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THE EFFECT OF HYDRODISTILLATION TECHNIQUE ON THE YIELD AND COMPOSITION OF ESSENTIAL OIL FROM THE SEED OF *Petroselinum crispum* (Mill.) Nym. ex. A.W. Hill

The essential oil was isolated from the seed of *Petroselinum crispum* (Mill.) Nym. ex. A.W. Hill by using different techniques of Clevenger-type hydrodistillation. The highest yield of oil, after five consecutive hydrodistillation runs (3.9 mL/100 g of plant material), was obtained by the technique in which water from the still flask was separated by filtration and used together with fresh water for immersing the plant material in a subsequent distillation. Regardless of the technique used, the oil contained different amounts of α -pinene, β -pinene, limonene, 2,3,4,5-tetramethoxy-1-allylbenzene, apiole and 1,2-benzenedicarboxylic acid.

Parsley (*Petroselinum crispum* (Mill.) Nym. ex. A.W. Hill) is a biennial herb of the genus *Petroselinum* of the family *Apiaceae* (*Umbelliferae*). In the first season the root and leaves are formed, while it flowers and brings seed in the second [1]. It grows on all types of soil. It is used in nutrition as a good source of vitamin A, thiamin, riboflavin, niacin and vitamin B₆ [2]. It is also well known as a medicinal herb [3,4] with antimicrobial, hypotensive [5], diuretic [6,7], laxative [8], spasmolytic [9], tonic and bactericidal effects [5,6].

The essential oil is contained in all parts of the herb and gives parsley its scent and flavor [10–13]. The highest content of oil is in the seed, 3–7%, while the leaves contain only 0.16–0.3% and the root 0.1% [11,12]. The main components of parsley seed essential oil are apiole, myristicin, safrole and 2,3,4,5-tetramethoxy-1-allylbenzene [14–16] (Figure 1). The seed also contain fatty oil up to 20%, with a petroselinic acid content of more than 75% of all the fatty acids and a protein content of up to 14% [17–19].

Aqueous, aqueous-vapour and vapour distillation can be used to separate the oil from the plant material [14,15]. The yield, taste and flavour of the oil depend on the technique used, i.e. on the hydrodistillation conditions [16,20]. The influence of different hydrodistillation techniques on the hydrodistillation kinetics, yield and composition of the parsley seed essential oil was investigated in this paper with aim to chose the optimal technique for obtaining the maximal oil yield.

EXPERIMENTAL

Plant material. The parsley (*Petroselinum crispum* (Mill.) Nym. ex. a.W. Hill) seed (*Petroselini fructus*) was

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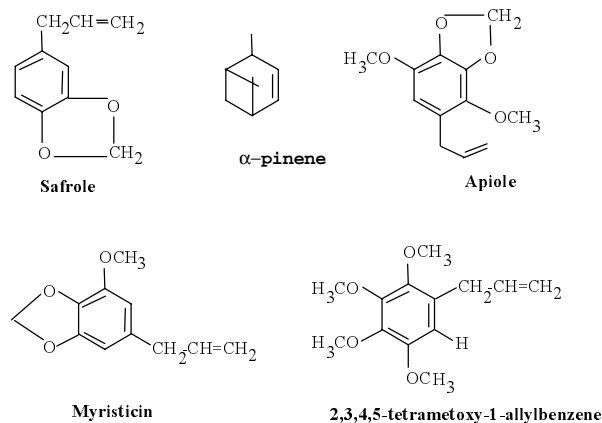


Figure 1. The main components of parsley seed essential oil

obtained from the "Dr. Josif Pančić" Institute for Medicinal Plant Research.

Essential oil content in the plant material. The initial oil content in the parsley seed was determined by the method presented in Ph. Jug. V [21].

Hydrodistillation

Technique I. Clevenger-type hydrodistillation (cohobation). The plant material was immersed in 2000 mL of water in the still flask, and the oil was isolated using a Clevenger-type apparatus. The distillation was stopped after 270 minutes. The oil was collected in the phase separation funnel, extracted with diethyl ether and dried over anhydrous sodium sulfate. The average oil yield was calculated from a series of five consecutive runs.

Technique II. The same as technique I, only the condensate water was not cohobated, it was saved and combined with fresh water to a volume of 2000 mL for immersing a new amount of parsley seed in the subsequent distillation.

Technique III. The same as technique I, only the water from the still flask (residue still water) after distillation was separated under vacuum using a

Buchner funnel and used together with fresh water (the residue still water and fresh water volume was 2000 mL) for immersing a new amount of parsley seed in the subsequent distillation.

Technique IV. The same as technique I, only the condensate and residue still water from the previous distillation were combined and used together with fresh water (the condensate water and residue still water and fresh water volume was 2000 mL) for immersing a new amount of parsley seed in the subsequent distillation.

Gas chromatography. To analyze the composition of the oil, an HP 5890 SERIES II (Hewlett Packard, USA) with A FID integrator (Integrator 3396 A, Hewlett Packard, USA) and Carbowax 20 M column (25 m x 0.2 x 0.2 μm) with hydrogen as carrier gas, were used. The column temperature was programmed at 60°C – 0.5 min, 100°C – 2 min, 15°C/min, 190°C – 5 min. The injector volume was 1 μL , the injector temperature 250°C and the detector temperature 280°C.

Determination of the refractive index. An Abbe refractometer AR3D (Krüss Optronic, Germany) was used to measure the refractive index.

Estimation of the mixing properties in ethanol. The oil, 1 mL, was added to a measuring cylinder and conditioned at 20 \pm 0.2°C. Ethanol, 80 vol. %, conditioned at 20 \pm 0.2°C, was added to the sample in 0.1 mL portions by a burette. Ethanol was added until a total of 20 mL was reached, mixing after each addition. If the mixture became opaque or opalescent before the total quantity was added, the volume of ethanol used was recorded.

RESULTS AND DISCUSSION

The initial oil content in the parsley seed was 4.9 mL/100 g plant material.

The maximum oil yields for different hydrodistillation techniques, for five hydrodistillation runs and hydrodistillation times are given in Table 1. Figure 2 shows the kinetics of oil hydrodistillation from the parsley seed by the four hydrodistillation techniques used, in the fifth hydrodistillation run, when the maximum yield was obtained.

The results of the oil hydrodistillation kinetics investigations (Figure 2) show that the oil yield depended on the hydrodistillation technique used. The highest oil yield of 3.9 mL/100 g (79.6% compared to the initial oil content in the plant material) was obtained in

Table 1. The effect of hydrodistillation technique on the essential oil yield from parsley seed in five consecutive hydrodistillation runs

	Time (min)	Run 1	Run 2	Run 3	Run 4	Run 5	Average yield
Technique I	270	2.20	2.00	2.20	2.15	2.30	2.17
Technique II	180	0.40	0.50	0.65	1.15	1.65	0.87
Technique III	270	2.20	2.70	3.20	3.60	3.90	3.12
Technique IV	180	0.50	0.90	1.25	1.60	1.95	1.24

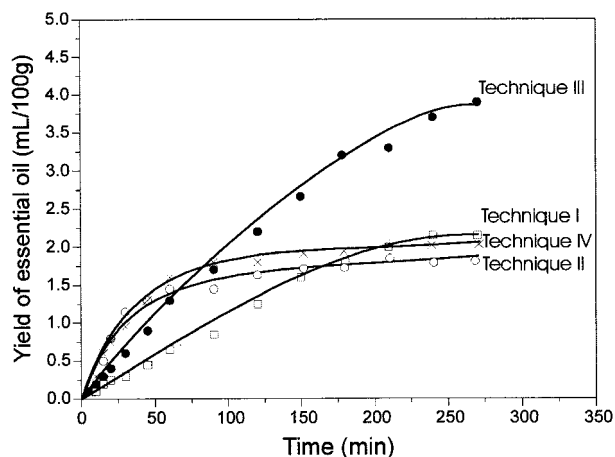


Figure 2. The kinetics of hydrodistillation of parsley seed essential oil in five consecutive hydrodistillation runs by different techniques

the fifth hydrodistillation run by technique III for 270 minutes of hydrodistillation.

By techniques I, II, III and IV, the average yield obtained from five hydrodistillation runs was 2.17, 0.87, 3.12 and 1.24 mL/100 g of plant material (44.3, 17.7, 63.7 and 25.3% compared to the initial oil content in the plant material), respectively (Table 1).

In five consecutive hydrodistillation runs by techniques II, III and IV the oil yield increased with increasing number of hydrodistillation runs. In technique II the increase of the oil yield was 0.10, 0.15, 0.40 and 0.50 mL/100 g of parsley seed in the subsequent distillation (an average of 0.287 mL/100 g of parsley seed). In technique III the increase of the oil yield was 0.50, 0.50, 0.40 and 0.30 mL/100 g of parsley seed in a subsequent distillation (average of 0.325 mL/100 g of parsley seed) and in technique IV it was 0.40, 0.35, 0.35 and 0.35 mL/100 g of parsley seed in the subsequent distillation (an average of 0.362 mL/100 g of parsley seed). According to these results, the total oil that can be obtained from the parsley seed, which is composed of three quantities is: the amount of oil recorded in the Clevengers graduated column at the end of the hydrodistillation by technique I (44.3% of the initial oil content in the plant material), the amount of oil retained in the condensate water (by technique II, average 5.86% of the initial oil content in the plant material per hydrodistillation run) and the oil retained in the water from the still flask (by technique III, average 6.63% of the initial oil content in the plant material per hydrodistillation run). By technique III 0.95 mL/100 g of parsley seed (about 20% of the initial oil content in the plant material) more were achieved than by technique I and this increase is due to the use of water from the still flask.

The variation of the average hydrodistillation velocity by different hydrodistillation techniques and time is shown (Figure 3). The average hydrodistillation velocity was calculated as $v = V_u/t$ where, V_u is the wehn oil volume for five hydrodistillation runs (mL) and t is the time of hydrodistillation (min). In the first 25 minutes the

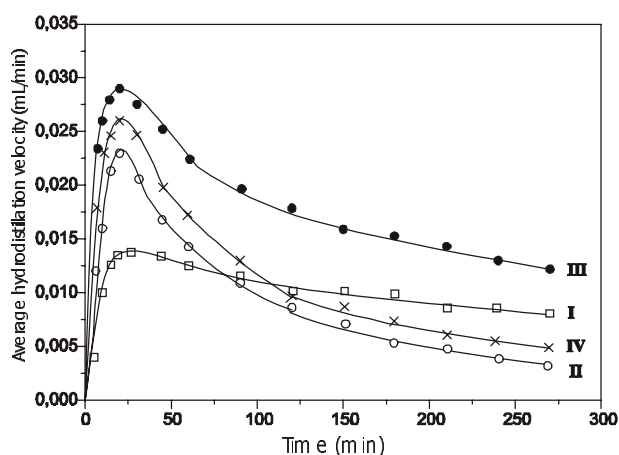


Figure 3. The variation of the average hydrodistillation velocity by different hydrodistillation techniques

Table 2. Qualitative and quantitative composition of the essential oil obtained by different techniques

Component	Technique			
	I	II	III	IV
α -Thujene	1.432 ¹⁾ 18.93 ²⁾	1.435 54.00	1.435 10.76	–
α -Pinene	1.561 2.03	1.565 4.78	1.568 4.36	1.570 3.30
β -Pinene	1.767 1.781	1.770 2.78	1.744 3.59	1.780 3.13
Sabinene	1.820 0.13	–	1.826 0.29	–
α -Terpinene	–	–	2.005 0.19	2.010 0.17
Limonene	2.195 10.42	2.199 0.39	2.143 0.10	2.180 0.14
Cineole	–	17.846 4.70	2.202 1.65	2.215 1.70
γ -Terpinene	–	–	–	2.428 0.05
p-Cimene	–	–	–	2.609 0.09
Myrtenal	7.911 0.38	–	7.925 0.48	7.498 0.57
Myristicin	17.876 7.62	–	17.915 9.70	–
2,3,4,5-Tetramethoxy-1-allylbenzene	18.028 2.16	18.016 1.17	18.060 0.19	18.045 17.03
Apiole	18.125 49.43	18.139 28.23	18.158 57.13	18.147 27.59
Safrole	–	–	–	18.330 36.59
1,2-Benzenedicarboxylic acid	19.809 6.22	19.801 3.53	19.835 8.92	19.931 9.51

¹⁾Retention time; ²⁾Content in the oil (%).

mean hydrodistillation velocity increased and the highest value was obtained by technique III (0.029 mL/min calculated per 100 g of plant material).

Table 2 show the results of the GLC analysis (components, retention times and component content in the oil) of oil samples obtained by different techniques.

Irrespective of the technique used, all the oils contained α -pinene, β -pinene, limonene, 2,3,4,5-tetramethoxy-1-allylbenzene, apiole and 1,2-benzenedicarboxylic acid. The identified components in the oils obtained by techniques I, II, III and IV represented 99.1, 99.6, 97.4 and 99.9% of the total oil, respectively. The oils obtained by techniques III and IV contained the largest number of components (12 in both oils), while the oils obtained by techniques I and II contained less components (10 and 8 components, respectively). Techniques III and IV, in which water from the still flask was used for immersing the plant material in the subsequent distillation, yielded oil with components ... as α -terpinene and myrtenal (these components were not detected in the oil obtained by technique I, where condensate water continuously came back in to the still flask and water from the still flask was not used).

Apiole was the major component in the oils obtained by techniques I and III (49.3 and 57.43%, respectively), while α -thujene and safrole (54.00 and 36.59%, respectively) were predominant in the oil obtained by techniques II and IV.

The content of hydrophobic (α -pinene, β -pinene, α -thujene, sabinene, α -terpinene, limonene, cineole, γ -terpinene and p-cimene) and hydrophilic (myrtenal, myristicin, 2,3,4,5-tetramethoxy-1-allylbenzene, apiole and 1,2-benzenedicarboxylic acid) components differed in oils obtained by various techniques. These differences were probably due to the different ratios of water, condensate water and water from the still flask used for immersing new amounts of parsley seed in the subsequent distillations in techniques I–IV. The content of hydrophobic components in the oils obtained by techniques I, II, III and IV was 33.2, 65.6, 20.9, and 8.58%, while the content of hydrophilic components was 65.9, 34.0, 76.5 and 91.3%, respectively. The fine oil droplets could be not effectively separated in the Clevenger graduated column. In the oil obtained by technique I the fraction of hydrophobic components was lower than in the oil obtained by technique II because the aqueous part of the condensate was continuously returned to the still flask. The high content of hydrophobic components in technique II was the result of using condensate water in which part of the finely suspended oil droplets were present, containing mainly hydrophobic components. The content of hydrophobic components was the lowest, in the oils obtained by techniques III and IV, due to the use of the aqueous phase suspension containing dissolved hydrophilic components.

As α -pinene, myristicin, safrole, 2,3,4,5-tetramethoxy-1-allylbenzene and apiole are mentioned in the literature as the main components of parsley oil [10–13], the components in the oils obtained by different techniques, α -thujene, β -pinene, sabinene, α -terpinene, limonene, cineole, γ -terpinene, p-cimene, myrtenal, and 1,2-benzenedicarboxylic acid, are newly identified components.

Table 3. Physical and chemical properties of the essential oils obtained by different techniques

Property	Technique I	Technique II	Technique III	Technique IV
d ₂₅ (g/mL)	1.06	1.01	1.04	1.01
n _D ²⁰	1.5115	1.5120	1.5190	1.5230
Solubility (volume parts of 80% vol. ethanol for 1 mL of oil)	6	8	7	6

All the oils are dark green with aroma and taste specific for parsley. The physical and chemical properties of the oils obtained by of different hydrodistillation techniques are given in Table 3. The density and refractive indexes of oils obtained by different techniques differed only slightly. The oil solubility was 6–8 parts of 80% vol. ethanol for 1 ml of oil.

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IZVOD

UTICAJ HIDRODESTILACIJE NA PRINOS I SASTAV ETARSKOG ULJA SEMENA PERŠUNA

(Naučni rad)

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Peršun (*Petroselinum crispum* (Mill.) Nym. ex. A.W. Hill) je poznata začinska i lekovita biljka roda *Petroselinum*, porodice *Apiaceae*. Poznata je i po antimikrobnim, hipotenzivnim, laksativnim i toničnim svojstvima. Etarsko ulje nalazi se u svim delovima biljke, a najviše ga ima u semenu (3–7%). Seme peršuna pored etarskog sadrži masno ulje, vitamin A i B₆, tiamin i niacin. U ovom radu ispitan je uticaj tehnike Clevenger–hidrodestilacije etarskog ulja iz semena peršuna na prinos ulja. Najveći prinos ulja (3,9 mL/100 g biljnog materijala), nakon pet uzastopnih hidrodestilacija, dobijen je tehnikom hidrodestilacije u kojoj se vodena faza suspenzije iz predhodne destilacije koristi za kvašenje biljnog materijala u narednoj destilaciji (Postupak III). GC analiza ulja je pokazala, da nezavisno od primenjene tehnike hidrodestilacije, ulje sadrži α–pinen, β–pinen, limonen, 2,3,4,5–tetrametoksi–1–alilbenzen, apiol i 1,2–benzendikarbonsku kiselinu.

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Ključne reči: *Petroselinum crispum* (Mill.) Nym. ex. A.W. Hill • *Apiaceae* • Seme • Etarsko ulje • Hidrodestilacija •

Key words: *Petroselinum crispum* • *Apiaceae* • Parsley seed • Essential oil • Hydrodistillation •