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## HYDRODISTILLATION KINETICS AND ESSENTIAL OIL COMPOSITION FROM FERMENTED PARSLEY SEEDS

The effect of fermentation conditions on the yield and composition of the essential oil from *Petroselinum crispum* (Mill.) Nym. ex. A.W.Hill seeds was studied. The yield of essential oil was determined by a Cleavenger-type apparatus, and the composition of the oil by GC analysis. The highest yield of essential oil (5.90 mL per 100 g, i.e. 96.7% in regard to the oil content in parsley seeds) was obtained from disintegrated parsley seeds fermented for 4 h at 30°C with a hydromodulus of 1:20 w/v, after the sixth hydrodistillation each lasting 240 minutes. The obtained oil contained  $\alpha$ -pinene,  $\beta$ -pinene, sabinene, myristicin, 2,3,4,5-tetramethoxy-1-allylbenzene, apiole and 1,2 benzene-dicarboxylic acid. The density of the oil ( $d_{25}$ ) was 1.039 g/mL<sup>3</sup>, the refractive index, ( $n_D^{20}$ ) was 1.5214 and the oil solubility was 8 volume parts of 80% vol. ethanol for 1 mL of oil.

**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*) [1]. Parsley grows on all types of soil. In the first season the root and leaves are formed, and in the second it flowers and bears seeds. It is our most familiar herb and has been widely employed as a culinary garnish for more than 2000 years. It is used in nutrition as a good source of Ca, Fe, Mg, K, Zn, vitamin A, B<sub>6</sub>, thiamin, riboflavin and niacin [2–4]. Parsley seeds contain fatty oil up to 20%, with a petroselinic acid content of more than 75% of all the fatty acids contained, and a protein content of up to 14% [5]. It is also well known as a medicinal herb [6,7] with antimicrobial, hypotensive and bactericidal [8], diuretic [9,10], laxative [11] and spasmolytic effects [12].

The essential oil is contained in all parts of the herb (the seeds contains 3–7%, the leaves 0.16–0.3% and the root 0.1%) [13]. The essential oil gives parsley its scent and flavour [14,15]. The essential oil from parsley seeds have mainly been obtained by steam distillation [16,17] or by a simultaneous steam distillation and extraction procedure [18,19]. The yield and composition of the essential oil depend on the seeds as a source: fresh or stored, non-disintegrated or disintegrated, fermented or non-fermented, as well as the regional and climate conditions of breeding, technique of hydrodistillation, hydromodulus [20], etc. The main components of parsley seed essential oil are apiole, myristicin, safrole and 2,3,4,5-tetramethoxy-1-allylbenzene [21], as well as santene,  $\alpha$ -thujene, camphene,  $\beta$ -pinene,  $\alpha$ -phellandrene,  $\beta$ -phellandrene, limonene,  $\gamma$ -caryophyllene [16],  $\alpha$ -pinene [18] and terpinolene [19]. In oil obtained from fresh parsley leaves components such as terpinene, 1-methyl-

4-isopropenylbenzene, 1,3,8-p-menthatriene, myrcene and thymol have also been identified [22].

In the literature there are no references about the effect of fermentation conditions and disintegration on the yield and composition of the essential oil from parsley seeds. In this paper the effect of temperature and time of fermentation, as well as the ratio of plant material to water for immersing (hydromodulus) on the yield and composition of the essential oil from parsley seeds, non-disintegrated and disintegrated, was studied. The aim of the paper was to determine the optimal fermentation conditions, obtain the essential oil and investigate its composition.

### EXPERIMENTAL

**Plant material.** Parsley (*Petroselinum crispum* (Mill.) Nym. ex. A.W. Hill) seeds (*Petroselinum fructus*), non-disintegrated and disintegrated were used. The seeds were obtained from the "Dr. Josif Pančić", Institute for Medicinal Plant Research Belgrade.

**Essential oil content in the plant material.** The parsley seeds (20 g) were placed into the still flask of a Cleavenger-type distillation apparatus, filled with water in 1:20 w/v ratio and distilled, by recirculating the condensed water. The oil volume was measured after 360 minutes.

**Effect of time of fermentation.** The plant material (20 g) was placed into the still flask, which was filled with water in a 1:15 w/v ratio and fermented at 28°C for 2, 4, 6 and 8 h. The contents were distilled in a Cleavenger-type distillation apparatus by recirculating the condensed water. The oil volume was measured after 15, 30, 60, 90, 120, 180, 240 and 360 minutes.

**Effect of temperature of fermentation.** The plant material (20 g) was placed into the still flask, which was filled with water in a 1:15 w/v ratio and fermented at 28, 30, 33, 35, 37 and 39°C for 4 h. The contents were then distilled in a Cleavenger-type distillation apparatus by recirculating the condensed water. The oil volume

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measured after 15, 30, 60, 90, 120, 180, 240 and 360 minutes.

**Effect of hydromodulus.** The plant material (20 g) was placed into the still flask, which was filled with water in 1:10, 1:15, 1:20 and 1:25 w/v ratios and fermented at 30°C for 4 h. The contents were then distilled in a Cleavenger-type distillation apparatus by recirculating the condensed water. The oil volume was measured after 15, 30, 60, 90, 120, 180, 240 and 360 minutes.

**Hydrodistillation.** The plant material (50 g) was placed into the still flask, which was filled with water in a 1:20 w/v ratio and fermented at 30°C for 4 h. The contents were then distilled in a Cleavenger-type distillation apparatus by a technique where the still water from the still flask (residual still water) was separated under vacuum using a Buchner funnel after distillation and used together with fresh water (the residual still water and fresh water volume was 1000 mL) to immerse the plant material in the subsequent distillation. Six hydrodistillation runs of 360 minutes each, were performed.

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

**Determination of the essential oil density.** The liquid density was determined in a standard way by using a pycnometer thermostated at 25°C. The density was determined as  $(m_2 - m_0) / (m_1 - m_0)$ , where  $m_0$  was 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.** The oil 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.** A Varian 3400 GC with a split/splitless injector (1:99) operated at 266°C was used to analyze the composition of the essential oil. Column: J&W Scientific DB-5 30m, 0.25 mm id, 0.25  $\mu\text{m}$  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 °/min. The detector temperature was 300°C.

## RESULTS AND DISCUSSION

The content of essential oil in the parsley seeds was 6.10 mL per 100 g of dry parsley seeds. The results of the hydrodistillation kinetics of the essential oil from non-disintegrated parsley seeds fermented at 28°C for different times of fermentation (2–8 h), by using a plant material to water ratio (hydromodulus) of 1:15 w/v, are shown in Fig. 1. The best yield of essential oil of 2.40 mL

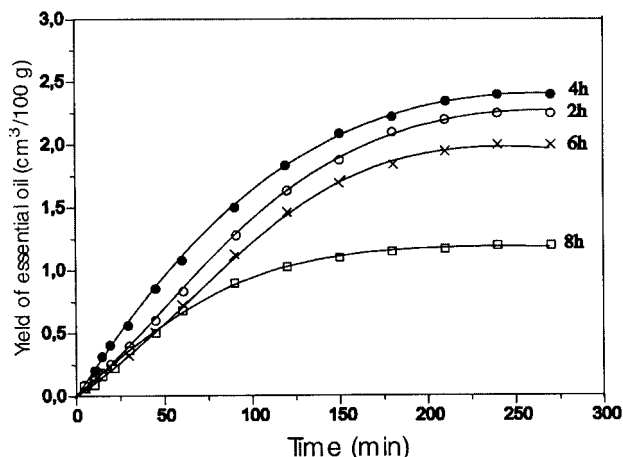


Figure 1. The kinetics of essential oil hydrodistillation from non-disintegrated parsley seeds fermented for different times (temperature of fermentation, 28°C; hydromodulus, 1:15 w/v)

per 100 g of parsley seeds (39.3% in regard to the oil content in the parsley seeds) was achieved by 4 h of fermentation and after 240 minutes of hydrodistillation. The decrease of the oil yield with longer fermentation time, is probably the consequence of enzyme inactivation or decomposition after a time of 4 h. In further investigations a fermentation time of 4 h was applied as optimal.

The kinetics of essential oil hydrodistillation from parsley non-disintegrated seeds fermented at different temperatures of fermentation (28–39°C) for 4 h and with a hydromodulus of 1:15 w/v, are shown in Fig. 2. The highest oil yield of 2.45 mL per 100 g of seeds (40.2% in regard to the oil content in parsley seeds) was obtained by seeds fermented at a fermentation temperature of 30°C after 240 minutes of hydrodistillation. By increasing the temperature above 30°C, the oil yield decreased, probably due to enzyme thermal instability. In further investigations, a temperature of 30°C was applied as

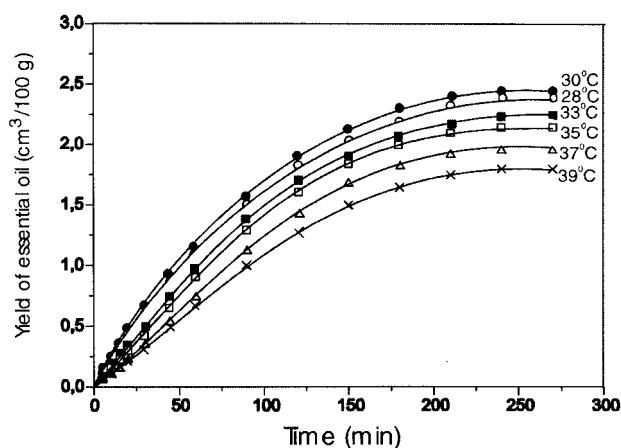


Figure 2. The kinetics of essential oil hydrodistillation from non-disintegrated parsley seeds fermented at different temperatures (time of fermentation 4 h; hydromodulus 1:15 w/v)

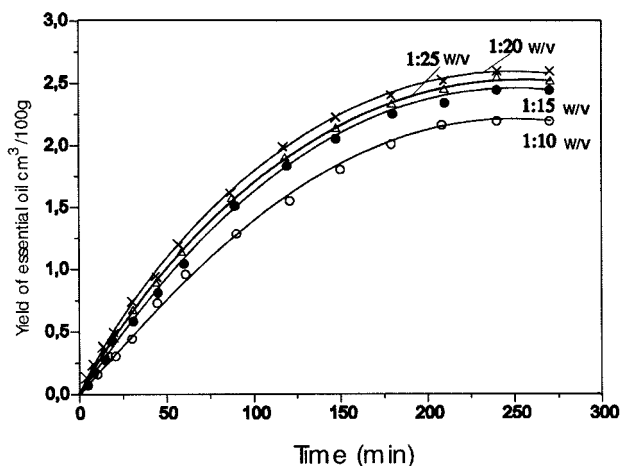


Figure 3. The kinetics of essential oil hydrodistillation from non-disintegrated parsley seeds fermented with different hydromoduli (temperature of fermentation 28°C; time of fermentation 4 h)

optimal for enzyme reactions in which the oil components were isolated from the plant material.

The results of the investigation of the oil hydrodistillation kinetics from non-disintegrated seeds fermented at the optimal fermentation temperature of 30°C for 4 h, the optimal fermentation time, using different hydromoduli are shown in Figure 3. The highest oil yield of 2.60 mL per 100 g of seeds (42.6% in regard to the oil content in parsley seeds) was obtained with a hydromodulus of 1:20 w/v, after 240 minutes of hydrodistillation. The oil yield increased with increasing hydromodulus up to 1:20 w/v, probably as a result of increasing the fraction of hydrophilic oil components in the steam phase and distillate. By further increasing the hydromodulus, the content of oil components decreased in the liquid phase due to its dilution. A hydromodulus of 1:20 w/v was used as optimal in further investigations.

Investigations of the optimal conditions for fermentation of disintegrated parsley seeds were also

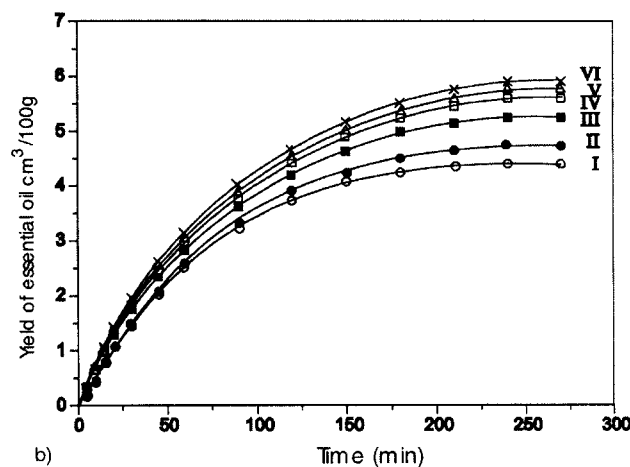
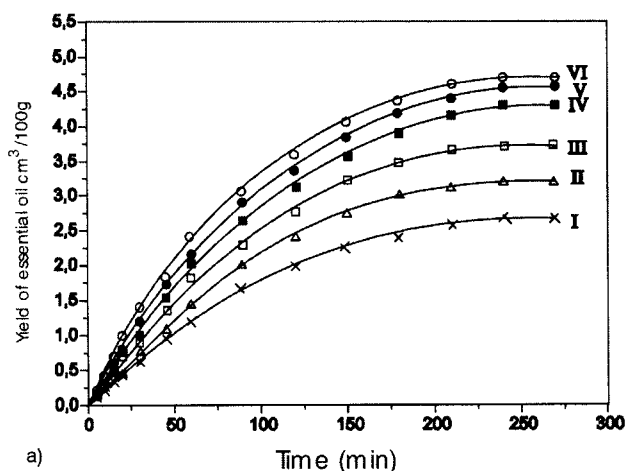


Figure 4. The kinetics of six hydrodistillation runs of oil from a) non-disintegrated and b) disintegrated parsley seeds (fermented at 30°C for 4 h, with a hydromodulus of 1:20 w/v)

Table 1. The yield of essential oil of six oil hydrodistillation runs from fermented parsley seeds under the optimal fermentation and hydrodistillation conditions by different techniques

Yield of essential oil	Technique I	Technique II	Technique III	Technique IV
mL/100 g of seeds	3.28 <sup>1</sup> 4.51 <sup>2</sup>	2.14 2.08	4.70 5.90	2.62 2.24
%	53.8 <sup>1</sup> 73.9 <sup>2</sup>	35.2 34.1	77.5 96.7	43.7 36.8

<sup>1</sup>Non-disintegrated seeds

<sup>2</sup>Disintegrated seeds

carried out. It was determined that the optimal fermentation conditions were same as for non-disintegrated seeds: fermentation temperature 30°C, time of fermentation 4 h, hydromodulus 1:20 w/v.

Fig. 4 shows the kinetics of six oil hydrodistillation runs with non-disintegrated (a) and disintegrated (b) seeds, under the optimal conditions of fermentation. The technique in which the still water from the still flask was used together with fresh water to immerse the plant material in the subsequent distillation (technique III) was applied [23]. This technique was found to be the best. By preliminary investigations of the effect of hydrodistillation technique on the oil yield from non-disintegrated and disintegrated seeds (Table 1).

The oil yield increased with increasing number of hydrodistillation runs from 2.60, 3.10, 3.65, 4.30, 4.55 to 4.70 mL per 100 g of non-disintegrated parsley seeds, and from 4.30, 4.65, 5.25, 5.60, 5.75 to 5.90 mL per 100 g of disintegrated seeds. The oil yield increased with increasing number of hydrodistillation runs due to the increase in the content of dissolved hydrophilic oil components in the residual still water which was used in the subsequent distillation for immersing the plant material.

The oil yield obtained from disintegrated seeds was nearly 20% higher than the oil yield obtained from

Table 2. Qualitative and quantitative composition of oil from parsley seeds fermented under the optimal conditions

Components	Retention time	Content in oil (%)
$\alpha$ -Pinene	6.908 <sup>1</sup>	15.24
	6.898 <sup>2</sup>	32.17
$\beta$ -Pinene	8.100	12.34
	8.086	22.28
Sabinene	9.670	4.97
	9.600	4.79
Myrtenal	15.210	0.75
	–	–
Myristicin	25.687	42.75
	25.527	25.08
2,3,4,5-tetramethoxy-1-allylbenzene	26.533	13.59
	26.433	8.67
Apiole	27.709	7.05
	27.630	5.38
1,2-Benzene dicarboxylic acid	30.083	3.31
	30.213	1.63

<sup>1</sup> Non-disintegrated seeds

<sup>2</sup> Disintegrated seeds

non-disintegrated seeds. The difference probably originates from the fact that during the fermentation of non-disintegrated seeds the cells in the plant material and seed pellicle were not fully decomposed and the process of oil component transfer in to the aqueous phase was slow or disabled.

It was calculated that the highest average oil hydrodistillation velocities from non-disintegrated and disintegrated seeds of  $3.2 \times 10^{-2}$  and  $5.7 \times 10^{-2}$  mL/min, respectively, were achieved in the twentieth minute of hydrodistillation.

The results of the GC analysis of the oil obtained by hydrodistillation from non-disintegrated and disintegrated parsley seeds under the optimal fermentation condition are presented in Table 2.

The oil from non-disintegrated seeds contained myristicin,  $\alpha$ -pinene, 2,3,4,5-tetramethoxy-1-allylbenzene,  $\beta$ -pinene, apiol, sabinene, 1,2-benzene dicarboxylic acid and myrtenal. The oil from disintegrated seeds contained the same components, except myrtenal. Myrtenal was probably transformed to some another component during seed disintegration, due to oxidation. The qualitative and quantitative composition of oils from non-disintegrated and disintegrated seeds showed that the content of hydrophobic ( $\alpha$ -pinene,  $\beta$ -pinene and sabinene) components was about 32% and 70%, while the hydrophilic components (myrtenal, myristicin, 2,3,4,5-tetramethoxy-1-allylbenzene, apiole and 1,2-benzene dicarboxylic acid) was about 68% and 30%, respectively. According to literature data,  $\alpha$ -pinene,  $\beta$ -pinene, myristicin, 2,3,4,5-tetramethoxy-1-allylbenzene and apiole have already been identified [16–22], while sabinene, myrtenal and 1,2-benzene dicarboxylic acid are new byidentified components.

The following results were obtained for the physical and chemical properties of oil from disintegrated seeds and non-disintegrated seeds: density ( $d_{25}$ ) 1.061 and 1.039 g/mL, refractive index ( $n_D^{20}$ ) 1.5273 and 1.5214, while oil the solubility was 8 volume parts of 80% vol. ethanol for 1 mL of oil, respectively. The results agree fairly well with the literature data [16].

## CONCLUSION

The oil yield and composition depend on the fermentation conditions and the disintegration of parsley seeds. The highest yield of essential oil (5.90 mL per 100 g, i.e. 96.7% in regard to the oil content in parsley seeds) was obtained from disintegrated parsley seeds fermented at 30°C, for 4 h, with a hydromodulus of 1:20 w/v, after the sixth hydrodistillation, each lasting 240 minutes. The technique of Clevenger hydrodistillation in which the still water from the still flask was used together with fresh water to immerse the plant material in the subsequent hydrodistillation was applied. The obtained oil contained  $\alpha$ -pinene,  $\beta$ -pinene, sabinene, myristicin, 2,3,4,5-tetramethoxy-1-allylbenzene, apiole and 1,2-benzene dicarboxylic acid. The density of the oil ( $d_{25}$ ) was 1.039 g/mL<sup>3</sup>, the refractive index, ( $n_D^{20}$ ) 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

### KINETIKA HIDRODESTILACIJE I SASTAV ETARSKOG ULJA FERMENTIRANOG SEMENA PERŠUNA

(Naučni rad)

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Peršun (*Petroselinum crispum* (Mill.) Nym. ex. A.W. Hill) je dvogodišnja biljka poznata po antimikrobnom, bakteri-cidnom, hipotenzivnom, laksativnom, diuretičnom i spazmolitičkom dejstvu. Seme peršuna sadrži 3–7% etarskog ulja koje se uglavnom dobija parnom destilacijom. U radu je ispitan uticaj uslova fermentacije na prinos i sastav etarskog ulja iz nesamlevenog i samlevenog semena peršuna. Seme je fermentirano na temperaturama 28–39°C u toku 2–8 sati i zatim praćena kinetika hidrodestilacije etarskog ulja. Prinos ulja određen je primenom Clevenger–aparature, a sastav ulja GC analizom. Prinos i sastav ulja zavise od uslova fermentacije i mlevenja semena. Najveći prinos ulja (5,90 mL/100 g biljnog materijala, odnosno 96,7% u odnosu na sadržaj ulja u semenu) ostvaren je sa samlevenim semenom peršuna fermentiranim na temperaturi 30°C, u toku 4 sati, pri hidromodulu 1:20 m/v i hidrodestilacijom u seriji od šest uzastopnih proba. Korišćen je postupak hidrodestilacije u kome se vodena faza suspenzije iz prethodnih destilacija koristi za kvašenje biljnog materijala u narednim destilacijama. U ulju su gasnom hromatografijom identifikovani  $\alpha$ -pinen,  $\beta$ -pinen, sabinen, miristicin, 2,3,4,5-tetrametoksi-1-alilbenzen, apiol i 1,2-benzendikarbonska kiselina. U literaturi se sve komponente izuzev sabinena, 1,2 benzendikarbonske kiseline i mirtenala, pominju kao komponente identifikovane u ulju semena peršuna. Gustina ulja ( $d_{25}$ ) je 1,039 g/mL, indeks refrakcije ( $n_D^{20}$ ) 1,5214, a 8 zapreminskih delova 80% vol. etanola je potrebno za rastvaranje 1 mL ulja. Vrednosti fizičko–hemijskih karakteristika dobijenih ulja pokazuju dobro slaganje sa literaturnim podacima.

Ključne reči: *Petroselinum crispum* (Mill.) Nym. ex. A.W.Hill, Seme, Etarsko ulje, Fermentacija, Hidrodestilacija, GC analiza.