Indian Journal of Natural Products and Resources
Vol. 9(1), March 2018, pp. 28-33

Comparison of ultrasonic assist head space solid-phase microextraction and classic hydrodistillation methods for the identification of essential oil in fruits and leaves of *Pistacia atlantica*

Mohammad Reza Nargesi, Fereshteh Bagheri, Soheila Shahriari* and Reza Tahvilian

Pharmaceutical Science Research Center, School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran

Received 7 December 2016; Revised 01 January 2018

Pistacia atlantica Desf. is one of the three species growing in Iran. The essential oil compounds from aerial (fruits and leaves) of *P. atlantica* were obtained by hydrodistillation (HD) and analyzed using GC-MS. The results of two different methods, hydrodistillation and ultrasonic assist headspace solid-phase microextraction (UA-HS-SPME) were compared. The GC-MS analysis identified 40 and 36 components of essential oils in fruits and leaves extracted using HD, respectively. The oil contained a high concentration of monoterpenes, and the main constituents were Germacrene D (9.29 %), Myrcene (9.3 %) in fruits and Myrcene (8.91 %), Germacrene D (7.89 %) in leaves. A total of 43 components were identified by UA-HS-SPME technique in both the fruits and leaves where the major components in fruits were Germacrene D (12.06 %), Myrcene (12.01 %). The volatile components, such as Germacrene D (10.53 %), Myrcene (10.18 %) were found to be major volatile constituents in leaves. UA-HS-SPME analysis showed more type and concentration of different compounds in the studied plant. For example, Germacrene D, Myrcene, Terpinen-4-ol compounds were well represented in the aerial parts (fruits and leaves). Moreover, HS-SPME allowed the occurrence of α-Cubebene, α-Ylangene and α-Farnesene as the main component in *P. atlantica* fruits and the leaves, but it was not detected in the HD method. Compared to the conventional technique, HD and UA-HS-SPME method were established short extraction time and high extraction efficiency.

Keywords: Hydrodistillation, Microextraction, *Pistacia atlantica*, UA-HS-SPME.

IPC code; Int. cl. (2015.01)−A61K 36/22, A61K 2236/33

Introduction

Anacardiaceae is a small family with about 400 species of mainly tropical trees and shrubs[1]. Among 15 known species of *Pistachios*, only 3 species grow in Iran, including, *Pistacia vera* L., *Pistacia khinjuk* Stocks and *Pistacia atlantica* Desf. They are the most important species of Pistachio and for this reason, Iran is known as the origin of *Pistachios*. The word 'Pistachio' is derived from a Persian name: "*Pisteh*"[2]. Pistachio trees are reported to possess antibacterial activity[3,4]. The Pistachio fruits are rich in oil and are used by the local inhabitants in many ways as an anti-diarrheal and also as a constituent of cattle feed[5]. The leaves of *P. atlantica* are used as a stomachic, while its fruits and oleoresin are used in medicine. *Pistacia* plants are known for their medicinal properties since antiquity. They have played important roles in folk medicine and are used in eczema treatment, coughs, sore throats, for its diuretic properties, renal stones, asthma and stomach ache, and as an astringent, anti-inflammatory, antipyretic, antibacterial, antiviral, pectoral, stimulant and kidney stones and jaundice[6-8].

The chemical composition of the essential oil of this plant reveals the presence of several main compounds: myrcene, limonene, terpinen-4-ol, a-pinene, b -pinene, a -phellandrene, sabinene, para-cymene and g-terpinene[9], a-pinene, limonene oxide, myrtenol and citral[10], a-pinene and bornyl acetate[11], a-pinene, a-thujene, spathulenol and bicyclogermacrene[12], α-Thujene, α-Pinene, Camphene, Sabinene, β-Pinene, d-3- careen and Limonene[13].

The essential oils of aromatic herbs are traditionally obtained using hydrodistillation. Increasing temperature in a traditional method like hydrodistillation causes degradation of some parts of volatile oils[14]. For these reasons, solid-phase micro-extraction (SPME) has been more and more employed in such cases, and HS sampling requires optimization of the extraction parameters to be carried out. SPME is a solvent-free extraction method in which analytes are directly absorbed into a fused silica fibre coated with a

*Correspondent author
E-mail: so.shahriari17@yahoo.com, rtahvilian@kums.ac.ir
Tel: +988334276482

polymer phase and then desorbed directly into a chromatographic injection port[15]. Several conditions regarding the time and temperature for equilibrium and extraction have been reported, according to the plant material analyzed[16-18].

Although, several workers have reported variations in chemical compositions of essential oils due to their origin, environmental conditions and the developmental stage of collected plant materials, no such information is available on the influence of extraction methods on the chemical composition of essential oil of *P. atlantica*. Therefore, to obtain a better understanding of the *P. atlantica* volatiles, we investigated the chemical composition of Iranian *P. atlantica* essential oils extracted using hydrodistillation (HD) from aerial parts (fruits and leaves) and the volatile fractions extracted using UA-HS-SPME from the same plant material. In both cases, the analysis was carried out using gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS).

Materials and Methods

Materials

The fruits and leaves of *P. atlantica* were collected in May 2015 from kerend of Kermanshah, Kermanshah province (western part of Iran). The plant samples were dried at room temperature (20-24 ^{o}C) in shade condition. PDMS coating was purchased from Supelco (Belefonte, PA, USA).

Extraction methods

Hydrodistillation

The dry plant materials (40 g) were ground into small pieces and subjected to hydrodistillation (HD) using a Clevenger-type apparatus (4 hours). Essential oils were dried over anhydrous sodium sulfate and stored at 4 ^{o}C in a sealed brown vial until analysis with GC and GC–MS.

Headspace solid- phase microextraction

The SPME extraction was performed for both samples (fruits and leaves). For the extraction, a 20 mL rubber septum-capped vial was used. About 1.07 g of ground material was placed in the glass vial, to which 200 µL double-distilled water (as matrix modifier) was added and the vial was shaken vigorously by hand for homogeneous dispersion of the spiked water. Ten mL of a 1 % nitrobenzene solution in methanol was added as internal standard to all samples. The vial was capped and sonicated in the thermostatted ultrasonic bath at 60 ^{o}C for 12.10 minutes for equilibration of volatiles between the headspace and the sample matrix. After sonication step, SPME extraction of the volatile compounds with the polydimethylsiloxane (PDMS) fibre was then accomplished during a 14.82 min period at 60 ^{o}C.

Instruments and GC-MS analysis procedure

UA-HS-SPME extraction was performed using commercial SPME fibre with 100 µm i.d PDMS coating purchased from Supelco (Bellefonte, PA, USA). The fibre was handled by manual SPME holder provided with Supelco. For essential oil extraction by Hydrodistillation method was applied. Ultrasonic irradiation was applied by means of Eurosonic 4D (EURONDA) equipped with a water bath where extraction vials were placed. All gas chromatography analysis was performed using Agilent 6890N GC instrument coupled to the 5973N mass detector from Agilent Technology, USA. Extracted analytes separated using a 30 m×250 µm HP5 capillary column coated with a 0.25 l m film of 5 % phenyl methylpolysiloxane, and were inserted directly into the ion source of the MS. The splitless injection was used for the samples. The column oven temperature was programmed at 5 ^{o}C/min from an initial temperature of 50 to 80 °C, then at 3 ^{o}C/min to 120 ^{o}C, finally at 15 °C/min to 260 °C. The injection and ion source temperatures were 250 and 230 ^{o}C, respectively. Helium (99.999 %) was used as carrier gas at a flow rate of 1 mL/min. The mass range scanned was 40–450 amu in full-scan acquisition mode. Analytes desorption from fibre was performed in the injection port for 3 min.

Results and Discussion

Composition of the essential oil by HD method

An analysis of the essential oils from the aerial part (fruits and leaves) of *P. atlantica* identified 40 and 36 components, respectively, which accounted for 88.1 and 75.96 % in fruits and leaves, respectively. Their retention indices and relative percentages are shown in Table 1. The essential oil was rich in monoterpenes, and the main constituents in the oils obtained by HD method were Germacrene D (9.29 %), Myrcene (9.3 %), Terpinen-4-ol (7.63 %), p-Cymene (5.98 %), (E)-Caryophyllene (4.86 %) and Camphene (4.28 %) in fruits and Myrcene (8.91 %), Germacrene D (7.89 %), Terpinen-4-ol (5.92 %), p-Cymene (5.59 %), (E)-Caryophyllene (4.04 %) and Camphene (3.94 %) in leaves.

Table 1 — Comparison of the essential oil of fruits and leaves in *Pistacia atlantica* by HD and UA-HS-SPME methods

Components	RI	HS-SPME techniques		Hydrodistillation method	
		Leaves (%)	Fruits (%)	Leaves (%)	Fruits (%)
Tricyclene	901	1.81	1.72	1.01	1.9
α-Thujene	907	1.06	0.7	1.09	0.83
α-Pinene	916	7.04	6.23	9.07	8.26
Camphene	929	4.28	3.94	6.03	5.21
Sabinene	942	4.54	4.09	6.07	5.18
β-Pinene	951	3.83	3.57	5.07	3.97
Myrcene	963	9.3	8.91	12.01	10.18
α-Phellandrene	976	0.96	0.42	0.34	0.39
p-Mentha-1(7),8-diene	981	0.04	0.02	0.05	0.08
α-Terpinene	984	2.33	2.08	4.02	3.14
p-Cymene	992	5.98	5.59	7.01	5.87
β-Phellendrene	996	2.1	2.3	2.14	2.39
(*Z*)-β-Ocimene	1004	2.76	2.22	1.08	2.26
Benzene acetaldehyde	1010	0.01	-	0.05	0.04
(*E*)-β-Ocimene	1018	0.69	1.24	0.1	1.35
γ-Terpinene	1029	3.69	2.77	3.27	2.95
Terpinolene	1052	1.9	1.14	1.09	1.58
p-Cymenene	1060	0.37	0.02	0.16	0.14
cis-*p*-Menth-2-en-1-ol	1093	-	-	0.02	0.02
Camphor	1114	1.18	1.01	1.26	1.24
Terpinen-4-ol	1139	7.63	5.92	9.03	7.78
p-Cymen-8-ol	1149	1.11	-	1.02	0.84
α-Terpineol	1161	1.98	1.24	2.02	1.81
cis-Piperitol	1173	0.6	0.23	0.44	0.61
Bornyl acetate	1237	0.04	0.31	0.042	0.39
α-Cubebene	1281	-	-	1.09	1.045
α-Ylangene	1324	-	-	1.05	1.015
α-Copaene	1336	0.04	-	0.05	0.02
β-Bourbonene	1346	0.36	0.29	0.047	0.12
β-Cubebene	1352	0.29	0.19	0.01	0.11
(*E*)-Caryophyllene	1376	4.86	4.04	5.21	4.46
cis-Muurola-3,5-diene	1401	0.13	-	0.04	0.028
trans-Muurola-3,5-diene	1409	0.32	0.25	0.09	0.13
α-Humulene	1420	1.29	1.94	0.03	0.34
allo-Aromadendrene	1436	0.05	0.41	0.02	0.27
Germacrene D	1447	9.29	7.89	12.06	10.53
Bicyclogermacrene	1466	0.21	0.19	0.28	0.22
(*E,E*)-α-Farnesene	1478	-	-	1.12	1.04
Cubebol	1489	0.31	0.25	0.26	0.3
α-Cadinene	1540	0.46	0.29	0.24	0.23
Germacrene D-4-ol	1545	0.87	0.86	0.43	0.61
epi-α-Muurolol	1596	2.13	1.9	1.88	1.03
α-Muurolol	1635	0.97	0.64	0.44	0.52
α-Cadinol	1648	1.28	1.15	1.01	1.08
		40	36	43	43
Identified components (%)			75.96	98.84	91.5

*RI, retention indices relative to C6–C24 n-alkanes on the HP-5 column

Some of the components isolated from the extracts of *P. atlantica* were monoterpenes and sesquiterpenes[12]. Delazar *et al*. reported that a-pinene (70 %), limonene oxide (9 %), myrtenol (5.31 %), and citral (5.72 %) were the main components of *P. atlantica* essential oil from Marivan of Kurdistan province (situated in Iran)[10]. In another study by Barrero *et al*., terpinen-4-ol (21.7 %) and elemol (20 %) were reported to be the major components in *P. atlantica* essential oil[19]. Therefore, the results of essential oil composition in

P. atlantica are different. The changes in the essential oil content compositions might arise from several environmental factors such as climatically, seasonal, geographical parameters and genetic differences[20,21].

Optimization of analysis conditions and SPME method

For the achievement of an appropriate chromatographic separation of the essential oil components from *P. atlantica*, some different conditions were used for the GC column. From obtained chromatograms, it was clear that the best oven temperature program was that mentioned in the experimental.

The optimization of the parameters was accomplished by using the sampling optimization method. This method was of paramount importance in order to select the best working conditions for the interrelated variables[22,23]. The optimal conditions were sample weight, extraction temperature, extraction time and sonication time. Three major peaks (Germacrene D, Myrcene, and Terpinen-4-ol) were considered as the target peaks and their total areas were considered as the response to be optimized. The presence of water in the matrix of samples, as a modifier, improves the release of volatile compounds and subsequently increases their concentrations in the headspaces of the sample[23,24]. PDMS based mixed fibres were used throughout this research. It is a non-polar coating that has been known very effectively for non-polar and semi-polar analysis. Hence, PDMS coating fibres are most commonly used for extraction of the volatile compounds in medicinal plants.

Table 2, summarizes the conditions for the five initial and seven subsequently designed experiments. As shown in Fig. 1, the maximum response was obtained for experiment no.10. Therefore, the optimal SPME extraction conditions were as fibres coating type: PDMS, sonication time 12.10 minutes, extraction time 14.82 minutes, extraction temperature 60 °C and water content 200 µL/1.07 g of ground sample.

Table 2 — The SPME extraction conditions for the five initial and seven subsequently designed experiments.

Experiment no.	Sample weight (g)	Extraction temperature (°C)	Sonicaion time (min)	Extraction time (min)
1	1.00	60.00	10.00	20.00
2	0.50	60.00	10.00	20.00
3	1.00	55.00	10.00	20.00
4	1.00	60.00	10.00	25.00
5	1.00	60.00	15.00	20.00
6 (R1)	1.50	57.40	12.40	22.50
7 (R2)	1.24	56.10	13.60	16.24
8 (R3)	1.36	60.00	15.40	19.36
9 (R4)	1.56	57.80	10.40	19.04
10 (R5)	1.07	60.00	12.10	14.82
11 (R6)	0.98	59.00	15.10	17.63
12 (R7)	1.32	60.00	18.20	15.01

Identification of essential oil constituents by UA-HS-SPME method

The oil composition isolated by UA-HS-SPME from the aerial part (fruits and leaves) is listed in Table 1, in which the percentage and retention index of components are given. A total of 43 components were identified by UA-HS-SPME technique in both of the fruits and the leaves which the major constituents of the fruits: Germacrene D (12.06 %), Myrcene (12.01 %), Terpinen-4-ol (9.03 %), p-Cymene (7.01 %), (E)-Caryophyllene (5.21 %) and Camphene (6.03). The volatile components, such as Germacrene D (10.53 %), Myrcene (10.18 %), Terpinen-4-ol (7.78 %), p-Cymene (5.87 %), (E)-Caryophyllene (4.46 %) and Camphene (5.21 %) were found to be major volatile constituents of the leaves (Table 1). The other composition of essential oil constituents of *P. atlantica* were shown in Table 1. The major components found in the essential oil of *P. atlantica* in two methods were Germacrene D, Myrcene, Terpinen-4-ol.

In another study conducted by Kendirci and Altug[25], the volatile compounds of different varieties of fresh pistachio nuts were extracted by using SPME-GC/MS and it was found that volatiles of fresh pistachio nuts was mainly composed of terpenes like α-pinene, α-terpinolene, limonene, β-myrcene.

Fig. 2 and 3 compare the peak areas of major components of *P. atlantica* which obtained by both extraction methods. The highest percentage of α-Humulene was obtained by HD method whereas the efficiency of a UA-HS-SPME method for extracting Germacrene D, Myrcene, Terpinen-4-ol, and p-Cymene was higher than HD obtained from. Also, the results

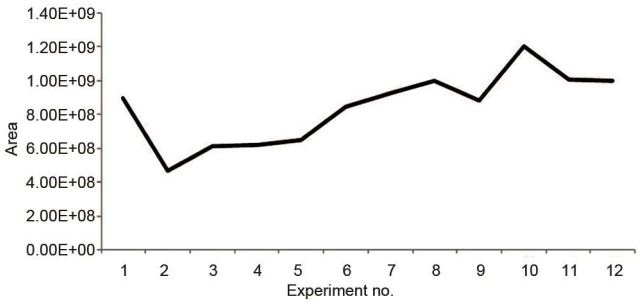

Fig. 1 — The response to the designed experiments using the simplex method mentioned in Table1.

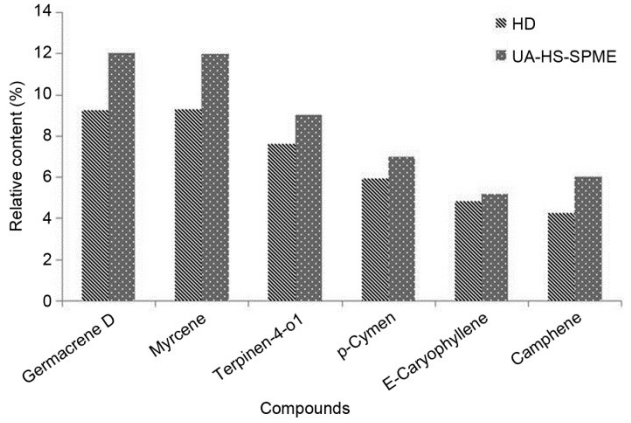

Fig. 2 — Comparison of six major relative content of UA-HS-SPME and HD extraction methods of the fruits of *Pistacia atlantica*.

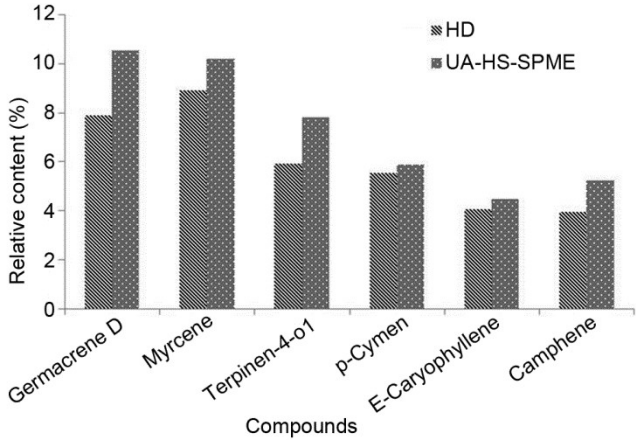

Fig. 3 — Comparison of six major relative content of UA-HS-SPME and HD extraction methods of the leaves of *Pistacia atlantica.*

show that UA-HS-SPME allowed the occurrence of α-Cubebene, α-Ylangene, and α-Farnesene as the main component in *P. atlantica* fruits and the leaves, but it was not detected in the HD method.

Our experience showed that SPME could not give the exact mass percentage of the constituents of volatile compounds in comparison with HD, due to the limited load capacity of micro-scale fibres especially for main components[26]. However, SPME is capable to analyze the volatiles with the least extraction time, sample amount and sample preparation step in addition to, the significant ability to trap and extracting of compounds which are more volatile [27].

Conclusion

According to the findings obtained from the two extraction techniques, the UA-HS-SPME seems to be the powerful technique for the extraction of the volatile compounds of *P. atlantica*. As above mentioned, by this technique the major volatiles which make up *P. atlantica* could be extracted and the percentages from unidentified compound were detected to be high in comparison with the HD technique. The several benefits such as rapidity, facility, as well as exploitation and exploration a large number of components with the lowest content of the sample and using a lower temperature in contrary to HD system can make the described system applicable for valuable volatiles in agriculture and food industries.

References

1 de Rougemont G M, A field guide to the crops of Britain and Europe, Collins, London, 1989, 111- 112.

2 Abrishami, M, "Iranian pistachio, historical recognition". Sahami Compony of Enteshar. Tehran, 1994, 669 (In Persian).

3 Tohidi M, Khayami M, Nejati V and Meftahizade H, Evaluation of antibacterial activity and wound healing of *Pistacia atlantica* and *Pistacia khinjuk, J Med Plants Res*, 2011, **5**(17), 4310-4314.

4 Koutsoudaki C, Krsek M and Rodger A, Chemical composition and antibacterial activity of the essential oil and the gum of *Pistacia lentiscus* Var, chia., *J Agric Food Chem*, 2005, **53**(20), 7681-7685.

5 Gardeli C, Vassiliki P, Athanasios M, Kibouris T and Komaitis M, Essential oil composition of *Pistacia lentiscus* L. and *Myrtus communis* L., Evaluation of antioxidant capacity of methanolic extracts, *Food chem*, 2008, **107**(3), 1120-1130.

6 Palevitch D, Yaniv Z, Dafni A and Friedman J, Medicinal plants of Israel: An ethnobotanical survey, in Craker, L. E. and Simon J. E. (Eds.) *Herbs, spices, and medicinal plants: Recent advances in botany, horticulture and pharmacology* (vol I), (USA: Oryx Press),1986, 281–345.

7 Ljubuncic P, Song H, Cogan U, Azaizeh H and Bomzon A, The effects of aqueous extracts prepared from the leaves of *Pistacia lentiscus* in experimental liver disease, *J ethnopharmacol*, 2005, **100**(1), 198-204.

8 Kordali S, Cakir A, Zengin H and Duru M E, Antifungal activities of the leaves of three Pistacia species grown in Turkey, *Fitoterapia*, **74**(1), 164-167.

9 Castola V, Bighelli A and Casanova J, Intraspecific chemical variability of the essential oil of *Pistacia lentiscus* L. from Corsica, *Biochem Syst Ecol*, 2000, **28**(1), 79-88.

10 Delazar A, Reid, R G and Sarker S D, GC-MS analysis of the essential oil from the oleoresin of *Pistacia atlantica* var. mutica, *Chem Nat Compd*, 2004, **40**(1), 24-27.

11 Derwich E, Manar A, Benziane Z and Boukir A, GC/MS analysis and *in vitro* antibacterial activity of the essential oil isolated from leaf of *Pistacia lentiscus* growing in Morocoo, *World Appl Sci J*, 2010, **8**(10), 1267-1276.

12 Gourine N, Yousfi M, Bombarda I, Nadjemi B and Gaydou E, Seasonal variation of chemical composition and antioxidant activity of essential oil from *Pistacia atlantica* Desf. Leaves, *J Am Oil Chem Soc*, 2010, **87**(2), 157-166.

13 Gabay C, and Kushner I, Acute-phase proteins and other systemic responses to inflammation, *N Engl J Med*, 1999, **340**(6), 448-454.

14 Dib M E A, Djabou N, Desjobert J M, Allali H, Tabti B, *et al.*, Characterization of volatile compounds of *Daucus crinitus* Desf. Headspace Solid phase microextraction as alternative technique to Hydrodistillation, *Chem Cent J*, 2010, **4**(1), 1.

15 Mardarowicz M, Wianowska D, Dawidowicz A L and Sawicki R, Comparison of terpene composition in Engelmann spruce (*Picea engelmannii*) using hydrodistillation, SPME and PLE, *Zeitschrift für Naturforschung C*, 2004, **59**(9-10), 641-648.

16 Bicchi C, Cordero C, Liberto E, Sgorbini B and Rubiolo P, Reliability of fibres in solid- phase microextraction for routine analysis of the headspace of aromatic and medicinal plants, *J Chromatogr A*, 2007, **1152**(1), 138-149.

17 Paolini J, Nasica E, Desjobert J M, Muselli A, Bernardini A F, *et al*., Analysis of volatile constituents isolated by hydrodistillation and headspace solid-phase microextraction from Adenostyles briquetii Gamisans, *Phytochem Anal*, 2008, **19**(3), 266-276.

18 Muselli A, Pau M, Desjobert J M, Foddai M, Usai M, *et al*., Volatile constituents of *Achillea ligustica* All. by HS-SPME/GC/GC-MS. Comparison with essential oils obtained by hydrodistillation from *Corsica* and *Sardinia*, *Chromatographia*, 2009, **69**(5-6), 575-585.

19 Barrero A F, Herrador M M, Arteaga J F, Akssira M, Mellouki F, *et al.*, Chemical composition of the essential oils of *Pistacia atlantica* Desf, *J Essent Oil Res*, 2005, **17**(1), 52-54.

20 Shahriari S, and Tahvilian R, The study of essential oil of *Hymenocrater longiflorus* Benth growing in Paveh, *J Rep Pharm Sci*, 2013 **2**(2), 111-115.

21 Perry N B, Anderson R E, Brennan N J, Douglas M H, Heaney A J, *et al.*, Essential oils from Dalmatian sage (*Salvia officinalis* L.) variations among individuals, plant parts, seasons, and sites, *J Agric Food Chem*, 1999, **47**(5), 2048-2054.

22 Valtiner S M, Bonn G K and Huck C W, Characterisation of different types of hay by solid-phase micro-extraction–gas chromatography mass spectrometry and multivariate data analysis, *Phytochem Anal*, 2008, **19**(4), 359-367.

23 Meshkatalsadat M H, Heravi M R P and Rashidipour F, Chemical characterization of organic components of *Salvia officinalis* using ultrasonic-assited head space solid-phase microextraction methods, *J Univ Chem Technol Metallurgy*, 2009, **44**(3), 291-296.

24 Xu H, Liao Y and Yao J, Development of a novel ultrasound-assisted headspace liquid-phase microextraction and its application to the analysis of chlorophenols in real aqueous samples, *J Chromatogr A*, 2007, **1167**(1), 1-8.

25 Kendirci P and Onoğur T A, Investigation of volatile compounds and characterization of flavor profiles of fresh pistachio nuts (*Pistacia vera* L.), *Int J Food Prop*, 2011, **14**(2), 319-330.

26 Priego-Capote F and De Castro M L, Analytical uses of ultrasound I. Sample preparation, *Trends Analyt Chem*, 2004, **23**(9), 644-653.

27 Ghiasvand A R, Setkova L and Pawliszyn J, Determination of flavour profile in Iranian fragrant rice samples using cold-fibre SPME–GC–TOF–MS, *Flavour Fragr J*, 2007, **22**(5), 377-391.