

## Aroma components of Iranian dried *Heracleum persicum* fruit (golpar) using solvent-assisted flavour evaporation technique

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### Summary

Aroma composition obtained from Iranian dried *Heracleum persicum* Desf. ex Fisch. was analysed by the gas chromatography-mass spectrometry (GC-MS) technique. For the first time in this plant aroma, the solvent-assisted flavour evaporation (SAFE) extraction method with dichloromethane was used prior to GC-MS. A total of 26 aroma compounds comprising esters, terpenes, acids, alcohol and aldehyde were identified and quantified in dried *H. persicum* fruit. Among the detected compounds, esters were present on highest levels, followed by terpenes. Hexyl butyrate was quantitatively the main aroma compound in this fruit, representing 65.6% of the total aroma compounds analysed, followed by octyl acetate (18.2%), hexyl isobutanoate (5.6%),  $\gamma$ -terpinene (1.6%) and *o*-cymene (1.3%).

### Keywords

*Heracleum persicum*; fruits; aroma; gas chromatography-mass spectrometry

The genus *Heracleum* L., with almost 125 species in the world and predominantly distributed in Asia, is one of the widespread members of Umbelliferae (Apiaceae) family [1]. This genus is represented by eight perennial aromatic species (*H. persicum* Desf. ex Fisch., *H. gorganicum* Rech.f., *H. rechingeri* Manden., *H. anisactis* Boiss. & Hohen., *H. pastinacifolium* K.Koch, *H. rawianum* C.C.Towns., *H. transcaucasicum* Manden., and *H. antasiaticum* Manden.) in the flora of Iran, three of which (*H. rechingeri*, *H. gorganicum* and *H. anisactis*) are vernacular to Iran [2]. The aromatic fruits of some *Heracleum* species, especially those of *H. persicum* Desf. Ex Fisher (syn. *H. pubescens* Rech. and *H. glabrescens* Boiss. & Hohen.) known as Persian hogweed or “golpar” [3], which is native to humid mountainous regions of Iran, Iraq and Turkey [4], are extensively used in the daily diet of the Iranian general population as flavouring agents and spices to enhance the flavour of food and making pickles in many parts of the country

[5]. In addition, its fruits are also used as carminative, antimicrobial tonic, digestive, aphrodisiac, anti-inflammatory, antioxidant and anticonvulsant herbal drug in Iranian folk medicine [4, 5].

Aroma is a complex mixture of a large number of low molecular weight volatile compounds, whose composition is specific to the species and often to the variety of fruit [6]. The plants belonging to Umbelliferae (Apiaceae) family are aromatic and have a distinctive flavour with various volatile compounds present in fruits and leaves [7]. Aromatic spices are the dried fruits of plants. Most spices are usually sold dried, due to the high water content in the fresh state, which causes severe deterioration by microbial growth and biochemical reactions. Water removal by dehydration microbiologically stabilizes spices by lowering the water activity ( $a_w$ ) values below the threshold for microbial growth (0.6) [8]. Some volatile compounds evaporate during air-drying, whereas others are partially retained, and some oxidation

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products appear during drying. When spices are used as marinades or as seasonings for foods, the dried vegetal product is directly added. The volatile oil composition of *H. persicum* fruits has been widely studied [4, 9–12]. Aliphatic esters constituted the main fraction of the oils (86.6–94.3%), with octyl acetate and hexyl butyrate as the major compounds [12]. Apart from the aliphatic esters, oil samples contained rather low amounts of monoterpene hydrocarbons (0.3–6.1%), oxygenated monoterpenes (0.1–6.7%) and carbonyls (0.5–4.9%). Scarce literature data are available on volatile profile compounds analysed directly in the spices [13]. Steam distillation (Clevenger apparatus) and organic solvent extraction (Soxhlet extractor) have both been widely used to extract volatile compounds from spices. However, these two extraction methods both tend to degrade the original spice flavour and lose volatile compounds due to the high temperatures used. In other words, most of the flavour compounds in spices are contained in their volatile oils and are changed or damaged upon heating or air oxidation [14]. Moreover, new flavours can be developed from non-volatile precursors by Maillard reaction, carotenoid degradation and/or lipid oxidation [15]. Thus, the first step in the study of aroma is the selection of an extraction technique for the volatile compounds, which provides an aromatic extract with an odour that is representative of the original product.

Under these circumstances, this research deals with aroma profile of air-dried fruits of *H. persicum* from Iran. In the present study, the aroma extraction method selected was the solvent-assisted flavour extraction method (SAFE). This method, due to low pressure used, extracts volatiles at low temperatures such as 40 °C, which prevents the formation of artifacts, and has already shown its reliability for the extraction of volatile compounds in Iranian saffron spice [16], orange juice [17] and coffee [18]. Gas chromatography (GC) coupled to flame ionization detection (FID) and mass spectrometry (MS) was used for quantification and identification of volatile compounds, respectively.

## MATERIALS AND METHODS

### Golpar sample and chemicals

The flat-oval shaped fruits of golpar were collected in August and September 2015 from northern region of Iran. The collected specimens were air-dried in the shade at room temperature (25 °C) for two weeks and protected from light for later analysis. The water used in the study was

purified by a Millipore-Q system (Millipore Billerica, Massachusetts, USA). Dichloromethane and sodium sulfate were obtained from Merck (Darmstadt, Germany). Dichloromethane was freshly distilled prior to use. Standard aroma compounds were obtained from Sigma Aldrich (St. Louis, Missouri, USA).

### Extraction of aroma compounds

The extraction of aroma compounds was performed in dichloromethane, which is an efficient solvent for the isolation of volatile compounds in fruits and plants [19]. The volatiles present in *H. persicum* were extracted using SAFE unit (Gläsbilerei Bahr, Manching, Germany) under vacuum ( $10^{-3}$  Pa; Vacuubrand DCP 3000, Wertheim, Germany). The extraction method was successfully applied in our previous research in Iranian saffron spice [16]. Briefly, the dried fruits of Iranian golpar sample were powdered to a uniform blend using porcelain mortar at room temperature. Before extraction, 3 g of powdered sample containing 100 ml dichloromethane was put into a 500-ml flask. The content was stirred at 4 °C for 30 min under nitrogen gas and then centrifuged at 4 °C for 15 min. The organic phase (solvent) was slowly fed into upper portion of the transfer head. Separation of the mixture occurred when content of the sample was dropped into the round bottom flask that was partially submerged in a warm (40 °C) water bath. Separated volatiles passed through the separation head into a receiving vessel, where they condensed and frozen because of the sudden drop in temperature. Once the separation was complete, the receiving vessel was removed and allowed to thaw at room temperature for 30 min [20]. After dehydration by anhydrous sodium sulfate, the pooled aromatic extract was reduced to 5 ml in a Kuderna Danish concentrator (Sigma Aldrich) fitted with a Snyder column (Supelco; Bellefonte, Pennsylvania, USA) and then to 200  $\mu$ l under a gentle stream of purified nitrogen. The extracts were subsequently stored at –20 °C in a 2 ml glass vial equipped with Teflon-lined cap until the analysis. Each sample was extracted in triplicate and the percentage of aroma compounds was calculated as means.

### GC-FID and GC-MS analyses of aroma compounds

The GC system consisted of an Agilent 6890 chromatograph (Agilent Technologies; Santa Clara, California, USA) equipped with a FID detector (Agilent) and an Agilent 5973-Network mass selective detector (MSD; Agilent). This system allowed us to simultaneously obtain an FID signal for quantification and an MS signal for

identification. GC effluent was split 1:1 between FID and MSD. Aroma compounds were separated on a DB-Wax column (30 m length  $\times$  0.25 mm i.d.  $\times$  0.5  $\mu$ m thickness; J&W Scientific, Folsom, California, USA). A volume of 2  $\mu$ l of the extract was injected in pulsed splitless mode (276 kPa; 0.5 min). This mode was chosen to minimize artifact formation by thermal degradation of analytes in the injection port. Injector and FID were set at 270 °C and 280 °C, respectively. The flow rate of carrier gas (helium) was 1.5 ml·min<sup>-1</sup>. The temperature of the column was first increased from 50 °C to 200 °C at a rate of 5 °C·min<sup>-1</sup> and then to 260 °C at 8 °C·min<sup>-1</sup> with a final hold at 260 °C for 5 min. The same oven temperature programs were used for MSD. The MS (electron impact ionization) conditions were as follows: ionization energy of 70 eV, mass range  $m/z$  of 30–300 Da, scan rate of 2.0 s<sup>-1</sup>, interface temperature of 250 °C, and source temperature of 180 °C.

The volatile compounds were identified by comparing their retention indices and mass spectra on the DB-Wax column with those of a commercial spectra databases (Wiley 6, Hoboken, New Jersey, USA and NBS 75k, Washington, District of Colombia, USA) and of the instrument's internal library created from previous laboratory studies [21]. Some of the identifications were confirmed by injection of chemical standards into the GC-MS system. Retention indices of the compounds were calculated by using an *n*-alkane series [22].

## RESULTS AND DISCUSSION

The aroma compounds and linear retention index values on DB-Wax column for these compounds are presented in Tab. 1. Isolation of aroma compounds by SAFE method resulted in an extract, which represented the characteristic aroma of the golpar, when a droplet of the aroma extract was vaporized on a strip of smelling paper. Mean values (percent) of the GC analyses of triplicate extractions and standard deviations are reported. As can be seen in Tab. 1, a variety of aroma compounds was found in the golpar. A total of 26 aroma compounds were identified, most of which were identified by previous studies in volatile oils of different *Heracleum* species and geographical origins [5, 12]. The aroma compounds of golpar included esters (10), terpenes (11), acids (3), alcohol (1) and aldehyde (1). Regarding the metabolic origin of these compounds, fatty acids are major precursors of aroma volatiles in most fruits [6]. Fatty acid-derived straight chain alcohols,

aldehydes, acids and esters ranging from C1 to C20 are important character-impact aroma compounds that are responsible for fresh fruit flavours with high concentrations, and are basically formed by three processes:  $\alpha$ -oxidation,  $\beta$ -oxidation and the lipoxygenase pathway [23].

Of all aroma compounds detected in golpar, esters were quantitatively the most abundant aroma compounds, accounting for 92.4% of total aroma compounds identified in sample, followed by terpenes (4.7%), acids (1.8%), alcohol (0.9%) and aldehyde (0.1%) (Tab. 1). Esters are important aroma compounds of many fruits. Hexyl butyrate was found a quantitatively main aroma compound in golpar, representing 65.6% of the total aroma compounds analysed, followed by octyl acetate (18.2%). These two compounds are also found as major aroma compounds in most *Heracleum* species and in different parts of this plant [3, 4, 9, 11, 24].

### Esters

Esters are important aroma compounds as they are responsible for both the mature flavour characteristic and fruity aromas [25]. Ten esters were identified and quantified in golpar fruit extracts and represented the main group of the aroma fraction compared to other aroma compounds, because they accounted for the largest proportion (92.4%) of the total aroma compounds. Although fruit aroma is generally a complex mixture of a wide range of volatile compounds, esters often have the major contribution. Hexyl butyrate was quantitatively (65.6%) the main ester in golpar, followed by octyl acetate (18.2%), hexyl isobutyrate (5.6%), butyl butyrate (0.9%), hexyl isovalerate (0.8%) and butyl isobutyrate (0.6%). The obtained results showed lot of similarities, but also slight differences in comparison with corresponding *H. persicum* fruit volatile oils from other different geographic regions by previous studies [4, 9]. Similarly, hexyl butyrate, along with octyl acetate, hexyl isobutyrate and hexyl 2-methyl butanoate/butanoic acid, 2-methyl-, hexyl ester were identified as potent aroma compounds among esters detected in volatile oils from *H. persicum* fruits [4, 9] and also in the majority of *Heracleum* species [26]. The main volatile substances in the most of volatile oils from *Heracleum* species are aliphatic esters such as octyl acetate and hexyl butyrate, hexyl-2-methylbutanoate and hexyl isobutyrate. In another study, HAJHASHEMI et al. [24] reported that hexyl butyrate (56.5%), octyl acetate (16.5%), hexyl 2-methylbutanoate (5.2%) and hexyl isobutyrate (3.4%) were identified as the major compounds of *H. persicum* fruit volatile oil. [24]. SANZ et al. re-

**Tab. 1.** Aroma compounds of dried Iranian *Heracleum persicum* Desf. ex Fisch. fruit.

Compounds		Linear retention index		Relative percentage [%]	Method of identification
		<i>LRI</i> <sub>Cal</sub>	<i>LRI</i> <sub>Ref</sub>		
Esters					
1	2-Methylpropyl acetate	1023	1013 [35]	0.1 ± 0.01	LRI, MS, Std
2	Butyl isobutyrate	1138	1144 [35]	0.6 ± 0.05	LRI, MS, Std
3	Isobutyl 2-methylbutyrate	1181	1167 [36]	0.1 ± 0.01	LRI, MS-tent
4	Butyl butyrate	1217	1209 [35]	0.9 ± 0.08	LRI, MS, Std
5	Butyl 2-methylbutanoate	1236	1240 [37]	0.5 ± 0.06	LRI, MS-tent
6	Hexyl isobutyrate	1325	1323 [35]	5.6 ± 0.64	LRI, MS, Std
7	Hexyl butyrate	1411	1424 [38]	65.6 ± 2.44	LRI, MS, Std
8	Hexyl isovalerate	1447	1457 [38]	0.8 ± 0.01	LRI, MS, Std
9	Octyl acetate	1465	1483 [38]	18.2 ± 0.45	LRI, MS, Std
10	Hexyl pentanoate	1482	1484 [36]	traces	LRI, MS, Std
	Total			92.4	
Terpenes					
11	α-Pinene	1012	1032 [39]	0.1 ± 0.01	LRI, MS, Std
12	α-Thujene	1017	1035 [39]	0.1 ± 0.00	LRI, MS-tent
13	β-Pinene	1106	1118 [39]	0.2 ± 0.02	LRI, MS, Std
14	Sabinene	1119	1132 [38]	0.1 ± 0.01	LRI, MS, Std
15	γ-Terpinene	1246	1255 [39]	1.6 ± 0.12	LRI, MS, Std
16	p-Cymene	1261	1280 [39]	0.4 ± 0.03	LRI, MS, Std
17	o-Cymene	1267	1187 [39]	1.3 ± 0.17	LRI, MS, Std
18	Linalool	1544	1553 [39]	0.5 ± 0.02	LRI, MS, Std
19	(E)-Anethole	1829	1845 [38]	traces	LRI, MS, Std
20	Thymol	2045	2198 [39]	0.4 ± 0.05	LRI, MS, Std
21	Carvacrol	2224	2239 [39]	traces	LRI, MS, Std
	Total			4.7	
Acids					
22	Butanoic acid	1623	1614 [40]	0.9 ± 0.08	LRI, MS, Std
23	Hexanoic acid	1842	1838 [40]	0.8 ± 0.07	LRI, MS, Std
24	Octanoic acid	2051	2060 [40]	0.1 ± 0.01	LRI, MS, Std
	Total			1.8	
Alcohol					
25	Octanol	1554	1562 [38]	0.9 ± 0.01	LRI, MS, Std
Aldehyde					
26	Octanal	1286	1296 [38]	0.1 ± 0.01	LRI, MS, Std

Results are the means ± standard deviation of relative percentages of the compounds from flame ionization detector peak area. *LRI*<sub>Cal</sub> – linear retention index calculated on DB-Wax capillary column; *LRI*<sub>Ref</sub> – literature linear retention index on the same column or equivalent stationary phase, MS – mass spectrometry, MS-tent – tentatively identified by MS, Std – chemical standard; When only MS or *LRI* is available for the identification of a compound, it must be considered as an attempt of identification.

ported that β-oxidation of fatty acids is the primary biosynthetic process providing alcohols and acyl coenzyme A (CoA) for ester formation [6]. Fatty acid acyl-CoA derivatives are converted to shorter-chain acyl-CoAs by losing two carbon units in every round of the β-oxidation cycle, requiring flavinadenine dinucleotide, nicotinamide adenine dinucleotide, and free coenzyme A. Acyl CoAs are

reduced by acyl CoA reductase to aldehyde that, in turn, is reduced by alcohol dehydrogenase to alcohol for use by alcohol acyl transferase to produce esters [27].

#### Terpenes

Terpenes quantitatively represent the second main group of aroma compounds in golpar fruits,

accounting for 4.7% of the total aroma compounds identified in the sample. Many of the terpene volatile compounds are direct products of terpene synthases, while others are formed through alterations of the primary terpene skeletons made by terpene synthases by hydroxylation, dehydrogenation, acylation and other reactions [28].  $\alpha$ -Pinene together with  $\alpha$ -thujene,  $\beta$ -pinene, sabinene,  $\gamma$ -terpinene, *p*-cymene, *o*-cymene, linalool, (*E*)-anethole, thymol and carvacrol were detected in this study as the terpene compounds. Most of these were previously identified in different parts (seeds, roots, stems and fruits) of golpar in previous studies [4, 10–12]. Generally, volatile oils have mainly the cytotoxic activity because of the presence of thymol, carvacrol,  $\alpha$ -pinene, *p*-cymene, (*E*)-anethole and linalool compounds [29–31]. Among the higher terpenes,  $\gamma$ -terpinene (1.6%) and *o*-cymene (1.3%) were found as the highest proportions in the aromatic extract, followed by linalool (0.5%) and *p*-cymene (0.4%). The proportion of  $\gamma$ -terpinene shown in Tab. 1 is in the range of the previous studies (0.1–3.9% [4] and 0.1–2.3% [32]), and much higher than the proportions of 0.3% and 0.8% found in seeds [3] and fruits [12] of *H. persicum*, respectively. *o*-Cymene (1.3%), the second main compound in terpene group, demonstrated a similar proportion (1.1%) as in *H. persicum* seed [3]. The proportion of linalool, another major compound in terpene group (Tab. 1), was much lower than the proportion reported in *H. persicum* fruit volatile oils (1.7%) [9], and much higher than the proportion in *H. persicum* root volatile oils (0.2%) [10]. It is clear that synthesis of linalool in vegetative and floral parts is common in plants and can have a key role whether as a part of the defence mechanism, such as in maize and soybean, or as an aroma compound that exists in numerous flowers and fruits [33]. *p*-Cymene was also determined in leaves and in the aerial part [34] of *H. persicum*. *p*-Cymene (0.1–4.8%),  $\gamma$ -terpinene (0.1–3.9%) and linalool (0.1–3.5%) were also detected in all oil samples or plants [4]. Based on the previous study [11], there are differences in chemical composition of oil from diverse parts of this plant.

In trace amounts, three acids (butanoic acid, hexanoic acid and octanoic acid) were also identified and quantified in our samples for the first time. Octanol was only detected as an alcohol in our study. Its percentage (0.9%) was higher than that reported in root (0.2%) [10], seed (0.4%) [3], fruit (0.5%) [33] and fruit with stem (0.7%) [9] volatile oils of *H. persicum*, and lower than the percentage in root (2.4%) [11] and fruit (1.4%) [24]. Octanal as an aldehyde was determined in

the sample and its proportion (0.1%) was lower than the proportion found in seed (0.2%) [3], root (1%) [10] and fruit (0.5%) [34] volatile oil.

## CONCLUSION

The present study was designed to determine the aroma compounds in dried fruits of Iranian golpar (*H. persicum* Desf. ex Fisch.). A total of 26 aroma compounds including esters, terpenes, acids, alcohol and aldehyde were identified and quantified in this fruit using GC-MS and GC-FID. The SAFE extraction method gave highly representative aromatic extract of the studied sample. Esters were determined at highest levels among the identified compounds, followed by terpenes. Within these, hexyl butyrate (65.6%) was quantitatively a major aroma compound in golpar, followed by octyl acetate (18.2%) and hexyl isobutanoate (5.6%).

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