber screening study, experimental conditions were set as follows: extraction temperature: 60 °C; equilibrium time: 30 min; extraction time: 15 min.

After sampling, the SPME was immediately inserted into the GC injector and the fiber thermally desorbed. A desorption time of 1 min at 250 °C was used in splitless mode. Before sampling, each fiber was reconditioned for 5 min in the GC injector port at 250 °C.

2.6. Experimental design

The optimization of the HS-SPME conditions was performed by the use of a central composite experimental design (CCD, with $\alpha = 1.682$) [34], which was based on a 2^3 factorial design plus six axial points plus six replicates in the center of the design. For HS-SPME optimization, the variables chosen were the extraction temperature (T, °C), the equilibrium time (t_{eq}, min) and the extraction time (t_{ext}, min). The factor levels and experimental domain are shown in Table 1.

Twenty experiments were performed in randomized order.

Statistical analysis was performed using Statistica v. 6.1 (Statsoft Inc., Tulsa, OK, USA).

2.7. GC–MS conditions

Analyses were carried out with a Varian 3400 gas chromatograph coupled to a Finnigan MAT-SSQ 710 A mass spectrometer.

Compounds were separated on a Rtx®-5 MS Crossbond® 5% diphenyl–95% dimethyl polysiloxane (30 m × 0.25 mm I.D., film thickness 0.25 μm) capillary column (Restek, Bellefonte, PA, USA). The column was maintained at 50 °C for 2 min after injection, then programmed at 8 °C min^{-1} to 280 °C, which was maintained for 2 min. Splitless injection was performed with helium as the carrier gas at a pressure of 13 p.s.i. at the column head. Injector, transfer line temperature and ion-source temperatures were 250, 280, and 160 °C, respectively. All mass spectra were acquired in electron-impact (EI) mode; the ionization voltage was 70 eV; the emission current was 350 μA; the mass range was 40–400 m/z.

A mixture of aliphatic hydrocarbons (C_8–C_{25}) in hexane (Sigma, Milan, Italy) was loaded onto the SPME fiber and injected under the above temperature program to calculate the retention index (as Kovats index, I) of each compound.

Table 1
Factor levels and experimental domain applied to optimize the HS-SPME experimental conditions

Factor	Experimental domain				
	$-\alpha$[a]	−1	0	1	α[a]
Extraction temperature (T, °C)	29.77	40	55	70	80.23
Equilibrium time (t_{eq}, min)	1.18	8	18	28	34.82
Extraction time (t_{ext}, min)	1.59	5	10	15	18.41

[a] $\alpha = 1.682$.

2.8. Qualitative and semi-quantitative analysis

Compounds were identified by comparing the retention times of the chromatographic peaks with those of authentic compounds run under the same conditions and by comparing the retention indices (as Kovats indices) with the literature data [35–42]. Peak enrichment on co-injection with authentic reference compounds was also carried out. The comparison of the MS fragmentation pattern with those of pure compounds and mass spectrum database search was performed using the National Institute of Standards and Technology (NIST) MS spectral database. Confirmation was also conducted using a laboratory-built MS spectral database collected from chromatographic runs of pure compounds performed with the same equipment and conditions. The relative amounts (RAs) of individual components are expressed as percent peak areas relative to total peak area.

3. Results and discussion

3.1. Choice of the extraction technique and fiber screening

Hydrodistillation has traditionally been applied for essential oil extraction from plant material. This technique presents some shortcomings, namely losses of volatile compounds, low extraction efficiency, and long extraction time. Also, high temperatures and water can cause degradation or chemical modifications of volatile constituents. In recent years, the most frequently used analytical techniques for the extraction and concentration of volatile compounds from aromatic and medicinal plants are those based on headspace analysis. Of the headspace methods, SPME represents a reliable tool for the analysis of organic volatile compounds.

In this study, two techniques were considered for the extraction of the volatile compounds from *Evodia* spp. fruits: hydrodistillation and HS-SPME. The amount of essential oil obtained by hydrodistillation was very small (<0.01 mL/40 g) and it was not possible to collect a quantity sufficient for a complete study of the composition by GC–MS. In view of the small amount of fruits of *Evodia* spp. available for this study and the very low yield of essential oil obtained by hydrodistillation, it was not possible to apply this technique for the extraction of the volatile fraction. In the present work, HS-SPME was therefore used for the extraction of the aroma compounds responsible for the significant flavour of the fruits of *Evodia* spp. Analysis of the volatiles was carried out by means of GC–MS.

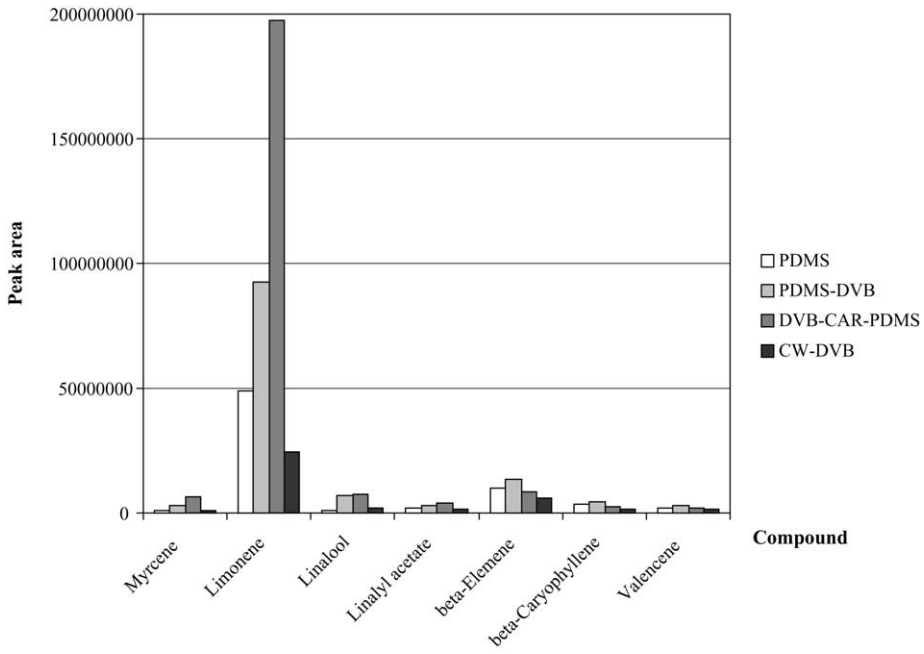

Fig. 1. Effect of the SPME fiber coating on the extraction of the aroma compounds from *Evodia* fruits.

Having chosen the more suitable extraction technique, the following step was the selection of the best fiber coating for HS-SPME. Bicchi et al. [43] evaluated the effect of the fiber coating on HS-SPME of volatile compounds from various aromatic and medicinal plants. Selection of the most appropriate SPME fiber depends on the compounds targeted and therefore on the plant material under study. The authors observed that the most effective fibres for HS-SPME were those characterized by two components: a liquid (PDMS) for the less polar compounds and a solid (DVB, CAR or both) polymeric coating for the more polar constituents.

In this study, four fibers, polydimethylsiloxane (PDMS, 100 μm), polydimethylsiloxane–divinylbenzene (PDMS–DVB, 65 μm), Stableflex divinylbenzene–carboxen–polydimethylsiloxane (DVB–CAR–PDMS, 50/30 μm) and Stableflex Carbowax–divinylbenzene (CW–DVB, 65 μm) were evaluated for the analysis of *Evodia* fruit aroma. Fig. 1 shows the results of the fiber screening.

Each fiber was exposed to the headspace for the same time at the same temperature, although these parameters are likely to vary as a function of the coating material. The peak area values obtained with the DVB–CAR–PDMS fiber were divided by a factor of two, since this fiber is twice as long as the other fibers tested [43]. As shown in Fig. 1, the results of the fiber screening confirmed that the PDMS–DVB and the DVB–CAR–PDMS fibers produced the best results for the compounds investigated. Of these two fibers, the DVB–CAR–PDMS showed a strong extraction capacity for monoterpenes, while the PDMS–DVB had a higher affinity for sesquiterpenes. In particular, the fiber based on the DVB–CAR–PDMS coating showed a very strong affinity to limonene. Given the better profile shown by this coating, this fiber was selected for the phytochemical characterization of the volatile compounds of *Evodia* fruits.

3.2. HS-SPME optimization

The analysis of headspace volatile constituents by HS-SPME is greatly influenced by the vapour pressure of flavour

Table 2
Experimental conditions and response value (total area) of the central composite design used to optimize the extraction conditions of *Evodia* spp. fruits by HS-SPME

Experiment number	T (°C)	t_{eq} (min)	t_{ext} (min)	Response value (total area[a])
1	70	28	15	268340625
2	70	28	5	284101041
3	70	8	15	254206860
4	70	8	5	243918222
5	40	28	15	48786504
6	40	28	5	219232436
7	40	8	15	148907645
8	40	8	5	233103194
9	80.23	18	10	263461839
10	29.77	18	10	58898984
11	55	34.82	10	225965823
12	55	1.18	10	61749079
13	55	18	18.41	252228163
14	55	18	1.59	179806788
15	55	18	10	196516550
16	55	18	10	256804605
17	55	18	10	232174758
18	55	18	10	239821164
19	55	18	10	296481947
20	55	18	10	282818574

Experimental conditions as in Section 2.7.
[a] Total area is expressed in arbitrary units.

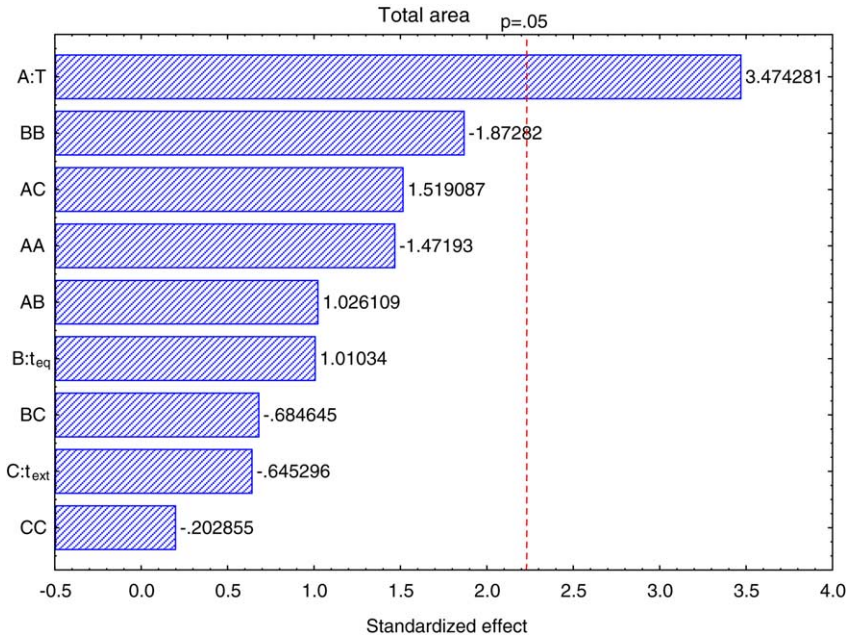

Fig. 2. Pareto chart for the response (total area of the GC analysis of the HS-SPME of *Evodia* fruits at the different conditions tested) considered in this study.

compounds in the vial. Extraction temperature (T, °C), equilibrium time (t_{eq}, min) and extraction time (t_{ext}, min) are three of the most important variables (factors) influencing the vapour pressure and equilibrium of the aroma compounds in the headspace. These three factors were therefore chosen and optimized. Traditional methods of optimization evaluate the effect of one variable at a time, keeping all the others constant during experiments with the exception of the one being studied. However, this type of experiment does not allow one to determine what would happen if the other variables also change. The experimental design enables the effects of several variables to be estimated simultaneously. In particular, response surface methodology coupled with a central composite design is an effective tool for optimizing a process [34] and was therefore applied in this study.

In the literature, the optimization of the SPME conditions is based either on the peak areas of some compounds present in the chromatogram [36,44,45] or on the sum of the peak areas of all the compounds identified in the sample [46–48]. A response based on the sum of the peak areas is one of the most frequently used parameter to optimize the SPME extraction conditions [46–48]. In this work, a response based on the sum of the areas of the GC–MS analysis of the compounds extracted by HS-SPME from *Evodia* fruits under the conditions of the design was studied. This response gives information on the intensity of the aroma compounds extracted. The experimental conditions of the central composite experimental design used to optimize the extraction conditions of *Evodia* fruits by HS-SPME had a strong influence on the peak area values, but the number of chromatographic peaks was not influenced. Therefore, a response based on the number of chromatographic peaks was not considered significant in this study.

The experimental values of the factors used to optimize the SPME conditions were selected to cover a wide range of conditions. In particular, the extraction temperature varied from 30 to 80 °C; the latter is a high temperature, which allowed the extraction of the less volatile organic compounds from the plant material. Several experiments were performed at higher extraction temperatures, but no changes in the qualitative and semi-quantitative composition of the aroma compounds extracted from *Evodia* fruits were observed. Therefore, the maximum value of the extraction temperature was set at 80 °C.

The 20 experiments of the experimental design were performed at random and the responses are shown in Table 2.

The data obtained were evaluated by ANOVA, the level of significance being set at 5%. This technique allowed us to evaluate the statistical significance of each factor and interactions between the different factors. Fig. 2 shows the Pareto chart for the response.

The extraction temperature was the most significant parameter (at $P < 0.05$), having a strong positive influence. The effect of temperature can be accounted for in that it can influence the partition coefficients of the compounds both between the sample and the headspace and between the headspace and the fiber, as well as the change in the vapour pressure of the compounds in the sample.

Since it was not possible to plot simultaneously the response as a function of the factors that control the extraction process, the effects of two factors on the response were considered separately. Fig. 3a shows the response surface plot obtained by plotting equilibrium time versus extraction temperature, with an extraction time equal to 10 min. Fig. 3b shows the response surface developed for extraction time and extraction temperature, maintaining an equilibrium time of 18 min.

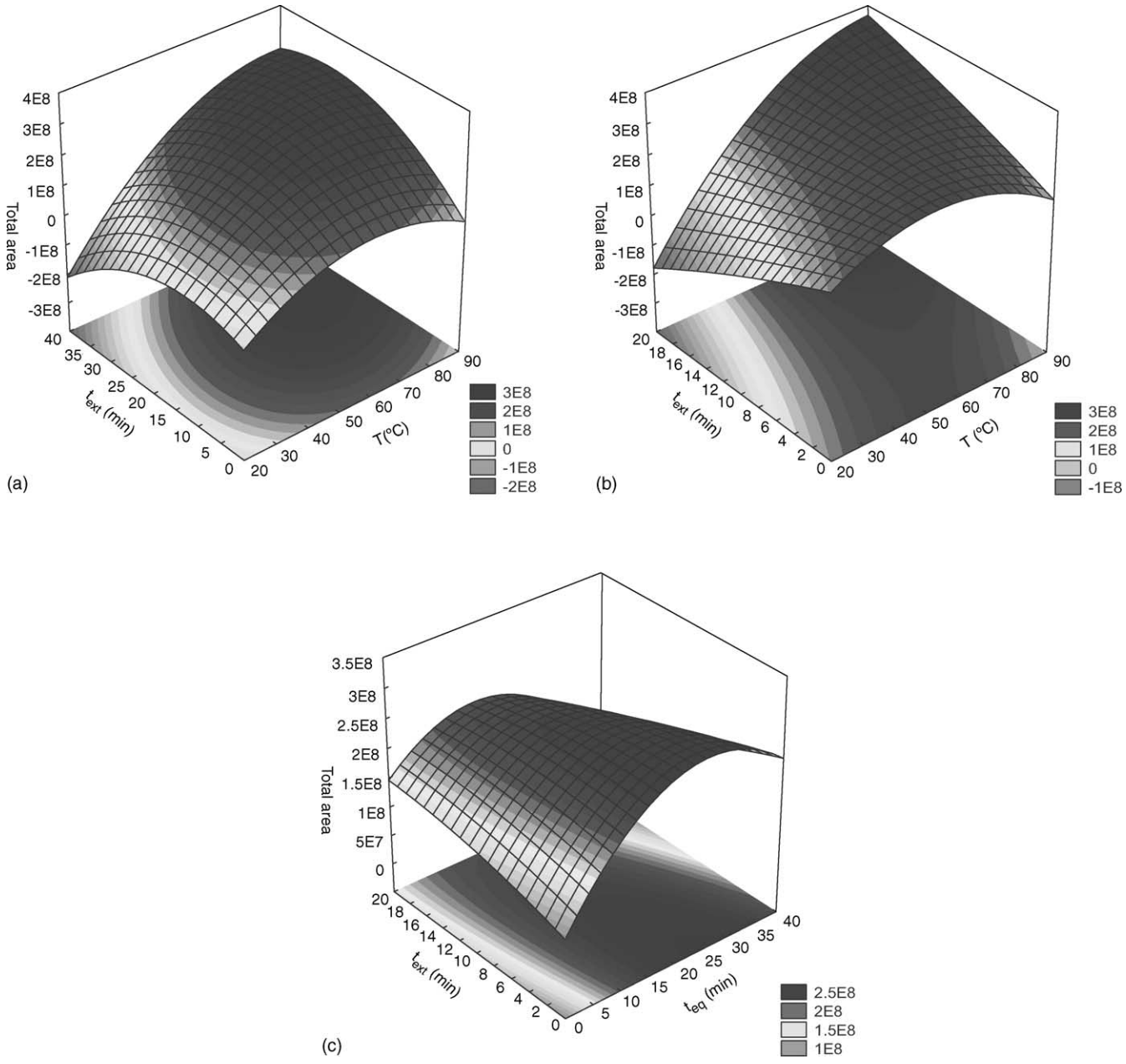

Fig. 3. Response surface plot for: (a) total area versus equilibrium time (t_{eq}, min) and extraction temperature (T, °C), with a constant extraction time equal to 10 min; (b) total area versus extraction time (t_{ext}) and extraction temperature (T, °C), with a constant equilibrium time equal to 18 min; (c) total area versus extraction time (t_{ext}, min) and equilibrium time (t_{eq}, min), with a constant extraction temperature (T, °C) equal to 55 °C.

Fig. 3c shows the response surface obtained for extraction time and equilibrium time, with an extraction temperature of 55 °C.

These graphs are useful for interpreting graphically the effect on the response of each pair of independent variables. These graphs indicate that the best responses can be obtained at high temperatures. It is well-known [49] that an increase in sampling temperature increases the headspace concentration of the aroma compounds, favouring their extraction. However, SPME involves an exothermic process and the extraction of compounds decreases as the temperature increases. In this study, a high temperature increased the experimental responses studied. This could be due to an increase in less volatile compounds in the headspace that might compensate for the decrease in adsorption induced by this high temperature. No decomposition of the aroma compounds from *Evodia* fruits was observed at high temperatures.

In conclusion, the experimental results show that the best global response, within the range studied, was reached when the extraction temperature was around 80 °C, equilibrium time near 25 min and extraction time close to 18 min. These values were therefore selected to extract the

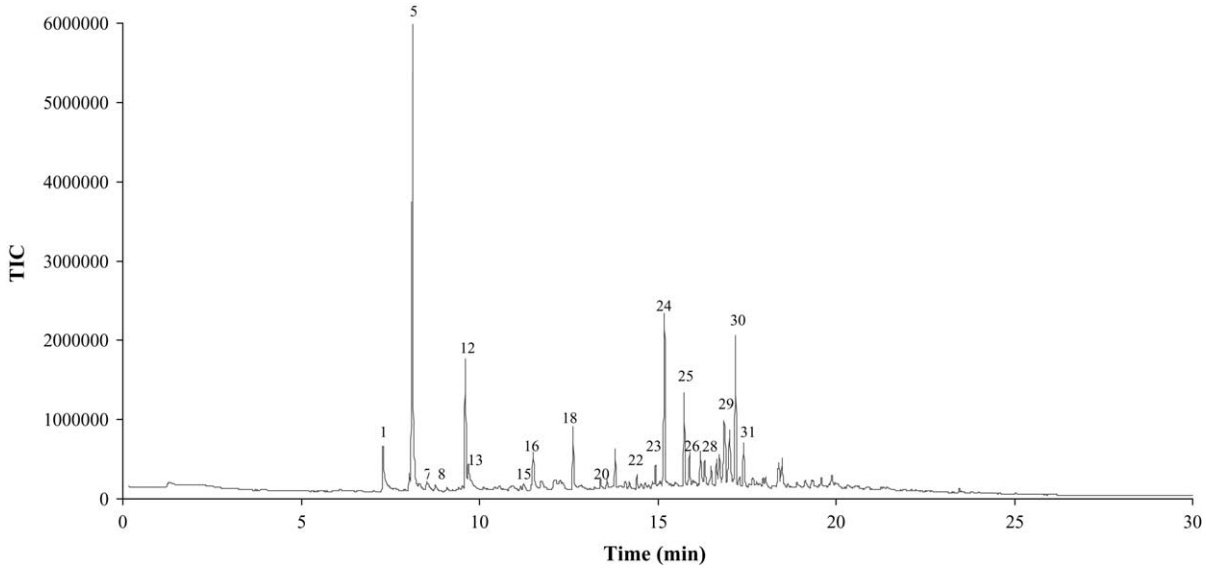

Fig. 4. Total ion current (TIC) chromatogram of the HS-SPME volatile compounds of *E. rutaecarpa*. Chromatographic conditions: Rtx®-5 MS Crossbond® 5% diphenyl–95% dimethyl polysiloxane capillary column (30 m × 0.25 mm I.D., film thickness 0.25 μm); oven temperature program: initial 50 °C (2 min constant), then to 280 °C at 8 °C min^{-1}, final 280 °C (2 min constant); splitless injection; carrier gas: helium at 13 p.s.i.; injector, transfer line temperature and ion-source temperatures: 250, 280, and 160 °C. The main peaks were assigned as in Table 3.

Table 3
Volatile aroma components of *Evodia* spp. obtained by HS-SPME

Peak number	Compound[a]	Kovats index (*I*)[b]	*E. rutaecarpa*		*E. officinalis*		Method of identification[d]
			%RA[c]	SD	%RA	SD	
1	Myrcene	993	**5.83**	0.45	**32.79**	2.16	a, b, c, d
2	δ-3-Carene	1010	0.11	0.03	0.67	0.04	a, b, c, d
3	α-Terpinene	1022	–	–	0.12	0.04	a, b, c, d
4	*p*-Cymene	1029	0.52	0.06	0.10	0.04	a, b, c, d
5	Limonene	1034	**33.79**	5.10	**18.36**	0.30	a, b, c, d
6	*cis*-β-Ocimene	1040	0.71	0.07	2.06	0.21	a, b, c, d
7	*trans*-β-Ocimene	1052	1.08	0.10	**6.04**	0.84	a, b, c, d
8	γ-Terpinene	1063	0.56	0.06	–	–	a, b, c, d
9	*cis*-Linalool oxide	1079	0.20	0.05	0.06	0.01	b, d
10	Terpinolene	1091	–	–	0.12	0.03	b, d
11	*trans*-Linalool oxide	1095	0.17	0.02	–	–	b, d
12	Linalool	1104	**8.15**	0.47	5.88	0.53	a, b, c, d
13	Nonanal	1109	2.83	0.37	–	–	b, d
14	Borneol	1175	–	–	0.18	0.01	a, b, c, d
15	4-Terpineol	1185	0.51	0.06	0.40	0.03	a, b, c, d
16	α-Terpineol	1198	**3.99**	0.15	0.21	0.01	a, b, c, d
17	Citronellol	1234	–	–	0.34	0.01	b, d
18	Linalyl acetate	1257	**4.13**	0.15	0.16	0.05	a, b, c, d
19	Geraniol	1261	–	–	0.25	0.01	b, d
20	Tridecane	1298	0.84	0.03	0.42	0.03	b, d
21	δ-Elemene	1343	0.47	0.04	0.24	0.02	b, d
22	α-Cubebene	1356	0.65	0.09	0.35	0.02	a, b, c, d
23	α-Copaene	1385	1.21	0.42	0.43	0.06	a, b, c, d
24	β-Elemene	1401	**10.78**	1.11	**7.85**	0.96	b, d
25	β-Caryophyllene	1431	**4.62**	0.83	**9.92**	0.83	a, b, c, d
26	γ-Elemene	1442	2.05	0.33	1.20	0.11	b, d
27	α-Guaiene	1447	0.07	0.03	–	–	b, d
28	α-Humulene	1465	1.77	0.13	0.99	0.09	a, b, c, d
29	Valencene	1499	**4.73**	0.40	**4.62**	0.45	a, b, c, d
30	2,6-Ditert-buthyl-4-hydroxy toluene	1519	7.34	0.78	5.02	0.51	a, b, c, d
31	δ-Cadinene	1535	2.86	0.28	1.82	0.01	b, d

(–): Compound not detected; experimental conditions as in Section 2.7.
[a] Compounds are listed in order of elution.
[b] Retention index on Rtx-5 column.
[c] Percent relative area.
[d] *a*: retention time; *b*: retention index; *c*: peak enrichment; *d*: mass spectrum.

volatile compounds from *Evodia* fruits in all the subsequent analyses.

3.3. Analysis of volatile compounds of Evodia fruits

The HS-SPME is a very convenient technique for providing the headspace fingerprint of *Evodia* spp. volatile organic compounds as it is simple, fast and solvent-free.

Fig. 4 shows the total ion current (TIC) chromatogram of the HS-SPME of the aroma compounds of *E. rutaecarpa*. The chromatographic conditions were optimised with the aim of obtaining a good separation of adjacent peaks within a short analysis time.

The compounds identified are described in Table 3 with their relative percentages. A total of 31 components were characterized.

The first observation is that no peaks appeared in the blank runs, thus indicating that no compounds due to the fiber coating or contamination occurred during the extraction of the volatile compounds.

The typical aroma of *Evodia* fruits can be attributed substantially to the large amounts of monoterpenes and, to a lesser extent, to the content of sesquiterpenes. Although the qualitative profile of both *E. rutaecarpa* and *E. officinalis* was very similar, their relative abundance was different. The differences between the volatile compounds of *E. rutaecarpa* and *E. officinalis* are mainly due to the composition of monoterpenes. The results of this study showed that the major monoterpenes (concentration >3.00%) of *E. rutaecarpa* were limonene (33.79%), linalool (8.15%), myrcene (5.83%), linalyl acetate (4.13%) and α-terpineol (3.99%). Of the sesquiterpenes, the main components were β-elemene (10.78%), valencene (4.73%) and β-caryophyllene (4.62%). As for *E. officinalis*, the major monoterpenes were myrcene (32.79%), limonene (18.36%), *trans*-β-ocimene (6.04%) and linalool (5.88%), while the sesquiterpene composition was similar to that of *E. rutaecarpa*: β-caryophyllene (9.92%), β-elemene (7.85%) and valencene (4.62%).

The peak no. 30, identified in both *E. rutaecarpa* and *E. officinalis* as 2,6-ditert-butyl-4-hydroxy toluene (i.e., butylated hydroxy-toluene or BHT), is a well-known synthetic antioxidant that is usually added to plastics, elastomers, solvents and food items as well. It is readily released from plastic vials or coatings and can contaminate analytical samples, headspace included, thus giving rise to an extra peak in the chromatogram [50]. This compound is just a contaminant and not a plant secondary metabolite and it would be misleading to include it in the list of the volatile compounds of *Evodia* spp.

4. Conclusion

HS-SPME coupled with GC–MS is a rapid and simple method that, for the first time, enables the extraction and identification of the volatile compounds responsible for the typical aroma of *Evodia* fruits. Choosing a fiber with suitable polarity, depending on the nature of the target compounds, is a very important factor in headspace analysis. In this work, the most effective fiber for HS-SPME was the DVB–CAR–PDMS. The effects of several experimental parameters on the HS-SPME sampling from *Evodia* fruits were also studied. The results showed that sampling temperature is the dominant factor for the HS-SPME of the volatile compounds of *Evodia* fruits; this could be due to an increase in less volatile compounds in the headspace which might compensate for the decrease in adsorption induced by high temperatures.

The HS-SPME–GC–MS method, developed and applied in this work, proved to be a simple, speed and convenient tool for the purpose of characterizing the volatile fraction of different *Evodia* species. Large amounts of monoterpenes and lesser amounts of sesquiterpenes are responsible for the characteristic aroma of these fruits. The qualitative profile of the volatile compounds of *E. rutaecarpa* and *E. officinalis* was similar, but their relative abundance showed several differences. This work is a first step which opens the perspective of further studies on the aroma composition of *Evodia* fruits. On the basis of this study, it can be concluded that HS-SPME followed by GC–MS is eminently suited to the extraction and semi-quantitative analysis of volatiles from *Evodia* fruits.

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