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SCIENTIFIC PAPER

635.78+66.061:577.115

# THE EXTRACTION OF TOTAL LIPIDS FROM PARSLEY (*Petroselinum crispum* (Mill.) Nym. ex. A.W. Hill) SEEDS

*The kinetics of extraction of total lipids from ground parsley (Petroselinum crispum (Mill.) Nym. ex. A.W. Hill) seeds with a mixture of ethanol or methanol with non–polar organic solvents, chloroform, carbon tetrachloride, trichloroethylene and petroleum ether, at various temperatures were studied. The maceration technique with reflux was used. The kinetic parameters were determined in extraction kinetic equations, as well as the optimal operation conditions for total lipids extraction. The maximum total lipids yield under optimal conditions was 33.7 g per 100 g of dry parsley seeds. Nine lipid fractions of the total lipids were separated by thin layer chromatography, among which were phospholipids, sterol, mono–, di– and triacylglycerol, free fatty acids and carbohydrates.*

Parsley ((*Petroselinum crispum* **Mill.**) Nym. ex. A.W. Hill) is a biennial herb species from the genus Petroselinum of the family *Apiaceae* (*Umbelliferae*) [1]. It is our most familiar herb and has been widely employed as a culinary garnish for more than 2000 years. It is a good source of Ca, Fe, vitamin C, Mg, K, Zn, vitamin A, thiamine, riboflavin, niacin, and vitamin $B_6$ [2]. It is also well known as a medicinal herb [3–5] with antimicrobial, hypotensive [6], diuretic [7,8], laxative [9], spasmolytic [10], tonic and bactericidal effects [6,7].

Parsley seeds contain lipids with approximately 20% fatty oil with approximately 14% non–saponifiable substances, flavonoids including largely apiin, tannins, polysacharides, traces of furanocumarins (bergapten) and organic acids: petroselinic acid (up to the level of 50% of the total fatty acids contained), oleic, glycolic and palmitic acids [11–14]. The seeds also contain approximately 2–7% essential oil with the main compound being apiole, myristicine and 1–allyl–2,3,4,5, tetramethoxy benzene [15,16]. The fatty oil from parsley seeds is especially interesting because of its application in the culinary and food industries. It is obtained by extraction from the seeds, usually at room temperature and by using a chloroform and methanol mixture to decompose lipid complexes with proteins and enables the complete extraction of lipids [17].

In this paper the kinetics of total lipids extraction from parsley seeds were investigated by using ethanol and methanol mixtures with chloroform, carbon tetrachloride, trichloroethylene and petroleum ether at various temperatures. The aim the of paper was to investigate the influence of the solvent mixture and extraction temperature on the kinetics and composition of total lipids in order to determine the optimal extraction conditions.

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Paper received: April 30, 2004
Paper accepted: October 11, 2004

## MATERIALS AND METHODS

**Plant material**. Parsley ((*Petroselinum crispum* **Mill.**) Nym. ex. A.W. Hill) seeds (*Petroselini fructus*) ("Dr. Josif Pančić", Institute for Medicinal Plant Research, Belgrade).

**Total lipids content**. The parsley seeds (30 g) were put into an Erlenmeyer flask, 300 ml of a chloroform and ethanol mixture (2:1, v/v) were added and extracted for 30 minutes, with reflux and mixing (200 $min^{-1}$) at water bath boiling temperature. The extract was separated by filtration using a Büchner funnel under weak vacuum. The plant material was extracted two more times by the same method; the extracts were mixed together and eluted in the separation funnel (3 x 30 ml). The eluted chloroform extract volume was recorded and an aliquot (2 ml) was taken for the dry residue determination test.

**Dry residue content**. (2 ml) Lipid extract into the disk plate analyzer (Scaltec SMO 01, Scaltec Instruments, Germany) was poured and dried at 110°C to constant weight. The content of dry residue was read on the display.

**Extraction of total lipids by solvent mixtures**. The parsley seeds (10 g) were extracted by maceration with reflux and mixing (200 $min^{-1}$) with (2:1, v/v) the mixtures: chloroform:methanol, chloroform:ethanol, petroleum ether:methanol, petroleum ether:ethanol, trichloroethylene:methanol, trichloroethylene:ethanol, carbon tetrachloride:methanol, carbon tetrachloride: ethanol at 1:10 m/v, at room temperature for periods of 5, 15, 30, 60 and 90 minutes. Separate samples were taken for individual extraction periods. The extract was separated by filtration under vacuum (extraction I), and extraction repeated under the same conditions (extraction II). The extracts were eluted with water (3x30 ml) and the dry content determined.

**Extraction of total lipids at various temperatures**. The parsley seeds (10 g) were extracted by a carbon tetrachloride:methanol mixture (2:1, v/v) at l:10 m/v with reflux and mixing ($200^{-1}$) at 40, 50, and 60°C and at the

solvent mixture boiling point (the reaction mixture temperature being 37, 46, 54, and 61°C±2°C, respectively) for a period of 5, 15, 30, 60, and 90 minutes. The extract was separated by filtration under vacuum (extraction I), and extraction repeated under the same conditions (extraction II). The extracts were eluted with water (3x30 ml) and the dry content determined.

**Thin layer chromatography**. Silica gel G (Kiesel gel 60 $GF_{254}$. 15 μm, Merck) was mixed with 65 ml of distilled water and stirred vigorously to obtain a consistent mass. A 0.25 mm thick layer of suspension was applied on 5 glass plates by a thin layer application device. The plates were air dried for 20 minutes and then activated by heating in a dryer at 110°C for 30 minutes. Lipid filtrate (0.01 $cm^3$) was applied to the starting points. The chromatogram was developed by an ascending n–hexane–diethyl ether–glacial acetic acid (73:25:2 v/v/v) mixture until the mobile phase front reached 2/3 of the plate height. The plates were left at room temperature to evaporate the mobile phase. The spots were visualized by spraying with 50% vol. water diluted sulfuric acid and heated to 110°C for 30 minutes [17].

## RESULTS AND DISCUSSION

### The influence of solvent on the total lipids extraction kinetics.

The total lipids were 34.1%.

The results of the influence of the solvent mixture on the total lipids extraction kinetics at room temperature are shown in Figure 1 and Table1. The results indicate that during extraction I of the total lipids, lasting 30 minutes, with different solvent mixtures 43.1 to 53.1% of the total lipids content in the seed were extracted (14.7 to 18.1 g total lipids per 100 g of dry parsley seed) and in extraction II for the same period of time, 12.0 to 22.9% of the total lipids content in the seed (4.1 to 7.8 g total lipids per 100 g of dry parsley seed). The highest yield was obtained by a trichloroethylene:methanol (2:1, v/v) mixture in extraction I, and by chloroform:methanol (2:1, v/v) in extraction II.

The maximal total lipids yield obtained in extraction I at room temperature with a herb material to solvent ratio of 1:10 m/v (Table 1) was lower by 3.3 to 18.8% compared to the maximal yield of 18.1 g per 100 g of dry parsley seed, obtained with the chloroform:methanol mixture (2:1, v/v). Assuming that the upper acceptable tolerance limit for the yield was up to 5%, then for extraction I, the trichloroethylene:ethanol (2:1, v/v) and carbon tetrachloride:methanol (2:1, v/v) mixtures could also be used.

Compared to the maximum total lipids yield of 7.8 per 100 g of dry parsley seed, obtained in extraction II with the chloroform:methanol (2:1, v/v) mixture, the other yields were lower by 17.9 to 47.4%.

The total lipids yield from extractions I and II were lower by 4.7% to 12.4% (yield of 22.2 to 20.4 g per 100 g of dry parsley seed) compared to the maximum total lipids yield of 23.3 g per 100 g of dry parsley seed, obtained with the carbon tetrachloride:methanol (2:1, v/v) mixture in 30 minutes. Since the total yield of total lipids with the chloroform:methanol (2:1, v/v) mixture was 4.7% lower than the maximum total lipids yield of 23.3 g per 100 g of dry parsley seed, this mixture could be used for the extraction of total lipids from parsley seeds.

Figure 1 shows that higher total lipids yields were obtained with mixtures of non–polar solvents with methanol than with their mixtures with ethanol, both in extraction I and II. Thus, the total lipids yield with the chloroform:methanol (2:1,v/v) mixture in extraction I was higher by 0.4 g per 100 g of dry parsley seed compared to that obtained with the chloroform:ethanol (2:1, v/v) mixture, the yield with the petroleum ether:methanol (2:1, v/v) and trichloroethylene:methanol (2:1,v/v) mixtures was 1.3 g per 100 g of dry parsley seed higher compared to those obtained with ethanol mixtures, and with the carbon tetrachloride:methanol (2:1, v/v) mixture the yield was 0.7 g per 100 g of dry parsley seed higher compared to that obtained with the carbon tetrachloride:ethanol (2:1, v/v) mixture. In extraction II the total lipids yield was 2.1 g per 100 g of dry parsley seed higher with the mixture where chloroform was the first solvent, 0.7 g per 100 g higher with the mixtures where the first solvents were petroleum ether and trichloroethylene, and 1.6 g per 100 g of dry parsley

*Table 1. Maximum total lipids quantities extracted with various mixtures at room temperature*

| Mixture (2:1, v/v) | Extraction I | | Extraction II | | Total | |
|---|---|---|---|---|---|---|
| | Yield* | SE (%) | Yield* | SE (%) | Yield* | SE (%) |
| Chloroform:ethanol | 14.7 | 43.1 | 5.7 | 16.7 | 20.4 | 59.8 |
| Chloroform:methanol | 15.1 | 44.6 | 7.8 | 22.9 | 22.9 | 67.5 |
| Petroleum ether:ethanol | 14.8 | 43.4 | 6.4 | 18.8 | 21.2 | 62.2 |
| Petroleum ether:methanol | 16.1 | 47.2 | 5.7 | 16.7 | 21.8 | 53.9 |
| Trichloroethylene:ethanol | 17.3 | 50.7 | 4.8 | 14.1 | 22.1 | 64.8 |
| Trichloroethylene:methanol | 18.1 | 53.1 | 4.1 | 12.0 | 22.2 | 65.1 |
| Carbon tetrachloride:ethanol | 16.8 | 49.3 | 4.2 | 12.3 | 21.0 | 51.6 |
| Carbon tetrachloride:methanol | 17.5 | 51.3 | 5.8 | 17.0 | 23.3 | 74.6 |

* g per 100 g of dry parsley seed

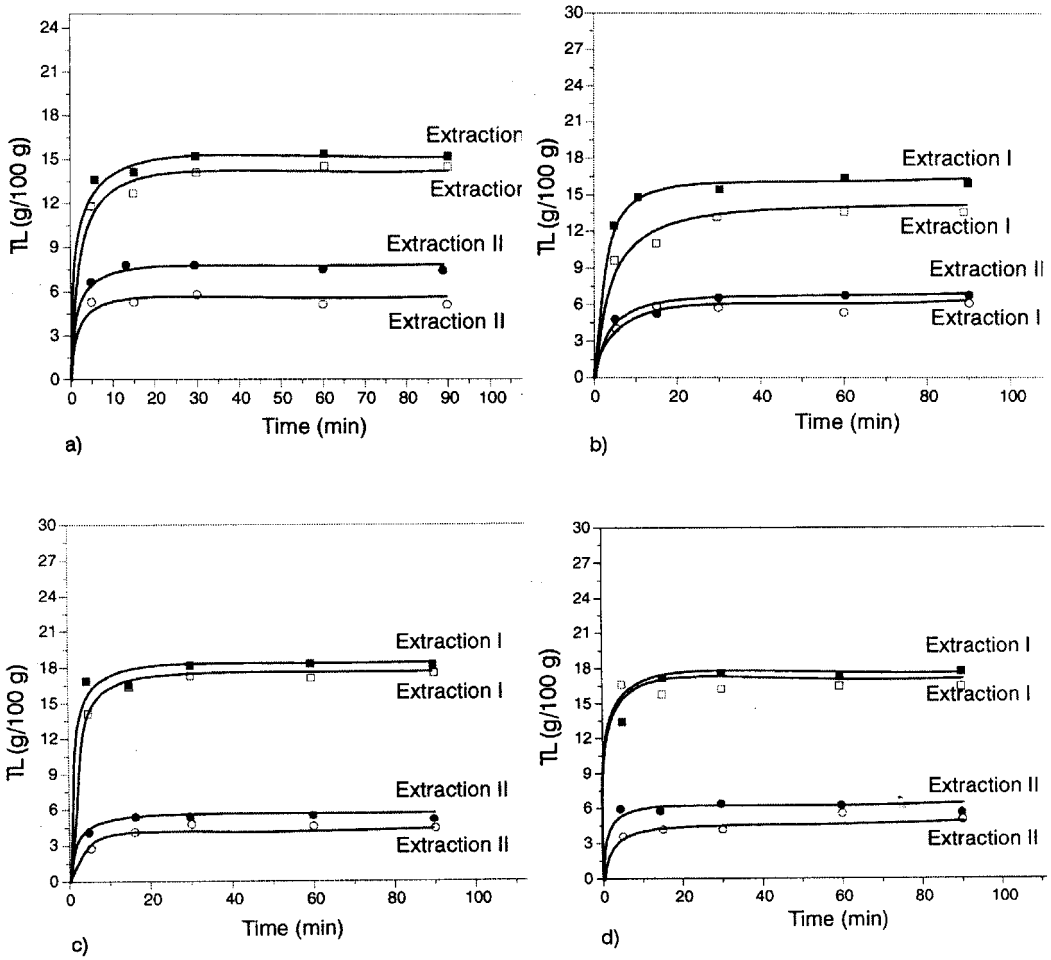


*Figure 1. Kinetics of extractions I and II of the total lipids at room temperature with various mixtures (2:1, v/v): a) chloroform:methanol (■, ●) and chloroform:ethanol (□, ○), b) ) petroleum ether:methanol (■ , ●) and petroleum ether:ethanol (□, ○), c) trichloroethylene:methanol (■, ●) and trichloroethylene:ethanol (□, ○), d) carbon tetrachloride:methanol (■, ●) and carbontetrachloride:ethanol (□, ○),*

seed higher with the mixture where carbon tetrachloride was the first and methanol the second solvent, as compared with the mixtures where the second solvent was ethanol.

The shape of the total lipids extraction kinetics curves, given in Figure 1, is characteristic for their model of extraction of extractive matter from the ground plant material. This model typically has fast and slow extraction periods [18]. According to Figure 1, after 30 minutes the quantity of extracted total lipids remains the same. The total lipids extraction kinetics for extraction I and II can be presented by the equation:

$$TL = a + kt \quad (1)$$

The total lipids quantity extracted by diffusion during the fast extraction period (b) is calculated by:

$$TL - a = b \quad (2)$$

The values of coefficients *a* and *k* in the total lipids extraction kinetics equations with different solvent mixtures at room temperature are given in Table 2. In the fast extraction period 33.1 to 51.6% of the total lipids

*Table 2. Values of the coefficients a, b, and k in the equations for the total lipids extraction kinetics with various solvent mixtures at room temperature*

| Mixture (2:1, v/v) | a* | b* | k x $10^{-2}$ (g $min^{-1}$/100 g) | PBE (min) |
|---|---|---|---|---|
| Chloroform: ethanol | 13.5[1)]<br>5.2[2)] | 1.2<br>0.5 | 4.00<br>1.67 | 25<br>15 |
| Chloroform: methanol | 13.7<br>7.3 | 1.4<br>0.5 | 4.67<br>1.67 | 20<br>15 |
| Petroleum ether: ethanol | 11.3<br>5.4 | 3.5<br>1.0 | 11.67<br>3.33 | 15<br>15 |
| Petroleum ether:methanol | 14.5<br>5.6 | 1.6<br>0.1 | 5.33<br>0.33 | 15<br>15 |
| Trichloroethylene: ethanol | 16.9<br>4.2 | 0.4<br>1.5 | 1.33<br>5.00 | 25<br>20 |
| Trichloroethylene: methanol | 17.6<br>3.8 | 0.8<br>0.3 | 2.67<br>1.00 | 25<br>25 |
| Carbon tetrachloride: ethanol | 16.7<br>4.1 | 0.1<br>0.1 | 0.33<br>0.33 | 20<br>15 |
| Carbon tetrachloride: methanol | 17.2<br>5.7 | 0.3<br>0.1 | 1.00<br>0.33 | 15<br>15 |

* g per 100 g of dry parsley seed
[1)]Extraction I
[2)]Extraction II

content in the seed were extracted (the value for *a* was 17.6 to 11.3 g per 100 g of dry parsley seed ) in extraction I, and 11.1 to 21.4% of the total lipids content in the seed (the value for *a* was 3.8 to 7.3 g per 100 g of dry parsley seed) in extraction II. This indicates a high cell destruction level in the plant material. In the slow extraction period of extraction I, 0.3 to 10.3% of the total lipids content in the seed (the *b* values being 0.1 to 3.5 g per 100 g of dry parsley seed), and in the slow extraction period of extraction II, 0.3 to 4.4% of the total lipids content in the seed (the *b* values being 0.1 to 1.5 g per 100 g of of dry parsley seed).

The value of coefficient *k* is between 0.33 x $10^{-2}$ and 11.67 x $10^{-2}$ g $min^{-1}$/100 g for extraction I, and between 0.33 x $10^{-2}$ and 3.33 x $10^{-2}$ g $min^{-1}$/100 g for extraction II. The fast extraction period at room temperature with various solvent mixtures lasts 15 to 25 minutes, for both extraction I and II (Table 2).

**The influence of extraction temperature on the total lipids extraction kinetics**. The investigation results on the influence of temperature on the total lipids extraction kinetics with the carbon tetrachloride:methanol mixture (2:1, v/v) are shown in Figure 2 and Table 3. The quantity of extracted total lipids in extraction I and II increased when the extraction temperature was increased. The quantity of lipids extracted at 40°C was higher 4.7% compared to the quantity extracted at room temperature; the quantity extracted at 50°C was 8.9% higher than that at 40°C, at 60°C it was 14.3% higher than that at 50°C, and the quantity extracted a thet

*Table 3. Maximum yields of the total lipids extracted with carbon tetrachloride:methanol (2:1, v/v) mixture at various temperatures*

| Mixture (2:1, v/v) | Extraction I | | Extraction II | | Total | |
|---|---|---|---|---|---|---|
| | Yield* | SE (%) | Yield* | SE (%) | Yield* | SE (%) |
| Room temperature | 17.5 | 51.3 | 5.8 | 17.0 | 23.3 | 68.3 |
| 40°C | 19.6 | 57.5 | 3.7 | 10.9 | 24.4 | 71.4 |
| 50°C | 21.7 | 63.6 | 4.9 | 14.4 | 26.6 | 78.0 |
| 60°C | 22.2 | 65.1 | 8.2 | 24.0 | 30.4 | 89.1 |
| Boiling temperature | 24.8 | 72.7 | 8.9 | 26.1 | 33.7 | 98.8 |

* g per 100 g of dry parsley seed

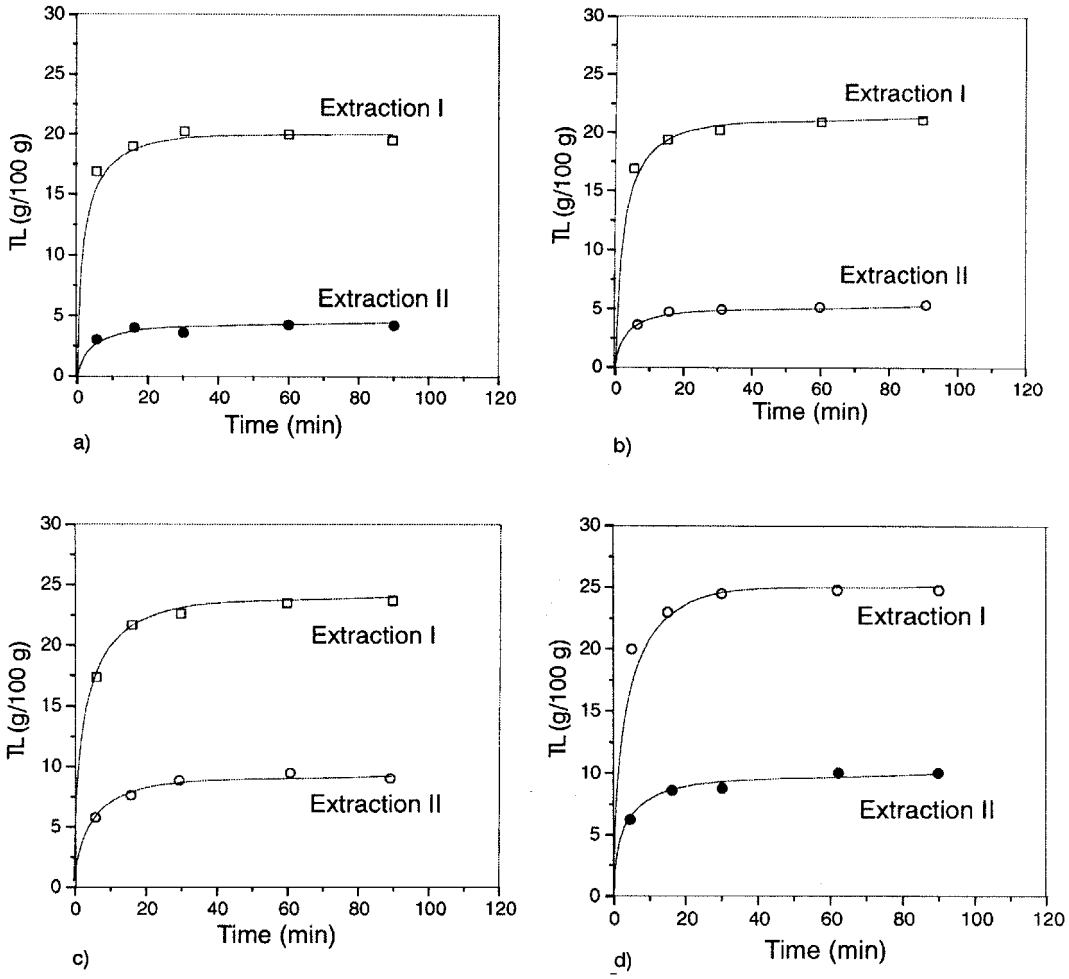


*Figure 2. Kinetics of extractions I and II of the total lipids with a carbon tetrachloride:methanol mixture (2:1, v/v) at various temperatures: a) 40°C, b) 50°C, c) 60°C, and d) boiling temperature*

*Table 4. Values of the coefficients a, b, and k in the equations for the total lipids extraction kinetics with a carbon tetrachloride: methanol (2:1, v/v) mixture at various temperatures*

| Mixture (2:1, v/v) | a* | b* | k x $10^{-2}$ (g $min^{-1}$/100g) | PBE (min) |
|---|---|---|---|---|
| Room temperature | 17.2[1)]<br>5.8[2)] | 0.3<br>0.1 | 1.00<br>0.33 | 20<br>15 |
| 40°C | 18.8<br>3.2 | 0.8<br>0.5 | 2.67<br>1.67 | 25<br>15 |
| 50°C | 18.7<br>4.2 | 3.0<br>0.7 | 10.00<br>2.33 | 25<br>20 |
| 60°C | 20.9<br>7.3 | 1.3<br>0.9 | 4.33<br>3.00 | 25<br>25 |
| Boiling temperature | 22.6<br>7.9 | 2.2<br>1.0 | 7.33<br>3.33 | 25<br>25 |

* g per 100 g of dry parsley seed
[1)]Extraction I
[2)]Extraction II

boiling temperature was 10.8% higher than the quantity extracted at 60°C. The highest quantity of total lipids (33.7 g per 100 g of dry parsley seed ) was extracted at the boiling point of the carbon tetrachloride:methanol mixture (2:1, v/v) (61±2°C). By the extraction of the total lipids at room temperature 51.3 and 17.0% of the total lipids content in the seed were extracted, at 40°C 57.5 and 10.9%, at 50°C 63.6 and 14.4%, at 60°C 65.1 and 24.0%, and at the mixture boiling temperature 72.7 and 26.1% of the total lipids content in the seed were extracted, after extraction I and extraction II, respectively.

In the fast extraction of the total lipids at various temperatures, in extraction I, 50.4 to 66.3% of the total lipids content in the seed were extracted (the *a* value was 17.2 to 22.6 g per 100 g of dry plant material) (Table 4), while in the fast extraction period of extraction II, 9.4 to 23.2% of the total lipids content in the seed were extracted (the *a* value was 3.2 to 7.9 g per 100 g of dry parsley seed). In the slow extraction period of extraction I, 0.9 to 8.8 % of the total lipids content in the seed were extracted (the *b* value was 0.3 to 3.0 g per 100 g of dry parsley seed), and in extraction II, 0.3 to 2.9% of the total lipids content in the seed (the value *b* was 0.1 to 1.0 g per 100 g of dry parsley seed).

According to Figure 2, the value of coefficient *k* for the extraction of the total lipids with the carbon tetrachloride:methanol (2:1, v/v) mixture at various temperatures (Table 4) is between 1.00 x $10^{-2}$ and 10.00 x $10^{-2}$ g $min^1$/100g for extraction I, and between 0.33 x $10^{-2}$ and 3.33 x $10^{-2}$ g $min^1$/100 g for extraction II, and the fast extraction period at various temperatures lasted 15 to 25 minutes for both extraction I and II.

The values of the kinetic parameters representing the qualitative characteristics of the fast and slow extraction periods are different and depend both on the type of solvent mixture and on the extraction temperature.

Thin layer chromatography detected nine spots with the following Rf values in the total lipids extract obtained under the optimal extraction conditions: 0.168, 0.223, 0.267, 0.329, 0.360, 0.422, 0.540, 0.671 and 0.937 originating from phospholipids, unidentified lipids, sterol, monoglyceride, diglyceride, free fatty acids, triglyceride, sterol and carbohydrates, respectively [16].

## CONCLUSION

The best extraction yield of the total lipids (33.7 g per 100 g of dry parsley seed) was achieved with a carbon tetrachloride:methanol (2:1, v/v) mixture at the boiling temperature after 30 minutes. In extraction I, 72.71% of the total lipids content in the seed were extracted, and in extraction II, 26.1%. Nine lipid fractions of the total lipids were separated by thin layer chromatography, among which there were phospholipids, sterol, mono–, di– and triacylglycerol, free fatty acids and carbohydrates. Regardless of the nature of the solvent mixture and the extraction temperature, the extraction kinetics of the total lipids from parsley seeds was characterized by periods of fast and slow extraction. The values of the kinetic parameters as the qualitative characteristics of the periods of fast and slow extraction depended on the solvent mixture and the extraction temperature.

## ACKNOWLEDGEMENTS

This work was supported under project MHT 2.11.0029.B. by the Ministry of Science and Technology of the Republic of Serbia, Serbia and Montenegro.

## NOTATION

TL – total lipids yield, (g per 100 g of dry parsley seed)
a – coefficient of the fast extraction period, i.e. part of the lipids extracted during the fast extraction period by the elution and dissolution of the total lipids from the surface of crushed seed cells, (g per 100 g of dry parsley seed)
b – part of the total lipids extracted during the period of slow extraction by diffusion from non–crushed plant cells, (g per 100 g of dry parsley seed)
k – coefficient of the slow extraction period, (g $min^{-1}$/100g)
t – extraction time, (min)

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## IZVOD

EKSTRAKCIJA UKUPNIH LIPIDA IZ SEMENA PERSUNA (*Petroselinum crispum* (Mill.) Nym. ex. A.W. Hill)

(Naučni rad)

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Masno ulje iz semena peršuna *Petroselinum crispum* (**Mill.**) **Nym. ex. A.W. Hill** ima veliku primenu u kulinarstvu i prehrambenoj industriji. Seme peršuna sadrži oko 20% masnog ulja, sa petroselinskom oleinskom, glikolnom i palmitinskom kiselinom kao glavnim komponentama. Cilj rada bio je da se ispita uticaj prirode smeše rastvarača i temperature ekstrakcije na kinetiku i sastav ekstrakta ukupnih lipida iz semena peršuna i izaberu optimalni uslovi ekstrakcije.

Za ekstrakciju lipida korišćene su smeše: hloroform:etanol, trihlorietil:metanol, petroletar:etanol, petroletar:metanol, trihloretilen:etanol, trihloetilen:metanol, ugljentetrahlorid:etanol i ugljentetrahlorid:etanol, u odnosu 2:1 v/v. Ekstrakcija je vršena primenom tehnike maceracije uz refluks i mešanje. Najveća količina ukupnih lipida (33,7 g po 100 g suvog samlevenog semena) ekstrahovana je smešom ugljentetrahlorid:metanol (2:1 v/v), na temperaturi ključanja reakcione smeše, za 30 minuta.

Prvom ekstrakcijom ekstrahovano je 72,7%, a drugom 26,1% ukupnih lipida. IR spektri ukazuju na prisustvo zasićenih i nezasićenih masnih kiselina, sterola i fosfolipida i isti kvalitativni sastav ekstrakta prve i druge ekstrakcije. Nazavisno od prirode smeše rastvarača i temperature ekstrakcije, kinetiku ekstrakcije ukupnih lipida iz semena peršuna karakteriše period brze i spore ekstrakcije i može se aproksimirati jednačinom kinetike ekstrakcije tipa UL = a + kt, karakterističnom za samleven biljni materijal. Vrednosti kinetičkih parametara koji su kvalitativne karakteristike perioda brze i spore ekstrakcije, zavise od prirode smeše rastvarača i temperature ekstrakcije.

Key words: Parsley seeds • *Petroselinum crispum* **Mill.** Nym. ex. A.W. Hill • Total lipids • Extraction •

Ključne reči: Seme peršuna • *Petroselinum crispum* **Mill.** Nym. ex. A.W. Hill • Ukupni lipidi • Ekstrakcija •