COMPOSITION OF TOBACCO SEED OILS*

M. Zlatanov, M. Angelova, G. Antova

University of Plovdiv "P. Hilendarski", Department of Chemical Technology, Plovdiv, Bulgaria

The content and composition of lipids isolated from the seeds of seven Bulgarian tobacco species were investigated. Glyceride oil in the seed was found to be 30.1–41.3%. The biological active substances – fatty acids, phospholipids, sterols and tocopherols were studied. Palmitic (16.4–27.7%), oleic (17.6–39.5%) and linoleic acids (5.2–51.9%) predominated in the oils. The content of phospholipids, sterols and tocopherols in the oils was 1.0–1.7%, 0.3–0.8% and 5–72 mg/kg respectively. Phosphatidylcholine (35.2–41.8%), phosphatidylinositol (23.8–29.8%) and phosphatidylethanolamine (11.6–21.1%) were found to be the main components in the phospholipid fraction. β-sitosterol (59.4–65.9%), stigmasterol (10.4–13.9%) and kampesterol (9.6–14.8%) predominated in the sterol fraction. γ-tocopherol (76.5–96.6%) was the main component in the tocopherol fraction.

tobacco seed oil; fatty acids; phospholipids; sterols; tocopherols

INTRODUCTION

Tobacco seeds are a by-product of leaves production of tobacco (*Nicotiana tabacum* L., family Solanaceae). The seeds can give glyceride oil, which is used as a raw material in the coating industry, for the preparation of printing inks, dyes, etc. The content of oil in the seeds is about 30.0%, mainly trilinolein and palmitodiolein (Frega et al., 1991; Giannelos, 2002; Mukhtar et al., 2006). According to Zhang et al. (2005) the content of oil in mature seed of transgenic lines reduced by 9 to 49%. The chemical composition of glyceride oil is important for the finding of new materials for industry and alternative applications (Patel et al., 1998; Talaqani et al., 1986). On the other hand, the composition of the oil depends considerably on temperature conditions and humidity in the fatty acids and some biologically active substances, such as sterols, phospholipids and tocopherols. The chemical characterization of *Nicotiana tabacum L.* seeds is important in the search of alternatives of oil and meal. The knowledge of the lipid composition of the seeds has taxonomic significance for plant classification and is useful for preserving seed purity.

Tobacco is growing in large areas in Bulgaria and a significant amounts of seeds are recovered as a by-product. The utilization of these seeds presents a great interest for tobacco manufacturing industry.

The present investigation provided the content of seed oil and the composition of fatty acids, phospholipids, sterols and tocopherols of the oils in seven Bulgarian tobacco species.

MATERIAL AND METHODS

Fruit material. The investigated tobacco seeds were provided by the Plovdiv Institute for investigation of tobacco. The investigations were carried out with air dried seeds.

Glyceride oil isolation. The oils were extracted in Soxhlet apparatus with n-hexane for 8 h. After rotation vacuum distillation of the solvent the extracted oils were weighed.

Fatty acid composition. The fatty acid composition of triacylglycerols was identified by capillary gas chromatography of their methyl esters. The esterification was carried out by the Metcalfe and Wang technique (Metcalfe, Wang, 1981). Methyl esters were purified by thin-layer chromatography. Determination was accomplished on a Pye Unicam 304 unit, provided with flame-ionization detector, 30 m capillary column "Innowax" impregnation (Scotia Pharmaceuticals Ltd) under the following conditions: column temperature 165 °C to 225 °C, with a change 4 °C/min, detector temperature 300 °C, injector temperature 280 °C, gas-carrier – nitrogen.

Phospholipid composition. The lipids were extracted from the seeds by the Folch procedure (Folch et al., 1957). Polar lipids were divided from unpolar ones by column chromatography (Kates, 1972). The phospholipid constituents were separated by two-directional thin-layer chromatography on Silica gel 60 G "Merck", impregnated with 1 g per 100 g $(NH_4)_2SO_4$ water solution (Beshkov, Ivanova, 1972). The first direction was carried out in chloroform: methanol: ammonia 65:25:5 v/v/v and second in chloroform: methanol: ammonia: acetic acid: water 50:20:10:10:5 v/v/v/v/v. The spots of the separated individual phospholipids were identified by spraying with specific reagents (Kates, 1972). In addition, R_F and standard spots were used for definitive identification. The quantitative evaluation was carried out spectrophotometrically at 700 nm (Beshkov, Ivanova, 1972).

Sterol composition. The free and esterified sterols were separated from the other oil constituents by preparative TLC on Silica gel 60 G "Merck" and mobile phase n-hexane: diethyl ether 1:1 v/v. The esterified sterols were saponified with ethanolic KOH, extracted and purified by TLC. The quantitative evaluation and individual composi-

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Table 1. Content of oil in the seeds and composition of the glyceride oils*

Tobacco species	Glyceride oil in seeds (%, wt)	Phospholipids in oils (%, wt)	Sterols in oils (%, wt)	Tocopherols in oils (mg/kg)
1. *Elenski 817*	38.6	1.4	0.4	72
2. *Nevrokop 1140*	38.2	1.7	0.4	44
3. *Nevrokop 261*	30.1	1.0	0.3	52
4. *Krumovgrad 988*	37.8	1.2	0.4	63
5. *Plovdiv 7*	38.4	1.1	0.4	52
6. *Djebel 81*	41.3	1.5	0.6	5
7. *Djebel 576*	37.9	1.4	0.8	37

*Average of three determinations

Table 2. Fatty acid composition of the oils (% wt)*

Tobacco species	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
1. *Elenski 817*	0.8	1.6	25.5	15.3	0.3	11.1	36.8	8.1	0.5
2. *Nevrokop 1140*	0.9	1.5	23.4	14.6	0.3	12.0	39.5	7.5	0.3
3. *Nevrokop 261*	1.1	1.3	27.5	15.8	0.4	8.5	35.7	9.3	0.4
4. *Krumovgrad 988*	1.5	1.7	26.3	16.8	0.2	10.0	36.6	6.6	0.3
5. *Plovdiv 7*	2.7	1.6	27.7	21.1	0.2	5.5	35.6	5.2	0.4
6. *Djebel 81*	0.3	0.2	20.6	1.6	–	4.6	20.5	50.2	2.0
7. *Djebel 576*	0.2	0.4	16.4	9.3	0.2	2.7	17.6	51.9	1.3

*Average of three determinations

tion were determined by gas chromatography (Homberg, Bielefeld, 1989), using HP 5890 A unit with FID, 25 m capillary column impregnated with OV-17 under the following conditions: column temperature 260–300 °C, with a change 6 °C/min, detector temperature 320 °C, injector temperature 300 °C, gas-carrier – nitrogen.

Identification was confirmed by retention time comparison of the individual constituents with those of authentic samples. Betuline was used as internal standard for quantitative evaluation of total sterols.

Tocopherol composition. Tocopherols and tocotrienols were analyzed directly in the oil by HPLC with fluorescence detection (Ivanov, Aitzetmüller, 1995). "Merck-Hitachi" unit fitted with column "Nucleosil" Si 50-5 250 x 4 mm and fluorescent detector "Merck-Hitachi" F 1000 was used. The operating conditions were as follows: excitation 295 nm, emission 330 nm, mobile phase n-hexane: dioxan 94:4 v/v, rate of mobile phase 1 cm^3/min. The peaks were identified using authentic individual tocopherols.

RESULTS AND DISCUSSION

Content of oil in the seeds and general composition of the vegetable oils are presented in Table 1.

The seeds of all investigated tobacco species have been found rich in glyceride oil (30.1–41.3%). The content of phospholipids and sterols in the oils was closed and was similar to those of other common oil-bearing seeds as sunflower (FAO/WHO Codex Stan., 1999). The quantity of tocopherols in the oils was found to be several times lower than in other oils (5–72 mg/kg only), in comparison with 870–950 mg/kg in sunflower oil (FAO/WHO Codex Stan., 1999).

Table 2 shows the fatty acid composition of the glyceride oil. Oleic acid was found to be the main component in the oils with the exception of the *Djebel 81* and *Djebel 576* seed oil, where linoleic acid predominated. High concentration of saturated fatty acids, mainly palmitic (16.4–27.7%) and stearic (2.7–12.0%) in all studied oils were established. Relatively high concentration of unusual widespread palmitoleic acid (1.6–21.1%) was identified. The content of linoleic acid (5.2–51.9%) is lower in comparison with data reported in other investigations, from different geographic regions, where its percentage was 45.4–76.9% (Koivai et al., 1983; Gofur et al., 1993).

Table 3 presents sterol composition of the oils. β-sitosterol (59.4–65.9%) was the main component in sterol fraction, followed by stigmasterol (10.4–13.9%) and kampesterol (9.6–14.8%). Unusual high amount of cholesterol (7.2–9.8%) was identified, while in the other vegetable oils this content was found to be 0.1–0.5% only (FAO/WHO Codex Stan., 1999). Similar content of cholesterol in the tobacco seed oils was reported by other authors (Frega et al., 1991).

The composition of phospholipids presented in Table 4 shows that phosphatidylcholine (35.2–41.8%) was found to be the main constituent followed by phosphatidylinositol (23.8–29.8%) and phosphatidylethanolamine (11.6–21.1%). The other components were identified in negligible quantities. These pictures were closed to the composition of other tobacco seed oils (Zlatanov,

Table 3. Sterol composition of the oils (% wt)*

Tobacco species	Cholesterol	Brassicasterol	Kampesterol	Stigmasterol	β-sitosterol	Δ^5-avenasterol	Δ^7-stigmasterol	Δ^7-avenasterol
1. *Elenski 817*	9.1	1.4	14.8	13.9	59.4	0.7	0.4	0.3
2. *Nevrokop 1140*	7.2	1.4	12.2	12.1	65.8	0.5	0.4	0.4
3. *Nevrokop 261*	8.6	1.3	11.8	11.1	65.9	0.3	0.4	0.6
4. *Krumovgrad 988*	8.9	1.7	13.6	13.0	61.5	0.4	0.4	0.5
5. *Plovdiv 7*	8.4	1.3	12.6	12.5	63.8	0.5	0.4	0.5
6. *Djebel 81*	9.8	1.4	9.9	10.4	63.4	3.8	1.0	0.3
7. *Djebel 576*	7.6	1.0	9.6	11.2	63.2	2.9	4.1	0.4

*Average of three determinations

Table 4. Phospholipid composition of the oils (% wt)*

Tobacco species	PC	PI	PE	PA	LPC	LPE	SM	PS
1. *Elenski 817*	39.8	29.8	18.7	2.4	1.3	2.2	4.1	1.7
2. *Nevrokop 1140*	35.6	23.8	17.8	6.0	7.8	1.9	2.0	5.1
3. *Nevrokop 261*	35.2	26.3	16.9	7.1	5.8	2.4	3.6	2.7
4. *Krumovgrad 988*	35.4	27.8	21.1	5.7	2.4	1.8	3.7	2.1
5. *Plovdiv 7*	41.8	27.4	17.1	4.3	4.3	1.1	2.6	1.4
6. *Djebel 81*	40.3	23.8	12.7	5.2	10.9	1.3	4.6	1.2
7. *Djebel 576*	40.6	29.2	11.6	1.9	7.7	2.3	4.1	2.6

*Average of three determinations
PC – phosphatidylcholine, PI – phosphatidylinositol, PE – phosphatidylethanolamine, PA – phosphatidic acids, LPC – lysophosphatidylcholine, LPE – lysophosphatidylethanolamine, SM – sphingomyeline, PS – phosphatidylserine

Table 5. Tocopherol composition of the oils (% wt)*

Tobacco species	α-T	α-T-3	β-T	γ-T	δ-T
1. *Elenski 817*	6.1	–	1.2	90.7	2.0
2. *Nevrokop 1140*	6.1	–	–	93.9	–
3. *Nevrokop 261*	9.8	–	3.7	80.4	6.1
4. *Krumovgrad 988*	10.6	–	3.2	84.2	2.0
5. *Plovdiv 7*	8.7	–	0.3	91.0	–
6. *Djebel 81*	17.4	6.1	–	76.5	–
7. *Djebel 576*	2.7	–	0.2	96.6	0.5

*Average of three determinations
T – tocopherol, T-3 – tocotrienol

Menkov, 2000) and vegetable oils as sunflower and safflower (FAO/WHO Codex Stan., 1999).

Tocopherol composition of the oils is presented in Table 5. γ-tocopherol (76.5–96.6%) predominated in all tobacco oils, followed by α-tocopherol. Unsaturated α-tocotrienol (α-T-3) was found to be in *Djebel 81* seed oil only.

Although tobacco seed oil is a non-edible oil, it can be utilized for bio-diesel production as a new renewable alternative engine fuel. The addition of tobacco seed oil methyl esters to the diesel fuel reduced CO and SO_2 emissions (Usta, 2005a, b). On the other hand, high percentage of saturated and monosaturated fatty acids (mainly palmitic and oleic acid) leads to preparing of the fatty acid methyl esters with comparatively high freezing point and high viscosity. This fact makes it difficult to use the methyl esters in pure form as biodiesel fuel. A more rational method for utilization is addition of the methyl esters to diesel fuel to remove this disadvantage.

REFERENCES

BESHKOV, M. – IVANOVA, M.: Determination of phospholipids in lipid mixtures. Sci. Works of High Inst. Food and Flavour Ind., Plovdiv (Bulg.), *20*, 1972: 231–234.

FAO/WHO Codex-Standards, 1999. Food standards 210.

FOLCH, M. – KEES, M. – STANLEY, G.: A simple method for isolation and purification of total lipids from animal tissues. J. Biol. Chem., *226*, 1957: 497–498.

FREGA, N. – BOCCI, F. – CONTE, L. – TESTA, F.: Chemical composition of tobacco seeds (*Nicotiana tabacum* L.). JAOCS, *68*, 1991: 29–33.

GIANNELOS, P. N. – ZANNIKOS, F. – STOURNAS, S. – LOIS, E. – ANASTOPOULOS, G.: Tobacco seed oil as

an alternative diesel fuel: physical and chemical properties. Ind. Crops Prod., *16*, 2002: 1–9.

GOFUR, M. A. – RAHMAN, M. S. – AHMED, G. M. – HOSSAIN, A. – HAGUE, M. E.: Studies on the characterisation and glyceride composition of tobacco (*Nicotiana tabacum* L.) seed oil. Bangladesh. J. Sci. Ind. Res., *28*, 1993: 25–31.

HOMBERG, E. – BIELEFELD, B.: Sterinzusamensetzung und Steringehalt in 41 verschiedenen pflanzlichen und tierichen Fetten. Fat. Sci. Technol., *91*, 1989: 23–27.

IVANOV, S. – AITZETMÜLLER, K.: Untersuchungen über die Tocopherol- und Tocotrienolzusammensetzung der Samenöle einiger Vertreter der Familie *Apiaceae*. Fat. Sci. Technol., *97*, 1, 1995: 24–29.

KATES, M.: Technique of Lipidology. New York, American Elsevier Pub. Co. 1972.

KOIVAI, A. – SUZUKI, F. – MATSUZAKI, T. – KAWASHIMA, N.: The fatty acid composition of seeds and leaves of Nicotiana species. Phytochemistry, *22*, 1983: 1409–1412.

METCALFE, L. – WANG, C.: Rapid preparation of fatty acid methyl esters using organic base catalyzed transesterification. J. Chromatogr. Sci., *19*, 1981: 530–533.

MUKHTAR, A. – ULLAH, H. – MUKHTAR, H.: Extraction and characterisation of tobacco seed oil. Asian J. Chem., *18*, 2006: 20–24.

PATEL, J. A. – PATEL, B. K. – CHAKRABOTY, M. K.: Production potential and quality aspects of tobacco seed oil. Tobacco Res., *24*, 1998: 44–49.

TALAQANI, T. E. – SHAFIK, J. – MUSTAFA, F. K.: Fatty acids composition of the seed oil of certain tobacco varieties cultivated in Northern Iraq. Indian J. Agric. Chem., *19*, 1986: 147–154.

USTA, N.: An experimental study on performance and exhaust emissions of a diesel engine fuelled with tobacco seed oil methyl ester. Energy Conv. Manag., *46*, 2005a: 2373–2386.

USTA, N.: Use of tobacco seed oil methylester in turbocharged indirect injection diesel engine. Biomass & Bioenergy, *28*, 2005b: 77–86.

ZHANG, F. Y. –YANG, M. F. – XU, Y. N.: Silencing of DGAT1 in tobacco causes a reduction in seed oil content. Plant Sci., *169*, 2005: 689–694.

ZLATANOV, M. – MENKOV, N.: Phospholipid and fatty acid composition of tobacco seeds. Rostl. Výr., *46*, 2000: 429–430.

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Složení rostlinných olejů v tabáku.

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Byl zkoumán obsah a složení lipidů izolovaných ze semen sedmi bulharských odrůd tabáku. Obsah glycerinového oleje v semenech byl 30,1 až 41,3 %. Byly sledovány biologicky aktivní látky – mastné kyseliny, fosfolipidy, steroly a tokoferoly. V olejích převládala kyselina palmitová (16,4–27,7 %), kyselina olejová (17,6– 39,5 %) a kyselina linolová (5,2–51,9 %). Obsah fosfolipidů, sterolů a tokoferolů v olejích byl 1,0–1,7 %, 0,3–0,8 % a 5–72 mg/kg. Hlavními součástmi fosfolipidové frakce byly fosfatidylcholin (35,2–41,8 %), fosfatidylinositol (23,8–29,8 %) a fosfatidyletanolamin (11,6–21,1 %). Beta-sitosterol (59,4–65,9 %), stigmasterol (10,4–13,9 %) a kampesterol (9,6–14,8 %) převládaly ve sterolové frakci. Gama-tokoferol (76,5–96,6 %) byl hlavní součástí v tokoferolové frakci.

olejniny tabáku; mastné kyseliny; fosfolipidy; steroly; tokoferoly

Contact Address:

Magdalen Z l a t a n o v, University of Plovdiv "P. Hilendarski", Department of Chemical Technology, 24 Tzar Assen Str., 4000 Plovdiv, Bulgaria, e-mail: magzlat@pu.acad.bg