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IDENTIFICATION OF ESSENTIAL OIL CONSTITUENTS OF CARAWAY (*CARUM CARVI*) USING ULTRASONIC ASSIST WITH HEADSPACE SOLID PHASE MICROEXTRACTION (UA-HS-SPME)

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Ultrasonic assist with headspace solid phase microextraction (UA-HS-SPME) have been applied, for the first time, for the extraction of volatile organic compounds of *Carum carvi* L. from Iran. The oils obtained were analyzed by GC–MS. Different experimental parameters such as fiber's coating type, sonication time, extraction time and temperature, and desorption time were investigated. Results showed that in the presence of UA-HS-SPME the highest extraction efficiency was achieved with a 100 μm polydimethylsiloxane (PDMS) fiber. Essential oil analysis showed that 10 compounds were identified through UA-HS-SPME-GC/MS method .The major component were carvone (57.7 %) and limonone (35.5 %).

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1. Introduction

The genus *Carum* belong to the *Apiaceae* or *Umbelifereae* family includes 25 spices, only Caraway (*Carum carvi* L.) has an economical importance, being used and cultivated in several regions (from Northern Europe to the Mediterranean regions, Russia, Iran, Indonesia and North America). In numerous countries it is a very common species and, as a result, an integral part of their folk medicines. *C. carvi* essential oil has been used as a flavouring for liquors and toothpaste, while the seeds have been used as a spice and flavouring. *C. carvi* essential oils or their pure constituents (such as carvone) showed several biological activities including insecticidal or insect repellent effects, antibacterial and antifungal effects, inhibition of seed germination and inhibition of sprouting of potatoes [1-2]. The percentages of essential oil available in *C. carvi* seeds were reported in different studies between 1% (v/w) and 6% (v/w). Caraway seeds contain a several components. Carvone and limonene are the main components available in their oil. Also, their seeds contain trace amounts of other compounds including acetaldehyde, furfural, carveole, pinene, thujone, camphene, phellandrene, etc [3].

Hydrodistillation (HD), steam distillation, steam and water distillation are the conventional methods to obtain essential oils from the essential oil-bearing plants. Among these methods, HD has been the most common approach to extract the essential oils from the medicinal herbs/plants. All conventional methods to isolate valuable compounds from aromatic plants have important drawbacks, such as low yields, formation of by-products – owing to degradation of thermally unstable and unsaturated compounds by temperature or hydrolytic effects, respectively –

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large extraction times and the presence of the extraction, usually a toxic organic solvent. In the last decade there has been an increasing demand for new extraction techniques, amenable to automation, with shortened extraction times and reduced organic solvent consumption, preventing pollution and reducing sample preparation costs [4].

Solid-phase microextraction (SPME) is a solvent-free sampling and effective technique that introduced in the early 1990s as a new sampling and sample-preparation method. In this method require only small amounts of sample and the aromatic oils from the sample are adsorbed directly onto an adsorbent coated fused-silica fiber and then either thermally desorbed directly into a gas chromatograph (GC) injection port or high performance liquid chromatography (HPLC) injection valve. In addition, SPME has been widely applied to the sampling and analysis of environmental, food, aroma, forensic, and pharmaceutical samples [5]. Headspace SPME (HS-SPME) and ultrasonic-assisted (UA) extraction has been successfully applied to determine volatile compounds from plant materials [6-10].

The aim of this research work was to extract essential oil from *C. carvi* as a famous Iranian essential oil-bearing material by UA-HS-SPME method.

2. Experimental

The *Carum carvi* L. fruits at ripening stage were harvested from plants growing in the gardens of the Agricultural College of Razi University, during May 2009. About 100 g of fruits dried in room temperature by spreading them on a clean aluminum foil in laboratory. 10 g portions of air-dried sample were subjected to a household coffee grinder in order to make them to a coarse powder. The ground samples were stored in nylon bags and placed in refrigerator until use and further analysis.

The SPME fiber, 100 μm polydimethylsiloxane (PDMS) was purchased from Supelco (Bellefonte, PA, USA) and conditioned prior to use according to suppliers prescriptions. Helium, 99.999%, used as carrier gas, was purchased from Roham Gas Company (Tehran, Iran). The alkane mixture consisting of the C8-C26 alkanes (concentration of 40 mg/mL in hexane) was purchased from Fluka. All other chemicals were of the highest purity available from Merck or Fluka. Doubly distilled deionized water was used.

The aerial parts of the plant were dried at room temperature by spreading them on clean aluminum foil in the laboratory. 10-g portions of air-dried sample were ground to a coarse powder using a household coffee grinder. The ground samples were stored in nylon bags and placed in a refrigerator prior to analysis. Extraction of the volatiles from the plant sample using SPME fibers was achieved by placing 0.5 g of ground sample into a 40-ml vial to which 500 μl double-distilled water was added as a matrix modifier, the vial was then vigorously shaken by hand to ensure homogeneous dispersal of the spiked water. The sample vial was then placed into an ultra-sonicator and incubated for 15 min to allow the volatiles to equilibrate between the headspace and sample matrix, during which time the sample was heated to 70 ºC. The actual SPME extraction of volatile compounds was accomplished by incubation with a polydimethylsiloxane (PDMS) fiber at 70 ºC for 40 min. Ultrasonic irradiation (18 kHz, 450 W) was applied by means of a PFO100 5RS Series ultrasonicator (Italy) equipped with a water bath in which the extraction vials were placed. The samples were sonicated to create stress in the sample matrix to facilitate the release of the analytes, and control the temperature during the extraction process. The alkane mixture (C_8-C_{26}), 40 mg/mL in hexane) was used for the calculation of retention indices (RIs). Loading the alkane mixture onto the fiber was carried out using a 5-min head space extraction from the 10-ml SPME vial including 1 ml double-distilled water spiked with 10 μl of the above-mentioned mixture.

Gas chromatography was performed with a Shimadzu model GC-17A (Kyoto, Japan) instrument equipped with a Shimadzu Quadropole-MS (QMS) model QP5050 detector. Separation was achieved using a 30 m × 0.25 mm I.D capillary fused silica column 6 coated with a 0.25 μm film of DB5-MS (5% Phenyl-95% Polydimethyl Siloxane), and a split/splitless injector with a 1 mm internal diameter glass liner. Ten-, 20- and 40-milliliter sample vials sealed with PTFE coated silicone septa (Supelco) were used for extraction. SPME fibers with PDMS (100 μm, non-bonded) coating, were used as commercial fibers for the extraction of analyses. The fibers were handled using a manual SPME fiber holder provided by Supelco (Bellefonte, PA, USA). Analyses

extracted onto the fiber were injected into the injection port of the GC system. The fiber was kept in the injection port for additional 2 min after injection to ensure the complete adsorption of the compounds from the fiber. Every 10 analyses on a GC run were carried out in the presence of the fiber but without sampling to assure complete adsorption. The injector was set at 220 ºC. The carrier gas was helium and flowed at a rate of 1 ml/min. The GC was operated in a splitless mode. The column temperature was initially set at 40 ºC and increased to 200 ºC at a rate of 4 ºC/min, and remain at 200 ºC for 1 min, resulting in a total GC run time of 45 min. The temperature of the ion source was kept at 220 ºC, and the transfer line temperature at 250 ºC. The mass fragments were collected in the range from m/z 40 to 450 with an acquisition rate of 1000 to provide a satisfactory number of points per peak for effective spectral resolution. The ionization energy of 70 eV and the detector voltage of 1700 V were applied to the QMS detector.

Most constituents were identified by gas chromatography through comparison of their retention indices (RIs) with those of the literature [11] or with those of authentic compounds available in our laboratories. The retention indices (RIs) were determined in relation to a homologous series of n-alkanes (C_8–C_{26}) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST 98 and Wiley 5 Libraries or with mass spectra from literature [11-13]. Component relative concentrations were calculated based on GC peak areas without using correction factors.

3. Results and discussion

The chemical composition of the oil of the *C. carvi* is listed in Table 1, in which the percentage and retention indices of components are given. The components were listed in order of their elution on the DB-5 column.

Table 1. Chemical composition of C. carvi oil extracted by UA-HS-SPME.

No.	RT (min) [a]	RI_{cal} [b]	Compounds	Relative content (%)
1	14.05	1000	Myrcene	0.48
2	16.29	1055	Limonene	35.5
3	18.68	1112	α-Terpinolene	0.1
4	20.40	1157	Trans Limonene Oxide	2.01
5	23.26	1231	Trans Dihydro Carvone	0.33
6	24.59	1266	Trans Carveol	0.31
7	25.68	1295	Carvone	57.7
8	26.31	1312	Perilla Alcohol	1.48
9	26.74	1324	Carvacrol	0.34
10	31.04	1447	β-Caryophyllene	0.41
Total content fraction of determinates compounds				98.66

[a] RT: retention time [b] RI_{cal}: calculated retention indices using kovats formula

Ten components were identified in the essential oil of *C. carvi* extracted using UA-HS-SPME method, represented 98.66% f the total components, the major compounds were carvone (57.7%) and limonene (35.5%). These results were accordance to previous studies that reported carvone and limonene were the main components of C. *carvi* oil [3, 4, 15]. Also, the essential oil of C. *carvi* was characterized by high contents of oxygenated monoterpenes (62.17%), monoterpenes (36.08%) and sesquiterpenes (0.41%), respectively (Figure 1). The fraction of oxygenated monoterpenes and monoterpene were enriched, mainly due to an increase in carvone and limonene percentage.

In order to provide a complete peak separation of extracted compounds, some preliminary SPME-GC/MS experiments were performed using ground *C. carvi* sample utilizing a PDMS fiber. From different recorded chromatograms, it becomes clear that the best GC program was as which mentioned previously (the column initial temperature: 40 ºC, with a rate of 4 ºC/min increased to 200 ºC, and remain at 200 ºC for 1 min). The affecting experimental parameters such as fiber's coating type, sonication time, extraction time and temperature, desorption time, and water content

of sample were optimized. The optimization of different affecting parameters was accomplished by using simplex method. The optimal conditions were as fiber's coating type: PDMS, sonication time: 15 min, extraction time: 40 min, extraction temperature: 70 ºC, adsorption time: 2 min, and water content: 500 µl per 0.5 g of ground sample. The use of a simplex optimization method was of paramount importance in order to select the best working conditions of interrelated variables.

A number of SPME fibers of different polarity and coating thickness are commercially available and have been used for extraction of the volatile compounds in medicinal plants [13]. Among the fibers, PDMS or PDMS-based mixed fibers are most commonly used. Using ultrasonic assist with headspace solid phase microextraction, the highest extraction efficiency was achieved with a 100 µm polydimethylsiloxane (PDMS) fiber and it was found that the oxygenated monoterpene fractions increased but the amount of sesquiterpenes and monoterpenes decreased.

In order to get access to the absolute mass percentage of the identified compounds, the essential oil of *C. carvi* was analyzed after extraction by SPME. Our experience showed that SPME could give the exact mass percentage of the constituents of volatile compounds in comparison with reported hydrodistillation method [3, 4, 15].

In conclusion, the study of the essential oil from *C. carvi* seeds showed the presence of carvone and limonene after extraction by SPME. Thus, the experimental parameters such as extraction time, irradiation power and ultra sound effects can be optimized for the particular aim of the SPME, either to obtain a high yield of essential oil, or to obtain essential oils of differing composition [4]. However, SPME is capable to analyze the volatiles with the least extraction time, sample amount, and sample preparation steps. In addition, significant ability of trapping and extracting of compounds which are more volatile.

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