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SCIENTIFIC PAPER

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THE EXTRACTION OF OIL FROM TOBACCO (*Nicotiana tabacum* L.) SEEDS

The classical extraction (maceration) of oil from the seeds of a tobacco (Nicotiana tabacum L.) plant strain, type Otlja, was carried out using n–hexane and petroleum ether as extractimg solvents at different temperatures (25 °C, 40 °C and the boiling temperature) and seeds–to–solvent ratios (1:3, 1:5 and 1:10 w/v). The effects of the process factors on the kinetic parameters and the oil yield were assessed using the full factorial experiments 2^4. The oil yield was increased if the seeds were grounded before the extraction and by increasing the extraction temperature and by decreasing the seeds–to–solvent ratio. n–Hexane was somewhat more efficient than petroleum ether in the tobacco seed oil (TSO) extraction. The oil yield of 23.5 g/100 g of seeds (based on dry weight) was achieved using n–hexane at the seeds–to–solvent ratio of 1:10 w/v and the boiling temperature for the time of 60 minutes. The maceration was found to be less efficient in recovering the TSO than the Soxhlet extraction where the maximum oil yield of 31.1 g/100 of dry seeds was obtained. The kinetics of the oil extraction was described using the model of unsteady diffusion through plant material. The major fatty acid (FA) of oil recovered from the TSO was linoleic acid. The high acid value (37 mg KOH/g) indicated that the content of the free FA in the oil was quite high.

Key words: Extraction, Full factorial experiment, Maceration, Nicotiana tabacum L, Tobacco, Tobacco seed oil .

Tobacco seeds (TS), a by–product of tobacco leaf production, contain oil in a wide range of 33 to 40 % of the total seed weight [1], depending on the type of the tobacco plant and plantation area. The major triglycerides accounting for about 90% of the composition of the oil are trilinolein and palmitodilinolein [2], and the main fatty acids (FA) of tobacco seed oil (TSO) are linoleic (66 to 76 %), oleic, palmitic and stearic acid [1]. This oil is free from nicotine [3], but it contains a high content of cholesterol [4]. Its nutritional value is better than that of groundnut and cotton seed oil and simmilar to that of safflower oil [3].

There is no existing usage of TSO except in soap production as well as in paint and varnish industries as a solvent [5]. All over the world, an effort is currently being made to develop alternative uses of TSO, such as in medicine [6] or biodiesel production [7,8]. Recent studies have suggested that TSO, as a renewable source of energy, may be an appropriate substitute for diesel fuel in both raw [7] and chemically modified [8] forms. The physical, chemical and fuel related properties of TSO are comparable to those of other vegetable oils and to current European specifications for automotive diesel fuel [7]. The methanolysis process, catalyzed by sodium hydroxide at the reaction temperature of 55 °C, was applied to produce biodiesel based on TSO, which was proven to be suitable for partial substitution for diesel fuel at most operating conditions [8].

The oil can be extracted from TS by either pressing, or solvent extraction. So far, the Soxhlet extraction technique has been applied for TSO extraction using n–hexane [1], petroleum ether [7] or diethyl ether [8] as extracting solvents. To the author's best knowledge, there is no available information on the kinetics of oil extraction from TS.

The present work deals with the classical extraction (maceration) of oil from the native and grounded seeds of a semi–oriental tobacco plant strain, type Otlja, using n–hexane and petroleum ether as extracting solvents at different temperatures (25 °C, 40 °C and the boiling temperature) and seeds–to–solvent ratios (1:3, 1:5 and 1:10 w/v). The main goals were to reflect the mechanism and kinetics of the extraction process, to compare the extraction efficiencies of two solvents from both the native and grounded tobacco seeds at different temperatures and seeds–to–solvent ratios and to analyze the fatty acid (FA) composition of the TSO recovered at different process conditions. The effects of the operating conditions on the kinetic parameters and the oil yield were assessed using the full factorial experiments 2^4. The aims were also to compare different extraction techniques (maceration versus Soxhlet) with respect to the yield and composition of the TSO, and, to define the optimum extraction conditions.

EXPERIMENTAL

Materials

The seeds of a semi–oriental strain of tobacco, type Otlja, were used in all experiments. The seeds were collected after the harvest of tobacco leaves from the fields in south Serbia, dried immediately after harvesting in a shady and well–aired place for 15 days, packed in

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paper bags and stored in a dark and dry place at room temperature. Before being used, the seeds were grounded by an electrical mill with a fast–rotating knife (15 000 rpm; 1 minute) to a fine powder. Moisture content, determined by drying the grounded TS at 105 °C to constant weight, was 5.5 %. The TSO content of 31.1 g/100 of dry seeds was determined by the Soxhlet extraction technique, using n–hexane or petroleum ether as extracting solvents at the seeds–to–solvent ratio of 1:10 w/v [9].

Petroleum ether (b.p. 50–70 °C) and n–hexane from Merck (Darmstadt, Germany) were used as extracting solvents. Sodium methoxide used for preparing the methyl ester of the FA present in the TSO was also from Merck. The standard for methyl esters, contained methyl esters of palmitic, stearic, oleic, linoleic and linolenic acids (20 % of each ester), was obtained from Sigma (St. Louis, MO). Concentric sulphuric acid and anhydrous sodium sulphate were purchased from Zorka–Pharma (Šabac, Serbia and Montenegro) and Merck–Alkaloid (Skopje, FYR Macedonia) respectively.

Extraction of TSO

The native or grounded TSO (5 g) was taken to Erlenmayer flasks (100 ml) placed in a thermostated water bath and the predetermined volume of extracting solvent was added; the seeds– to–solvent ratio was 1:3, 1:5 or 1:10 w/v. The extraction was performed at 25 °C, 40 °C and boiling temperature for 2.5, 5, 10, 20, 40 and 60 minutes. The temperature was controlled and maintained at the desired level (±0.1 °C). At the end of the extraction cycle, the liquid extract was separated from the solid residue by vacuum filtration. The seed cake was washed twice with fresh solvent (20 ml). The filtrates were collected and the solvent was evaporated to the constant weight of the oil using a rotary vacuum evaporator at 50 °C. The yield of oil (in g/100 g of dry seeds) after certain extraction time was calculated from the mass of recovered oil and the mass of seeds.

Successive extraction of TSO

In order to increase the extraction efficiency, six successive extractions of TSO were performed using n–hexane. In this process, the residual seed material, after separation of the liquid extract, from one extraction batch was treated with the fresh solvent at 25 °C for 20 minutes. The seeds– to–solvent ratio was 1:3 w/v. Each filtrate was evaporated to the constant weight of using a rotary vacuum evaporator at 50 °C.

Fatty acid composition of TSO

FA composition of TSO was analyzed by gas chromatography after the FA had been transformed into methyl esters [10]. The methyl esters were prepared by treating the oil (1 g) with sodium methoxide (10 ml) under reflux for 1 h. Then, distilled water (10 ml) was added, followed by 3–4 drops of concentrated sulphuric acid. The methyl esters of the oil were extracted with chloroform and the solvent was removed by evaporation. Finally, the water present in the oil was removed by treating the oil with anhydrous sodium sulphate.

Methyl esters of TSO were analysed using an Agilent 6850 Gas Chromatograph, equipped with a DB WAX capillary column (30 m x 0.25 mm x 0.25 μm) and a flame ionization detector. The following temperature program was used: column, 50 °C, 1 min, 25°C/min to 50–200°C and 3°C/min to 230 °C; injector, 250°C; and detector, 280°C [11]. Nitrogen, hydrogen and air at flow rates of 20, 30 and 400 ml/min, respectively, were used for the analysis. The volume of oil injected was 0.2 μL. The methyl esters of palmitic, stearic, oleic, linoleic and linolenic acids were identified by comparison of retention times of the oil components with those of standard methyl esters. By using the standard mixture of FAME (Sigma, St. Louis, MO), calibartion curves were generated and used for the quantification of the methyl esters of TSO present in the samples.

Physical and chemical properties of TSO

Physical and chemical properties of the TSO, extracted using *n*–hexane at the seeds–to–solvent ratio of 1:3 w/v and 25 °C for 20 minutes, were analyzed using standard procedures. The density, viscosity and refraction index of the TSO were measured at 20 °C by a pycnometer [12], a viscosimeter [13] and a refractometer [14], respectively. The iodine and saponification values were determined by the AOCS methods [12]. The acid value of the reaction mixture was determined by the acid–base titration technique [12], except that a standard solution of NaOH was used instead of the standard solution of KOH.

RESULTS AND DISCUSSION

The mechanism, kinetics and efficiency of TSO extraction using *n*–hexane and petroleum ether as extracting solvents, together with the FATSO composition and the physico–chemical properties of TSO were studied. Various factors, which can affect the recovery of oil from native and grounded TS, were considered in the study. These parameters included: the extraction techniques (maceration, successive maceration and Soxhlet extraction), seed comminution, type of organic solvent, the seeds–to–solvent ratio and the temperature of extraction. The effects of these factors on the kinetic parameters and the oil yield were analyzed using the full factorial experiments 2^4.

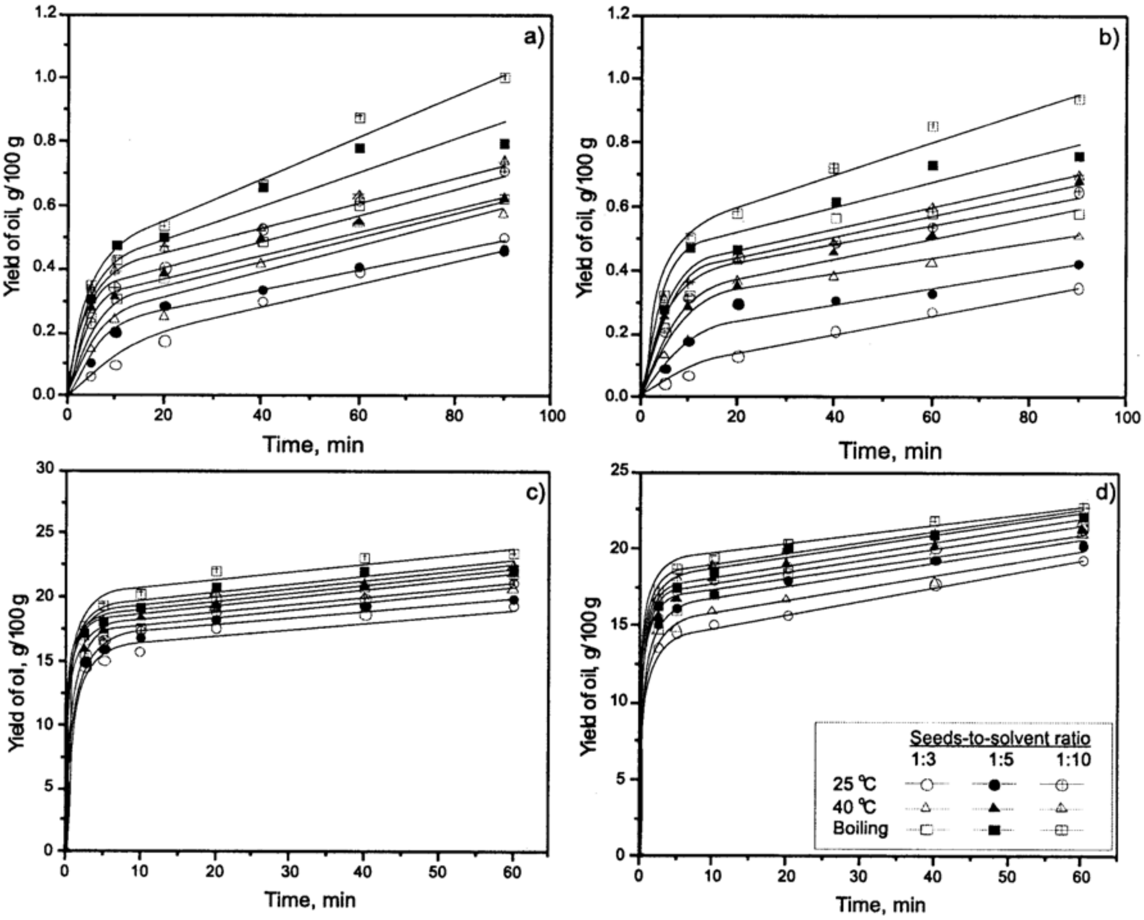

Figure 1. Variation of the oil yield with the progress of maceration at the seeds-to-solvent ratios of 1:3, 1:5 and 1:10 g/ml at 25 °C, 40 °C and boiling temperature using native seeds and a) n-hexane or b) petroleum ether as well as grounded seeds and c) n-hexane or d) petroleum ether

Mechanism of extraction of TSO

The extraction of the oil from native and grounded TS using *n*-hexane and petroleum ether at seeds-to-solvent ratios of 1:3, 1:5 and 1:10 w/v and 25 °C, 40 °C and boiling temperature was studied. The time-course of change of the oil yield in the liquid extracts during the extraction is shown in Fig. 1. Curves are typical for extraction of extractive substances from plant materials. For both native (Fig. 1a and b) and grounded (Fig. 1c and d) TS, two periods of extraction are observed: washing, characterized by a rapid increase in the yield of oil at the early beginning of the process, and slow extraction (approximately after the first 10 minutes and 2.5 to 5 minutes for native and grounded tobacco seeds, respectively), characterized by a slow increase in the oil yield with the progress of extraction. The optimum time for extraction is approximately 60 and 20 minutes, for native and grounded TS respectively, ensuring that the equilibrium oil yield in liquid extracts was nearly achieved.

Kinetics of extraction of TSO

The kinetics of the extraction process of bioactive substances from plant materials has most often been modelled using the unsteady diffusion through plant material [15,16], the film theory [17,18] and the empirical equation of Ponomaryov [15,19]; derivation of model equations can be found elsewhere [18]. The models are based on the two-stage extraction mechanism and all three are two-parametric. One parameter characterizes the washing stage (so called washing coefficient) and the second one characterizes the slow extraction (so called slow extraction coefficient).

For the purpose of mathematical modeling of the extraction of TSO, the first model, as fitting the experimental data satisfactory, being physically based and relatively simple, was applied in the present study. The basic equation of this kinetic model is as follows [18]:

$$\frac{q}{q_o} = (1 - b')\, e^{-k't} \qquad (1)$$

or its linearized form

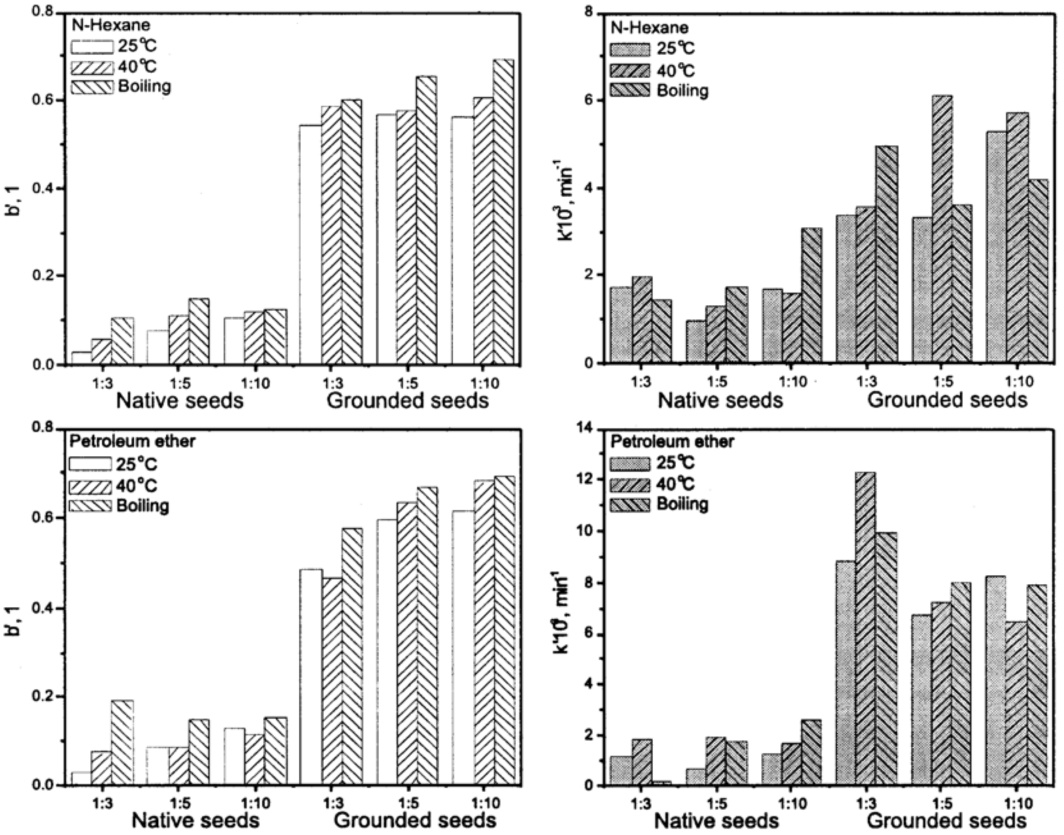

Figure 2. Dependence of kinetics parameters of the model of unsteady diffusion through plant material on the seeds-to-solvent ratio for the extraction of oil from native and grounded TS

$$\ln\left(\frac{q}{q_0}\right) = \ln(1 - b') - k't \qquad (2)$$

where b' is the washing coefficient, 1; k' is the slow extraction coefficient, $\min^{-1}$; q is the oil content in the seeds during the extraction, g/100 g; q_o is the oil content initially present in the seeds, g/100 g; and t is time in minutes. The difference (q_o – q) is the amount of oil dissolved in the solvent. The oil yield recovered from the grounded TS by the Soxhlet extraction was adopted as the initial content of TSO.

The parameters of the kinetic model were calculated from the experimental data by means of the linear regression method using the equation (2). The values of both kinetic parameters are presented in Fig. 2 (the coefficient of linear correlation was higher than 0.90). Both kinetic parameters depended on the comminution of the seeds, the type of solvent, the seeds–to–solvent ratio and the extraction temperature.

The values of the washing coefficient were approximately 3 to 18 times higher for the grounded seeds than for the native ones. By seed comminution, not only the protective seed coat but also many cells from the seed interior were destroyed, enabling more oil to come into direct contact with the solvent and dissolve. The washing coefficient increased with increasing the extraction temperature, due to the increase of the oil solubility, and with decreasing the seeds–to–solvent ratio, probably due to the increase of the driving force for oil mass transfer from the surface of seed particles into the bulk of liquid extract. The effect of solvent type on the washing coefficient depended on the seeds–to–solvent ratio. For the seeds–to–solvent ratio of 1:3 w/v, the washing coefficient was higher for n–hexane (about 4 to 20 %), while for the seeds–to–solvent ratios of 1: 5 and 1:10 w/v, it was higher for petroleum ether (about 2 to 12 %). The slow extraction coefficient was about 10 times higher for the grounded seeds than for the native ones, due to shorter distances which the oil should diffuse from the interior of seed particles to the surface.

Oil yield

The yield of oil from the TS, recovered after 60 minutes of extraction, depended on the comminution of the seeds, the type of solvent, the seeds–to–solvent ratio and the working temperature. As can be concluded from Fig. 3, the importance of these factors decreased in the following order: comminution of seeds, working temperature, seeds–to–solvent ratio and type of solvent.

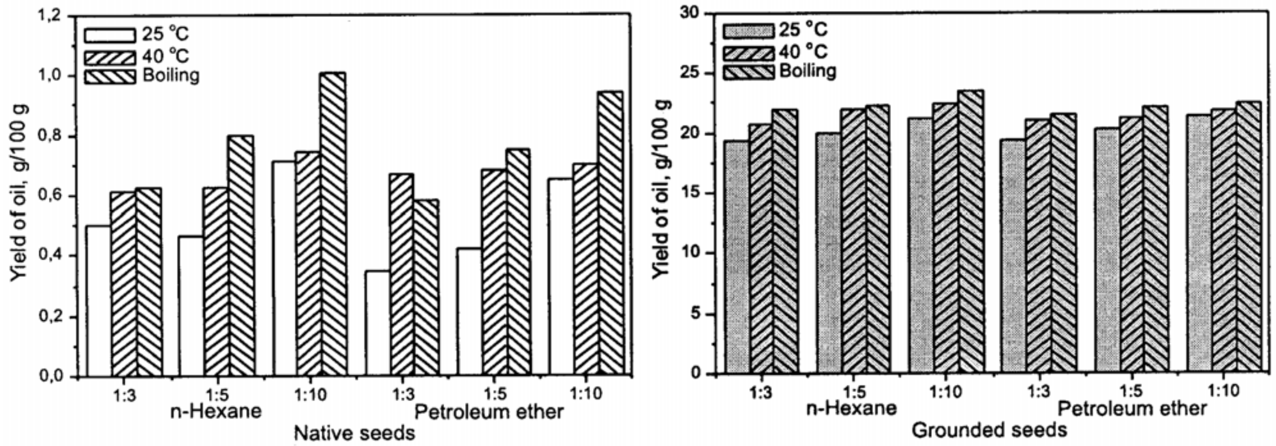

Figure 3. Effects of seed comminution, solvent type, seeds-to-solvent ratio and temperature on the yield of TSO

Effect of seed comminution

The oil yield from the grounded seeds was 24 to 56 times higher than from the native ones. This was attributed to destroying both the protective seed coat and the cells from the interior of seeds, so that more oil was able to come in the direct contact with the solvent and to dissolve. The reduced resistance to the mass transfer through plant particles, due to shorter distances which the oil should diffuse from the interior of seed particles to the surface, contributed also to raise the oil yield with the seed comminution. The effect of the seed comminution on the oil yield depended on the other operating conditions, the combined effect of the seed comminution with the temperature being the most effective, the oil yield being in average 39, 36 and 29 times higher at 25 °C, 40 °C and boiling temperature, respectively, if the seeds were grounded before extraction. The effect of the seed comminution decreases with increasing the seeds–to–solvent ratio.

Effect of temperature

The oil yield increased with increasing the extraction temperature. This was attributed to the enhanced solubility of the oil in the extracting solvent at higher temperatures. Also, the higher the extraction temperature, the lower the viscosity of extracting solvent decreased. This would cause the increase in the oil diffusion coefficient, contributing to the increase of oil mass transfer from the interior of plant particles into the solution. The extraction temperature affected significantly the yield of oil from the native seeds, but only little that from the grounded ones. The yield of oil from the native and grounded TS was twice to four times and about 8 to 17 % higher at boiling temperature than at 25 °C, respectively.

Effect of seeds–to–solvent ratio

The oil yield increased with decreasing the seeds–to–solvent ratio from 1:3 to 1:10 w/v, independently of the other extraction conditions, due to a higher driving force in the more diluted solution. This effect was more important for the native seeds, where the increase was 25 to 42 %, than for the grounded ones, where the oil yield was increased only 1 to 9 %.

Effect of extracting solvent

The oil yields, obtained by using n–hexane and petroleum ether, were similar, although n–hexane was somewhat more efficient than petroleum ether. This effect was more important for the native seeds than for the grounded ones.

Effect of extraction technique

Figure 4 compares the oil yields obtained from single–extraction of 20 minutes, of six 20–minutes successive extraction cycles and the Soxhlet extraction using *n*–hexane. The repeated extraction of TSO, which

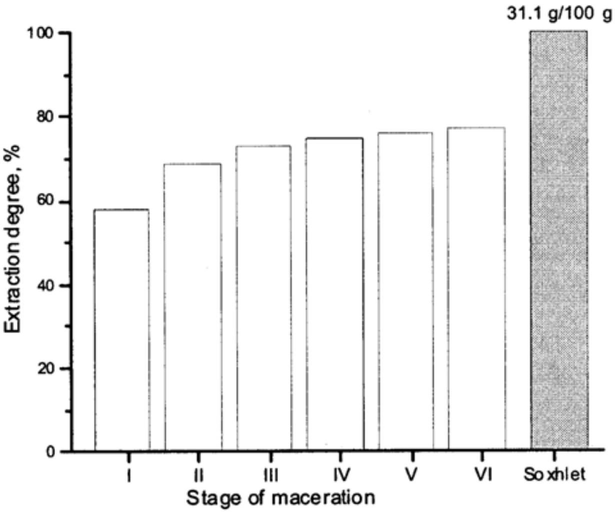

Figure 4. Efficiency of successive extraction of TSO compared to the Soxhlet extraction (grounded seeds; n-hexane; seeds-to-solvent ratio of 1:3 w/v; 25 °C; and duration of an extraction cycle: 20 minutes)

was applied in order to increase the extraction efficiency, was performed by treating the residual seed material from the previous extraction batch with the fresh solvent. The extraction conditions were as follows: the seeds–to–solvent ratio of 1:3 w/v and temperature of 25 °C. The oil yield increased in the repeated extraction, but it was only 77 % of that achieved by the Soxhlet apparatus.

The classical extraction was less efficient than the Soxhlet extraction technique in recovering the TSO. The values of the oil yield from the native and grounded seeds, achieved by using *n*–hexane and the seeds–to–solvent–ratio of 1:10 w/v at the boiling temperature, were only 3.3 and 75.7 % of the yield obtained by the Soxhlet extraction (31.1 g/100 g of seeds), respectively. The advantage of the extraction was relatively high oil yields at 25 °C using both solvents at all seeds–to–solvent ratios. Oil yields from the grounded seeds for n–hexane and petroleum ether were in the ranges between 62.4 to 75.7 % (19.4 to 23.5 g/100 g of seeds) and 62.1 to 71.9 % (19.3 to 22.4 g/100 g of seeds) of that obtained by the Soxhlet extraction, respectively.

Table 1. Process factors

Notation for factor	Process factor	Lower level (–)	Upper level (+)
A	Seed comminution	Native seeds	Grounded seeds
B	Type of solvent	*n*-Hexane	Petroleum ether
C	Seeds to solvent ratio (g/ml)	1:3	1:10
D	Temperature	25 °C	Boiling temperature

Table 2. Full factorial experiment 2^4

No. trials	Design matrix					Response		
	(X_0)	Factor A (X_1)	Factor B (X_2)	Factor C (X_3)	Factor D (X_4)	b 1	$k \cdot 10^3$ min^{-1}	Oil yield[a] g/100 g
1	+	–	–	–	–	0.028	1.73	0.42
2	+	+	–	–	–	0.542	3.38	19.41
3	+	–	+	–	–	0.026	1.15	0.27
4	+	+	+	–	–	0.482	8.82	19.31
5	+	–	–	+	–	0.107	1.70	0.62
6	+	+	–	+	–	0.561	5.29	21.21
7	+	–	+	+	–	0.127	1.26	0.54
8	+	+	+	+	–	0.616	8.23	21.31
9	+	–	–	–	+	0.104	1.43	0.60
10	+	+	–	–	+	0.603	4.94	21.94
11	+	–	+	–	+	0.191	0.14	0.58
12	+	+	+	–	+	0.578	9.92	21.52
13	+	–	–	+	+	0.126	3.06	0.93
14	+	+	–	+	+	0.692	4.16	23.53
15	+	–	+	+	+	0.151	2.63	0.86
16	+	+	+	+	+	0.693	7.87	22.37

[a] Oil yield achieved after 60 minutes of extraction

Full factorial experiment 2^4

The effects of four process factors (Table 1), namely the seed comminution (A), the type of solvent (B), the seeds–to–solvent ratio (C) and the temperature (D) on the kinetic parameters and the oil yield, were assessed using the matrix of full factorial experiment 2^4 with no replication, as shown in Table 2.

A linear first–order regression model was used to connect the response with the factors and their interactions:

$$\begin{aligned} y = {} & b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + \\ & + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{14}X_1X_4 + b_{23}X_2X_3 + \\ & + b_{24}X_2X_4 + b_{34}X_3X_4 + b_{123}X_1X_3 + b_{124}X_1X_2X_4 + \\ & + b_{134}X_1X_3X_4 + b_{234}X_2X_3X_4 + b_{1234}X_1X_2X_3X_4 \end{aligned} \qquad (3)$$

where y is the response value, X_i are the levels of *i*–factor, b_0 is the intercept, which corresponds to the response for $x_i = 0$, b_i are linear regression coefficients and b_{ij} are regression coefficients of two–, three– or four–way interactions. The values of all linear regression coefficients are given in Table 3.

The important factors were screened out by the analysis of variance, the main results being given in Table 4. For the washing coefficent, the important factors with the significance level of 0.05 are the comminution of seeds, the seeds–to–solvent ratio and the process

Table 3. Linear regression coefficients for the kinetic parameters and the oil yield

Regression coefficient	Washing coefficient	Slow extraction coefficient	Oil yield
b_0	0,352	4,11	11,00
b_1	0,244	2,47	10,33
b_2	0,006	0,90	-0,12
b_3	0,032	0,17	0,47
b_4	0,040	0,16	0,57
b_{12}	-0,010	1,24	-0,08
b_{13}	0,012	-0,36	0,31
b_{14}	0,005	-0,02	0,45
b_{23}	0,006	-0,17	-0,03
b_{24}	0,005	-0,02	-0,09
b_{34}	-0,009	-0,01	-0,07
b_{123}	0,011	-0,30	-0,04
b_{124}	-0,007	0,06	-0,11
b_{134}	0,016	-0,51	-0,10
b_{234}	-0,011	0,12	-0,07
b_{1234}	0,000	0,03	-0,05

Table 4. Results of analysis of variance

Source of variation	Degrees of freedom	Washing coefficient		Slow extraction coefficient		Oil yield		$F_{1;5;0.95}$
		Mean square	Test statistic	Mean square	Test statistic	Mean square	Test statistic	
A	1	0.954	552.28	97.57	82.24	1706.35	18122.55	6.61
B	1	0.001	0.39	12.83	10.82	0.23	2.41	
C	1	0.017	9.73	0.45	0.38	3.51	37.26	
D	1	0.026	15.18	0.42	0.35	5.14	54.61	
AB	1	0.002	0.92	24.53	20.67	0.10	1.05	
AC	1	0.002	1.39	2.04	1.72	1.56	16.52	
AD	1	0.000	0.24	0.00	0.00	3.21	34.12	
BC	1	0.001	0.38	0.48	0.40	0.01	0.14	
BD	1	0.000	0.21	0.01	0.01	0.14	1.44	
CD	1	0.001	0.77	0.00	0.00	0.08	0.85	
ABC+ABD+ACD+ BCD+ABCD	5	0.002		1.186		0.09		
Total	15							

temperature,while the effects of the solvent type are ignorable. For the slow extraction coefficient, the important factors with the significance level of 0.05 are the comminution of seeds, the type of solvent and the interaction of seed grounding and type of solvent, while the effects of the seeds–to–solvent ratio and the process temperature are not significant.

The experiment was done with no replication, so the residual variance was determined based on the variance of three– and four–way interactions. That these interactions could be included into the residual variance, it was checked by the Bartlett's test for homogeneity of variances. Bartlett test statistics of 6.17, 5.62 and 1.00 in the case of washing coefficient, slow extraction coefficient and oil yield, respectively were smaller than the upper critical value of the chi–square distribution with four degrees and a significance level of 0.05($\chi^2_{4;0.05} = 9.49$). Thus, the variances of three– and four–way interactions were judged to be equal.

If the factors and interactions, which are not statistically significant with the significance level of 0.05, are omitted, the linear regression equations for the washing coefficient, the slow extraction coefficient and the oil yield are as follows (coefficient of linear correlation, R^2 = 0.984, 0.935 and 0.999), respectively:

$$y = 0.352 + 0.244\,X_1 + 0.032X_3 + 0.040X_4 \qquad (4)$$

$$y = 4.11 + 2.47X_1 + 0.90X_2 + 1.24X_1X_2 \qquad (5)$$

and

$$y = 11.00 + 10.33X_1 + 0.47X_3 + 0.57X_4 + + 0.31X_1X_3 + 0.45X_1X_4 \qquad (6)$$

By analyzing the linear regression equations, one can conclude about the effects of each factor and interaction of factors on washing and diffusion processes during the extraction of TO as well as on the oil yield. As washing process depends on the availability of TO for dissolution and its solubility in the extracting solvent, it is normal that grounding, temperature and solvent volume primarily affect the washing coefficient. Obviously, the solubility of the TO is approximatelly the same in two extracting solvents used in this study. Equation (5) confirms that the slow extraction process is affected primarily by the resistence to diffusion of oil from the interior of seed particles to their surface, which depends on both the particle size (the major factor) and the type of solvent. The important factors with the significance level of 0.05 affecting the oil yield are the comminution of seeds, the process temperature and the seeds–to–solvent ratio, as well as the interactions of seed grounding with temperature and the with the seeds–to–solvent ratio. Either n–hexane or petroleum ether can be used as extracting solvent. Thus, to achieve a high yield of TO, TS must be grounded before extraction, and extraction should be carried out at the boiling temperature of the extracting solvent chosen and at higher seeds–to–solvent ratios.

FATSO composition

The FA composition of the oils recovered from the grounded seeds at 25 °C and boiling temperature, using *n*–hexane or petroleum ether in the classical and Soxhlet extractions, are compared in Table 5. With the method used for the FA composition determination, very good reproducibility was obtained and the standard deviation of the relative areas was smaller than 1 % for each FAME [11]. Independently of the extraction technique, the extracting solvent and the operating temperature, the FATSO composition was dominated by linoleic, oleic, palmitic and stearic acids (greater than 95%). The total

Table 5. Effects of extraction technique, solvent type and operating temperature on FATSO composition (in %)

Fatty acid	Maceration[a]				Soxhiet[b]	
	n-Heksane		Petroleum ether		*n*-Heksane	Petroleum ether
	25°C	BT[c]	25°C	BT		
Palmitic	8.50	8.52	8.51	8.50	8.42	8.69
Stearic	2.77	4.72	2.80	5.05	3.78	2.90
Oleic	12.49	14.72	12.36	14.29	11.78	12.54
Linoleic	71.28	69.97	70.3	69.92	72.22	72.92
Linolenic	0.93	1.06	0.98	1.03	0.84	0.89
Others	4.03	1.01	5.05	1.21	2.96	2.06
Saturated	11.27	13.24	11.31	13.55	12.20	11.59
Unsaturated	84.7	85.75	83.64	85.24	84.84	86.35
Mono–unsaturated	12.49	14.72	12.36	14.29	11.78	12.54
Poly–unsaturated	72.21	71.03	71.28	70.95	73.06	73.81

[a]Seeds–to–solvent ratio: 1:3 w/v; and duration of extraction: 20 minutes (this work).
[b]Seeds–to–solvent ratio: 1:10 w/v; and duration of extraction: 90 minutes (unpublished data). [c]BT – boiling temperature.

saturated (palmitic and stearic) and unsuturated (oleic, linoleic and linolenic) FA compositions were in the ranges between 11.3 to 13.5 % and 84.9 to 86.4 %, respectively. The FATSO composition for the Otlja tobacco is similar to those originated from different world regions, with nearly the same contents of major FA, as can be seen in Table 6.

Physical and chemical properties of TSO

Some physical and chemical properties of TSO of different origin are compared in Table 7. The values of the physical and chemical properties of the TSO are in the standard ranges for TSO as suggested by the AOCS [29]. The acid value of the oil (37 mg KOH/g of oill) obtained from the Otlja TS was quite high, indicating a high content of free FA (higher than 18 % of the oil).

CONCLUSIONS

This study deals with the mechanism, kinetics and efficiency of TSO extraction using n–hexane and petroleum ether as extracting solvents, as well as with the FATSO composition and the physical and chemical properties of TSO recovered.

Similarly to any extraction of bioactive substances from plant materials, the TSO extraction occurred via two steps, namely washing and diffusion. In the former step, the TSO extraction was rapid, due to dissolution of oil from surfaces of TS particles, while the latter one was slow, due to diffusion of oil from the interior of TS particles towards their free surface. Based on the mechanism observed, the kinetics of TSO extraction was described by the two–parametric model of unsteady state diffusion of the oil through TS particles.

The efficiency of TSO extraction was affected by the type of extraction technique, the TS comminution, the type of solvent, the seeds–to–solvent ratio and the operating temperature. The oil yield of 23.5 g/100 g of seeds (based on dry weight) was achieved using

Table 6. Review on FATSO composition

Reference	Tobacco type	Fatty acid[a]										
		(14:0)	(16.0)	(16:1)	(18:0)	(18:1)	(18:2)	(18:3)	Others	Saturated	Mono–unsaturated	Poly–unsaturated
This work[b]	Otlja	–	8.26	–	3.66	12.17	70.21	0.89	4.81	11.92	12.17	71.10
[2]	NA	–	7.9	–	3.1	17.1	70.6	1.1	0.2	11	17.1	71.7
[7]	NA[c]	0.09	10.96	0.2	3.34	14.54	69.49	0.69	0.69	14.39	14.74	70.18
[20]	P–23	0.02	10.13	0.09	2.67	13.71	72.34	0.94	0.1	12.82	13.71	73.38
[21]	NA	–	3.0	–	5.0	16.0	70.0	–	6	8	16	70
[22]	NA	0.17	8.87	–	3.49	12.4	67.75	4.20	3.12	12.53	12.4	71.95
[23]	NA	0.1	11.5	0.2	3.4	14.1	68.1	0.6	2	15	14.3	68.7
[24]	NA	0.7	9.4	–	–	13.7	70.7	0.9	4.6	10.1	13.7	71.6
[25]	Kentucky 104	–	9.5	0.1	2.8	10.6	74.9	1.1	1	12.3	10.7	76
	Bright Italia	–	9.2	0.1	2.5	9.5	76.1	1.4	1.2	11.7	9.6	77.5
	Bright V.	–	8.9	0.1	2.6	11.1	75.1	1.1	1.1	11.5	11.2	76.2
[26]	NA	–	6.1	–	–	7.2	85.2	–	1.5	6.1	7.2	85.2
[27]	Golsoor	0.02	12.0	–	3.30	12.04	72.60	–	0.04	15.32	12.04	72.6
	Rawandoz	–	10.60	–	3.90	14.90	70.20	–	0.4	14.5	14.9	70.2
[28]	NA	–	9.60	–	2.80	12.10	74.10	1.40	0	12.4	12.1	75.5

[a]Miristic (14:0); palmitic (16.0); palmitoleic (16:1); stearic (18:0); oleic (18:1); linoleic (18:2); and linolenic (18:3); [b]Maceration (*n*–hexsane, ratio of seeds to solvent 1:3, 20 minutes, 25°C); [c]NA – not available.

Table 7. Physical (20 °C) and chemical properties of TSO

Property	This work[a]	[2]	[5]	[20]	[21]	[26]	[29]
Density, kg/m^3	924.0	920.0	917.5	923.3	NA[b]	923.1	923– 925
Viscosity, mPas	93.3	NA	27.7[c]	NA	NA	54.28	NA
Index of refraction	1.4739	1.4740	NA	1.4758[d]	1.4690	1.4758	1.474–1.483
Iodine value, g J_2/100 g of oil	130	137	135	135	145	143.8	129–142
Saponification value, mg KOH/g of oil	193.9	189.6	193	197	190.9	193.56	186–197
Acid value, mg KOH/g of oil	37	NA	NA	1.34[e]	NA	NA	NA

[a]The oil obtained by maceration (*n*–hexsane, ratio of seeds to solvent 1:3, 20 minutes, 25°C); [b]NA; [c]At 40 °C; [d]AtAt 25 °C; [e]Free FA (%)

n–hexane at the seeds–to–solvent ratio of 1:10 w/v and the boiling temperature for 60 minutes, but it was lower than that obtained by the Soxhlet extraction (31.1 g/100 of dry seeds). The oil yield increased in the six 20 minute successive extraction cycles, but it was also smaller than that of the Soxhlet extraction.

The statistical analysis of the effects of the important process factors on the oil yield showed that, to achieve a high yield of TO, TS must be grounded before the extraction, and the process should be carried out at the boiling temperature of the extracting solvent chosen and at higher seeds–to–solvent ratios. Either *n*–hexane or petroleum ether can be used as extracting solvent, but the former is more acceptable than the latter as being less volatile and less flammable. For the economic reasons (such as smaller solvent cost), the ratio of grounded TS to extracting solvent of 1:3 w/v should be considered for industrial extraction process.

Independently of the extraction technique, the extracting solvent and the operating temperature, The FATSO composition was dominated by linoleic (70 to 73 %), oleic (12 to 15 %), palmitic (about 8.5 %) and stearic (2.8 to 4.7 %) acids. With increasing temperature, the content of saturated and mono–unsaturated FA increased, while the content of poly–unsaturated FA decreased.

Classical solvent extraction (maceration) using *n*–hexane gave reasonable oil yields from grounded TS. Due to its inherent disadvantages such as the need of solvent evaporation, adding extra cost, novel extraction techniques (such as CO_2–extraction) or pressing should be considered and tested to extract oil from TS.

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ABBREVIATIONS

FA	– Fatty acids
TS	– Tobacco seed
TSO	– Tobacco seed oil
FATSO	– Fatty acid tobacco seed oil

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