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THE EFFECT OF PARSLEY (*Petroselinum crispum* (Mill.) Nym. ex. A.W. Hill) SEEDS MILLING AND FERMENTATION CONDITIONS ON ESSENTIAL OIL YIELD AND COMPOSITION

Parsley (Petroselinum crispum (Mill.) Nym. ex. A.W. Hill) is well known as a medicinal herb with antimicrobial, hypotensive, diuretic, laxative and spasmolytic effects. Essential oil is present in all parts of the herb. In this paper the effect of parsley seeds milling and fermentation conditions on the essential oil yield and composition were studied. The essential oil yield was determined by a Clevenger-type apparatus, and the oil composition by GC analysis. The obtained oil contained (-)-pinene, (+)-pinene, sabinene, myristicin, 2,3,4,5-tetramethoxy-1-allylbenzene, apiol and 1,2 benzene-dicarboxylic acid. The physico-chemical properties of the isolated oil were determined and found to correspond to the standard values.

Key words: Petroselinum crispum (Mill.) Nym. ex. A.W.Hill, parsley seeds, essential oil, fermentation, hydrodistillation, GC analysis.

Parsley (*Petroselinum crispum* (Mill.) Nym. ex. A.W. Hill) is a biennial herb species from the genus *Petroselinum* of the family *Apiaceae* (*Umbelliferae*). In the first season the root and leaves are formed, while it flowers and produces in the second [1]. It is well known as a medicinal herb [2] with antimicrobial, hypotensive [3], diuretic [4], laxative [5] and spasmolytic effects [6].

Parsley is a good raw material for essential oil, resinoid, oleoresin and lipid production (especially for fatty oil and fatty acids such as palmitic, oleic, linolic and petroselinic acid) [7]. All of these products, especially essential oil (*Aetheroleum petroselinum*), are widely used in the pharmaceutical, cosmetic and food industries. Essential oil is present in all parts of the herb [3,8,9]. The oil content in the root is up to 0.1%, in the leaves it lies in the range 0.05–0.3%. The content in the seeds is the highest and it ranges from 2 to 7% [8,10]. The seeds also contain fatty oil in the range of 20–22% and proteins in an amount of 14% [11,12]. Parsley is a good source of Ca, Fe, vitamin C (150–180 mg per 100 g of dried leaves), carotene (up to 5 mg per 100 g of dried leaves) and it is widely used in nutrition [13].

The yield and composition of essential oil are dependent on the seeds as a source: fresh or stored, non-disintegrated or disintegrated, fermented or non-fermented seeds as well as regional and climate conditions of breeding, hydrodistillation technique, hydromodulus [14–16] etc. Also, more mature seeds produce a higher oil yield [17]. The main components of parsley seed essential oil are apiol, myristicin, safrole and 2,3,4,5-tetramethoxy-1-allylbenzene [17], as well as santene, α -thujene, camphene, β -pinene, α -phellandrene, β -phellandrene, limonene, γ -caryophyllene [18], α -pinene [19] and terpinolene [20].

The effect of parsley seed milling and fermentation conditions on the essential oil extraction kinetics and the

oil yield and composition were studied in this paper. The aims of the paper are to choose the optimal seeds (native or fermented), and determine the fermentation conditions for obtaining the maximal oil yield.

EXPERIMENTAL

Plant material. Non-disintegrated and disintegrated Parsley (*Petroselinum crispum* (Mill.) Nym. ex. A.W. Hill) seeds (*Petroselinum fructus*, verification number 99755), were used. The seeds were obtained from the Institute for medicinal plant research "Dr. Josif Pančić", Belgrade.

Essential oil content in the plant material

a) Native seeds.

The plant material (20 g) was placed into the still flask of a Clevenger-type distillation apparatus, filled up with water in a 1:20 w/v ratio and distilled, by recirculating the condensed water. The oil volume was recorded after 360 min.

b) Fermented seeds.

The plant material (20 g) was placed into the still flask, filled up with water in a 1:20 w/v ratio and fermented at 30°C for 240 min. The still flask was then connected with a Clevenger-type apparatus and the distillation procedure was repeated.

Effect of hydrodistillation hydromodulus. The plant material (20 g) was placed into the still flask of a Clevenger-type distillation apparatus, filled up with water in 1:10, 1:15, 1:20 and 1:25 w/v ratios and distilled by recirculating the condensed water. A separate sample was used for each hydromodulus. A new quantity of plant material was used for each hydromodulus. The oil volume was recorded after 270 min.

Effect of fermentation time. The plant material (20 g) was placed into the still flask, filled up with water in a 1:15 w/v ratio and fermented at 28°C, for 2, 4, 6 and 8 h. After fermentation, a distillation was carried out on a Clevenger-type apparatus by recirculating the condensed water. For each fermentation time a new quantity of plant material was used. The oil volume was

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recorded out after 15, 30, 60, 90, 120, 180, 240 and 270 min.

Effect of fermentation temperature. The plant material (20 g) was placed into the still flask, filled up with water in a 1:15 w/v ratio and fermented at 28, 30, 33, 35, 37 i 39°C for 4 h. After fermentation, a distillation was carried out on a Clevenger-type apparatus by recirculating the condensed water. A new quantity of plant material was used for each fermentation temperature. The oil volume was recorded after 15, 30, 60, 90, 120, 180, 240 and 270 min.

Effect of fermentation hydromodulus.

The plant material (20 g) was placed into the still flask, filled up with water in 1:10, 1:15, 1:20 and 1:25 w/v ratios and fermented at 30°C for 4 h. Afterwards, a distillation was performed using a Clevenger-type apparatus by recirculating the condensed water. A new quantity of plant material was used for each fermentation hydromodulus. The oil volume was recorded after 15, 30, 60, 90, 120, 180, 240 and 270 min.

Hydrodistillation.

a) Native seeds.

The plant material (20 g) was placed into the still flask, filled up with water in a 1:20 w/v ratio and distilled on a Clevenger-type apparatus by a technique where the still water from the still flask (residue still water) was separated under vacuum using a Büchner funnel after distillation and used together with fresh water (the residue still water and fresh water volume was 400 mL) for immersing the plant material in the subsequent distillation. For each subsequent distillation a new quantity of plant material of 20 g was used. Six hydrodistillation runs of 270 min each, were performed [14].

b) Fermented seeds.

Plant material (20 g) was placed into the still flask, filled up with water in a 1:20 w/v ratio and fermented at 30°C for 4 h. The still flask was connected with a Clevenger-type apparatus and the distillation procedure was performed as above. For each subsequent distillation a new quantity of plant material of 20 g was

used. Six hydrodistillation runs of 270 min each, were performed.

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

Determination of essential oil density. A standard method for determining liquid density was carried out by using a pycnometer thermostatted at 25°C. The density was determined as $(m_2 - m_0) / (m_1 - m_0)$, where m_0 is the mass of the empty pycnometer, m_1 the mass of the pycnometer with distilled water and m_2 the mass of the pycnometer with oil.

Estimation of essential oil solubility in ethanol. Oil (1 mL) was added into a measuring cylinder, conditioned at $20 \pm 0.2^\circ\text{C}$. Gradually 80% vol. ethanol, conditioned at $20 \pm 0.2^\circ\text{C}$, was added to the sample by a burette in 0.1 mL portions. Ethanol was added until a total volume 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.

Gas chromatography. To analyze the composition of the essential oil, two types of equipment were used. The first one was a VARIAN 3400 GC with a split/splitless injector (1:99) operated at 266°C . Column: J&W Scientific DB-5 30m, 0.25 mm id, 0.25 μm film; carrier gas: hydrogen, 1 mL/min measured at 210°C . The column temperature was linearly programmed from 60 to 285°C at 4.3 $^\circ\text{C}/\text{min}$. The detector temperature was 300°C . The second one was a HP 5890 SERIES II GAS-CHROMATOGRAPH with a FID detector and 3396 A HP integrator. The column used was CARBOWAX 20 M (25 m x 0.2 mm x 0.2 μm), with the temperature program 60°C , 0.5 min; $4^\circ\text{C}/\text{min}$; 100°C , 2min; $15^\circ\text{C}/\text{min}$; 190°C , 5 min. The detector temperature was 280°C .

RESULTS AND DISCUSSION

Oil content. The initial oil content in the native seeds was 5.10 and 6.10 mL per 100 g plant material in the fermented parsley seeds.

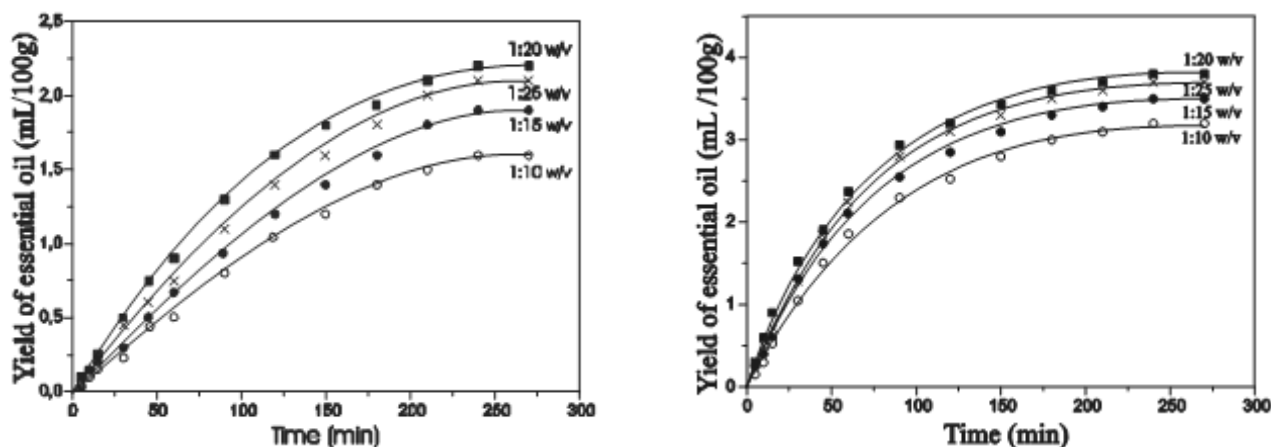


Figure 1. The kinetics of essential oil hydrodistillation from a) non-disintegrated and b) disintegrated native parsley seeds using different hydromoduli

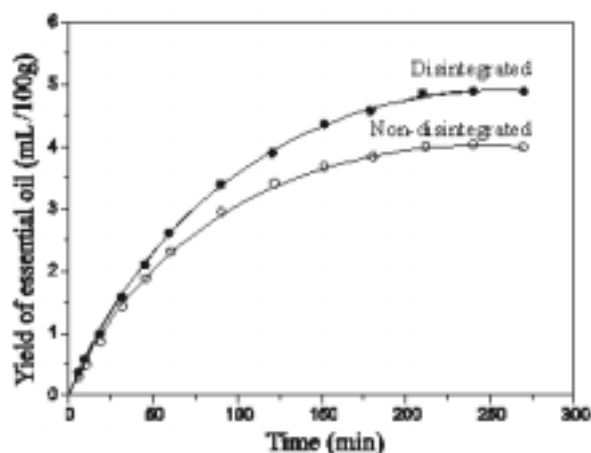


Figure 2. The kinetics of oil hydrodistillation from non-disintegrated and disintegrated native parsley seeds using hydromodulus of 1:20 w/v in the sixth run

Effect of hydrodisillation hydromodulus. The results of the investigation of the oil hydrodistillation kinetics from native parsley seeds using different hydromoduli are shown in Figure 1. The highest oil yields of 2.20 and 3.80 mL per 100 g of non-disintegrated and disintegrated seeds, respectively (43.1 and 74.5%, respectively, in regard to the oil content in the native parsley seeds), was obtained using a hydromodulus of 1:20 w/v for 240 min of hydrodistillation. The oil yield increased with increasing hydromodulus up to 1:20 w/v, probably as a result of the increasing fraction of hydrophilic oil components in the steam phase and distillate. By further increase of the hydromodulus, the content of oil components decreased in the liquid phase due to dilution. A hydromodulus of 1:20 w/v was used as the optimal one in further investigations.

Figure 2 shows the kinetics of oil hydrodistillation from non-disintegrated (a) and disintegrated (b) native parsley seeds with a hydromodulus of 1:20 w/v in the fifth hydrodistillation run. A technique in which still water from the flask was used together with fresh water for immersing the plant material in the subsequent distillation was applied [16].

The oil yield increased with the number of hydrodistillation runs and the maximal yield was achieved in the fifth run (3.9 and 4.9 mL per 100 g of seeds, i.e. 76.5 and 96.1% in regard to the initial content) after 240 min of hydrodistillation. The oil yield increased with increasing number of hydrodistillation runs due to the content of hydrophilic components increasing in the water from the still flask which was used to immerse the seeds in the subsequent hydrodistillation run. By investigating the effect of seed milling on the oil yield it may be shown, that the maximal oil yield obtained from disintegrated seeds was 20.04% higher than the yield obtained from non-disintegrated seeds.

The oil yield increased with increasing number of hydrodistillation runs due to the increasing content of dissolved hydrophilic oil components in the water from

Table 1. Effect of fermentation time on the essential oil yield

Fermentation time (h)	Essential oil yield			
	Non-disintegrated seeds		Disintegrated seeds	
	mL/100 g	%*	mL/100 g	%*
2	2.25	36.9	3.45	56.5
4	2.4	39.3	3.6	59.0
6	2.0	32.8	3.1	50.8
8	1.2	19.67	2.3	37.7

*In regard to the oil content in fermented seeds

the still flask, which was used in subsequent distillations to immerse the plant material.

Effect of fermentation time. The results of the effect of fermentation time on oil yield are shown in Table 1. Oil was obtained from disintegrated and non-disintegrated seeds fermented at 28°C, using a hydromodulus of 1:15 w/v for 2, 4, 6 or 8 h. The maximal oil yields of 2.4 and 3.6 mL per 100 g of non-disintegrated and disintegrated seeds, respectively, i.e. 39.3 and 59.0% in regard to the oil content in fermented seeds, respectively, were obtained from seeds fermented for 4 h and after 210 min of hydrodistillation. The oil yield increased with increasing fermentation time up to 4 h as a result of enzymatic transformation when the oil components from the plant material dissociate. After 4 h of fermentation the yield decreased probably due to enzymes becoming inactivate and unstable. Fermentation time of 4 h was adopted as the optimal value. The oil yield obtained from disintegrated seeds was higher than the oil yield obtained from non-disintegrated seeds by 33.4%.

Effect of fermentation temperature. The oil yield increased by increasing the fermentation temperature up to 30°C. The best yields of 2.45 and 4.0 mL per 100 g of non-disintegrated and disintegrated seeds, respectively, i.e. 40.2 and 65.6% in regard to the oil content in fermented seeds were obtained during fermentation at 30°C. The maximal oil yield from disintegrated seeds was higher by 38.75% than the maximal oil yield obtained from non-disintegrated seeds. By increasing the fermentation temperature up to

Table 2. Effect of fermentation temperature on the essential oil yield

Fermentation temperature (°C)	Essential oil yield			
	Non-disintegrated seeds		Disintegrated seeds	
	mL/100 g	%*	mL/100 g	%*
28	2.4	39.3	3.6	59.0
30	2.45	40.2	4.0	65.6
33	2.25	36.9	3.5	57.4
35	2.15	35.2	3.4	55.7
37	1.95	32.0	2.75	45.1
39	1.8	29.5	2.3	37.7

*In regard to the oil content in fermented seeds

Table 3. Effect of fermentation hydromodulus on the essential oil yield

Hydro-modulus (w/v)	Essential oil yield			
	Non-disintegrated seeds		Disintegrated seeds	
	mL/100 g	%*	mL/100 g	%*
1:10	2.20	36.1	3.7	60.6
1:15	2.45	40.2	4.0	65.6
1:20	2.60	42.6	4.3	70.5
1:25	2.50	40.1	4.2	68.8

*In regard to the oil content in fermented seeds

30°C, the oil yield decreased probably because the enzymes which catalysed processes of compound transformation to oil components soluble in oil, became thermally unstable. Consequently, a fermentation temperature of 30°C was chosen as the optimal one.

The maximal oil yields obtained from seeds fermented at different fermentation temperatures using a hydromodulus of 1:20 w/v and after 4 h of fermentation are presented in Table 2.

Effect of fermentation hydromodulus.

The results of the effect of the fermentation hydromodulus on the oil yield are presented in Table 3. By increasing the fermentation hydromodulus up to 1:20 w/v, the oil yield also increased. Maximal oil yields of 2.6 and 4.3 mL per 100 g of non-disintegrated and disintegrated seeds, respectively, i.e. 42.6 and 70.5% in regard to the oil content in fermented seeds, were obtained from seeds fermented at 30°C for 4 h and after 210 min of hydrodistillation using a hydrodistillation hydromodulus of 1:20 w/v. At this hydromodulus, the maximal oil yield from disintegrated seeds was 43.0% higher than the maximal oil yield obtained from non-disintegrated seeds. So, a fermentation hydromodulus of 1:20 w/v was accepted as the optimal value.

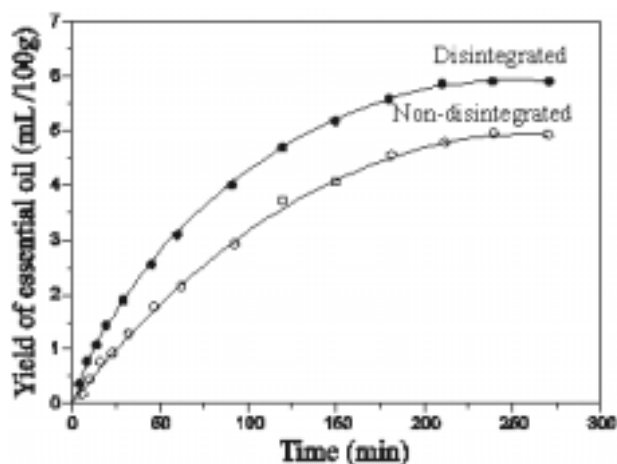


Figure 3. The kinetics of oil hydrodistillation from fermented seeds a) non-disintegrated and b) disintegrated parsley seeds in the sixth run

Table 4. The yield of essential oil obtained under optimal conditions

Parsley seeds		Hydrodistillation, time (min)	Yield of essential oil	
			mL/100 g	%*
Native	Non-disintegrated	240	3.9	76.51
	Disintegrated	240	4.9	96.1 ¹
Fermented	Non-disintegrated	210	4.7	77.1 ²
	Disintegrated	210	5.9	96.7 ²

¹In regard to the oil content in native seeds.

²In regard to the oil content in fermented seeds.

The kinetics of oil hydrodistillation in the sixth run from non-disintegrated and disintegrated seeds, under the optimal conditions of fermentation are presented in Figure 3.

The oil yield obtained from disintegrated seeds was nearly 20% higher than the oil yield obtained from non-disintegrated seeds. During the fermentation of non-disintegrated seeds, the cells in the plant material and seed pellicle are not fully decomposed and oil component transfer into the aqueous phase is slow or disabled, thus giving a lower oil yield.

The comparison of oil yields from native and seeds fermented under the optimal conditions (fermentation temperature 30°C, fermentation time 4 h, hydromodulus of 1:20 w/v) is presented in Table 4. It may be seen that the maximal oil yield of 5.9 mL per 100 g was obtained from disintegrated and fermented seeds after 210 min of hydrodistillation.

Different oil yields are the result of seed milling and fermentation. The results from Table 4 show that the hydrodistillation times needed for achieving the maximal oil yield from native seeds are 30 min longer than the hydrodistillation time for oil from fermented seeds for both disintegrated and non-disintegrated seeds. The maximal oil yield from non-disintegrated and fermented seeds was 17% higher than the maximal oil yield from non-disintegrated and fermented seeds. The maximal oil yield obtained from disintegrated and fermented seeds was also 17% higher than the maximal oil yield from disintegrated and fermented seeds.

The oil composition.

The results of GC analysis of the oil obtained by hydrodistillation from non-disintegrated and disintegrated parsley seeds (native and fermented under the optimal fermentation condition) are presented in Table 5.

The oil from non-disintegrated native and fermented seeds contained myristicin, α -pinene, 2,3,4,5-tetramethoxy-1-allylbenzene, β -pinene, apiol, sabinene, 1,2-benzene dicarboxylic acid and myrtenal. The oil from disintegrated fermented seeds contained the same components, except myrtenal. The oil from disintegrated native seeds contained the same components, except myrtenal and 1,2-benzene dicarboxylic acid. Myrtenal and 1,2-benzene dicarboxylic acid were probably transformed to another component

Table 5. The qualitative and quantitative composition of essential oil from parsley seeds, native and fermented

Compo-nents	Parsley seeds			
	Native seeds		Fermented seeds	
	Non-disin-tegrated	Disinteg-rated	Non-disin-tegrated	Disinteg-rated
α -pinene	6.9 ¹ 20.22	6.9 47.5	6.9 15.2	6.9 32.2
β -pinene	8.1 16.7	8.1 31.1	8.1 12.3	8.1 22.3
Sabinene	9.7 6.1	9.7 8.2	9.7 4.9	9.7 4.8
Myrtenal	15.2 1.1	– –	15.2 0.7	– –
Myristicin	25.6 36.7	25.5 7.8	25.7 42.7	25.5 25.1
2,3,4,5-Tetra-methoxy-1-allyl-benzene	26.5 11.04	26.5 3.78	26.5 13.59	26.4 8.7
Apiole	27.7 5.4	27.7 1.6	27.7 7.1	27.6 5.4
1,2-Benze-nedicarbo-nic acid	30.1 2.0	– –	30.1 3.3	30.2 1.6

¹ Retention time in min² Content in the oil in %

Table 6. Physical and chemical properties of essential oils obtained from parsley seeds

Parsley seeds		Property		
		d ₂₅ (g/mL)	n _D ²⁰	Solubility*
Native	Non-disintegrated	1.039	1.5295	6
	Disintegrated	1.038	1.5227	8
Fermented	Non-disintegrated	1.039	1.5273	7
	Disintegrated	1.040	1.5214	8

*In volume parts of 80% vol. ethanol

during seed disintegration due to oxidation. The qualitative and quantitative composition of oils from non-disintegrated and disintegrated native seeds show that the content of hydrophobic (α -pinene, β -pinene and sabinene) components was about 43% and 86%, while the content of hydrophilic components (myrtenal, myristicin, 2,3,4,5-tetramethoxy-1-allylbenzene, apiol and 1,2-benzene dicarboxylic acid) was about 57% and 14%, respectively. The qualitative and quantitative composition of oils from non-disintegrated and disintegrated fermented seeds show that the content of hydrophobic components was about 32% and 70%, while the content of hydrophilic components was about 68% and 30%, respectively. These components have already been identified in the literature [14,20].

The physical and chemical properties of the essential oils obtained from different seeds are presented in Table 6. The results agree fairly with the literature data [18].

CONCLUSION

The oil yield and composition are dependent on the milling and fermentation conditions of parsley seeds. The highest yield of essential oil of 5.90 mL per 100 g, i.e. 96.7% in regard to the oil content in fermented parsley seeds, was obtained from disintegrated parsley seeds fermented at 30°C for 4 h using a hydromodulus of 1:20 w/v and after the sixth hydrodistillation run, each lasting 210 min. The technique of Clevenger hydrodistillation where still water from the still flask was used together with fresh water for immersing the plant material in the subsequent hydrodistillation was applied. The obtained oil contained α -pinene, β -pinene, sabinene, myristicin, 2,3,4,5-tetramethoxy-1-allylbenzene, apiol and 1,2-benzene dicarboxylic acid. The density of the oil (d₂₅) was 1.04 g/mL, the refractive index (n_D²⁰) was 1.5214 and the oil solubility was 8 volume parts of 80% vol. ethanol for 1 mL of oil.

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IZVOD

UTICAJ MLEVENJA I USLOVA FERMENTACIJE SEMENA PERŠUNA NA PRINOS I SASTAV ETARSKOG ULJA

(Naučni rad)

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Peršun (*Petroselinum crispum* (Mill.) Nym. ex. A.W. Hill) je dvogodišnja začinska i lekovita biljka. Poznata je po antimikrobnim, hipotenzivnim, diuretičnim, laksativnim i spazmolitičnim svojstvima. Etarsko ulje nalazi se u svim delovima biljke, a najviše ga ima u semenu, 2–7%. U ovom radu ispitan je uticaj mlevenja i uslova fermentacije semena peršuna na prinos i sastav etarskog ulja. Sadržaj ulja određivan je Cleavenger metodom, a analiza sastava ulja izvršena je gasnom hromatografijom. Prinos ulja zavisi od mlevenja i uslova fermentacije. Najveći prinos etarskog ulja (5,9 cm³/100 g biljnog materijala, tj. 96,7% u odnosu na sadržaj ulja u fermentiranom semenu) dobijen je iz samlevenog semena fermentiranog 4h na 30°C, pri hidromodulu 1:20 m/v, u seriji od šest uzastopnih hidrodestilacija od 210 minuta. U ulju su identifikovane sledeće komponente: α-pinen, β-pinen, sabinen, mirtenal, miristicin, 2,3,4,5-tetrametoksi-1-alil benzen, apiol i 1,2-benzendi-karboksilna kiselina. Određene su fizičko-hemijske karakteristike izolovanog ulja i utvrđeno da odgovaraju propisanim vrednostima. Gustina ulja (d₂₅) je 1,04 g/mL, indeks refrakcije (n_D²⁰), 1,5214, a rastvorljivost ulja 8 zapreminskih delova 80% vol. ethanolaja je potrebno za rastvaranje 1 mL ulja.

Ključne reči: *Petroselinum crispum* (Mill.) Nym. ex. A.W. Hill, seme, etarsko ulje, fermentacija, hidrodestilacija, GC analiza.