

Lipid Composition of Tobacco Seeds

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Abstract

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The content and composition of lipids isolated from seeds of 7 Bulgarian tobacco species was investigated. 382-492g.kg⁻¹ glyceride oil in the seed was founded to be. The biological active substances - fatty acids, phospholipids, sterols and tocopherols were studied. Palmitic (126-367g.kg⁻¹), oleic (152-263g.kg⁻¹) and linoleic acid (176-627g.kg⁻¹) predominated in the oils. The content of phospholipids, sterols and tocopherols in the oils was 2-15g.kg⁻¹, 3-8g.kg⁻¹ and 2-195 mg/kg respectively. Phosphatidylcholine (247-405g.kg⁻¹), phosphatidylinositol (215-276g.kg⁻¹) and phosphatidylethanolamine (143-320g.kg⁻¹) were found to be the main constituents. b-sitosterol (433-682g.kg⁻¹), stigmasterol (102-188g.kg⁻¹) and kampesterol (93-149g.kg⁻¹) predominated in the sterol fraction. All of tocopherol derivatives were identified in the tocopherol fraction.

Key words: tobacco seed oil, fatty acids, phospholipids, sterols, tocopherols

Abbreviations: PC - Phosphatidylcholine, PI - Phosphatidylinositol, PE – Phosphatidylethanolamine, PA - Phosphatidic acids, LPC – Lysophosphatidylcholine, LPE - Lysophosphatidylethanolamine, SM – Sphingomieline, PS – Phosphatidylserine

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 raw material in the coating industry, for preparation of printing inks, dyes etc. The content of oil in the seeds is about 300 g.kg⁻¹, mainly trilinolein and palmitodiolein (Frega et al., 1991). The chemical composition of glyceride oil is important to look at new mate-

rials for industry and alternative applications (Patel et al., 1998; Talaqani et al., 1986). On the other hand the composition of the oil rests considerably with temperature conditions and wet in the fatty acids and some biologically active substances as sterols, phospholipids and tocopherols. The chemical characterization of *Nicotiana tabacum L.* seeds has been found important to look at alternative products of the crop i.e. oil and meal and find some uses of these products. The knowledge of lipid

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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 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 composition of fatty acids, phospholipids, sterols and tocopherols of the oils in 7 Bulgarian tobacco species.

Experimental

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

Methods

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 and 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) and conditions as follows: column temperature 165°C to 225°C, with a change 4°C/min, detector temperature 300°C, injector tem-

perature 280°C, gas-carrier - nitrogen.

Phospholipid composition. Lipids were extracted from the seeds by *Folch* procedure (Folch et al., 1957). Polar lipids were divided from unpolar lipids by column chromatography (Kates, 1972). The phospholipid constituents were separated by two-directional thin-layer chromatography on Silica gel 60 G "Merck", impregnated with 10g.kg⁻¹ (NH₄)₂SO₄ water solution (Beshkov and 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 and 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 composition was determined by gas chromatography (Homberg and Bielefeld, 1989), using HP 5890 A unit with FID, 25 m capillary column impregnated with OV-17 and conditions as follows: 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 and Aitzetmüller, 1995). "Merck-Hitachi" unit fitted with column "Nucleosil" Si 50-5 250x4 mm and fluorescent detector "Merck-Hitachi" F 1000 was used. The operating conditions were as follows: excitation 295nm, emission 330nm, mobile phase n-hexane: dioxan 94:4 v/v, rate of mobile phase 1cm³/min. The peaks were identified using authentic individual tocopherols.

Statistical analysis

Standard deviations and statistical significance of differences between the mean values were calculated using standard mathematical and statistical methods at 5% of significance level.

Results and Discussion

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

The seeds of all investigated tobacco species have been found rich in glyceride

oil (382-492 g.kg⁻¹). 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 time lower than other oils (2-195 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. Linoleic acid was found to be the main component in the oils of Barley species (619-627g.kg⁻¹). High concentration of oleic (152-161g.kg⁻¹) and palmitic (126-175g.kg⁻¹) in all studied oils of Barley species were established. Palmitic acid (211-367g.kg⁻¹) predominated in the oils of Virgin species and Koker 254, followed by oleic acid (198-263g.kg⁻¹) and linoleic acid (176-266g.kg⁻¹). The content of linoleic acid is lower in comparison with data reported in other investigations, from different geographic regions, where its percentage was 454-769g.kg⁻¹ (Koivai et al., 1983; Gofur et al., 1993). Relatively high concentration of unusual palmitoleic acid (2-127g.kg⁻¹), typical for seabuck-

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

Tobacco species	Glyceride oil in seeds, g.kg ⁻¹	Phospholipids in oils, g.kg ⁻¹	Sterols in oils, g.kg ⁻¹	Tocopherols in oil, mg/kg
1. Barley 21	409.00	10.00	8.00	2.00
2. Barley 1000	382.00	14.00	6.00	2.00
3. Barley 1317	383.00	15.00	6.00	10.00
4. Virgin 330	409.00	2.00	3.00	48.00
5. Virgin 454	382.00	4.00	5.00	195.00
6. Virgin 514	382.00	4.00	3.00	115.00
7. Koker 254	492.00	3.00	3.00	43.00

Table 2
Fatty acid composition of the oils, g.kg⁻¹

Tobacco species	Barley 21	Barley 1000	Barley 1317	Virgin 330	Virgin 454	Virgin 514	Koker 254
Fatty acids							
C12:0 (lauric)	11	9	4	7	1	19	102
C14:0 (miristic)	3	2	2	11	76	50	40
C16:0 (palmitic)	175	126	166	367	329	248	211
C16:1 (palmiticoleic)	2	41	8	111	107	127	105
C17:0 (margarinic)	17	1	4	6	6	3	5
C18:0 (stearic)	16	35	35	55	66	87	65
C18:1 (oleic)	153	161	152	263	238	215	198
C18:2 (linoleic)	619	624	627	179	176	248	266
C18:3 (linolenic)	2	1	2	1	1	3	8

Table 3
Sterol composition of the oils, g.kg⁻¹

Tobacco species	Barley 21	Barley 1000	Barley 1317	Virgin 330	Virgin 454	Virgin 514	Koker 254
Sterols							
Cholesterol	100	79	104	112	71	56	66
Brasikasterol	18	6	13	3	2	3	2
Kampesterol	97	93	101	147	149	141	127
Stigmasterol	102	128	104	188	160	140	144
β-sitosterol	672	682	643	433	487	493	519
Δ ⁵ avenasterol	7	7	25	100	115	144	125
Δ ⁷ stigmasterol	3	4	8	14	10	9	10
Δ ⁷ avenasterol	1	1	2	3	6	14	7

thorn fruit oils was identified.

Table 3 presents sterol composition of the oils. β-sitosterol (433-682g.kg⁻¹) was the main component in sterol fraction, followed by stigmasterol (102-188g.kg⁻¹) and kampesterol (93-149g.kg⁻¹). Unusual high amount of cholesterol (56-112g.kg⁻¹) typical for animal lipids were identified, while in the other vegetable oils these content

was found to be 1-5g.kg⁻¹ only (FAO/WHO Codex Stan., 1999). Similar content of cholesterol in the tobacco seed oils was reported by other authors (Frega et al., 1991). In the Virgin species and Koker 254 seed oils were determined high levels of nm Δ⁵-avenasterol (100-144g.kg⁻¹).

Almost all of phospholipids classes were identified in phospholipids fraction

Table 4
Phospholipid composition of the oils, g.kg⁻¹

Tobacco species	Barley 21	Barley 1000	Barley 1317	Virgin 330	Virgin 454	Virgin 514	Koker 254
Phospholipids							
PC	405	363	365	281	247	269	313
PI	241	215	276	263	228	244	237
PE	147	195	143	224	320	250	264
PA	34	26	13	31	45	57	50
LPC	103	94	103	60	63	52	42
LPE	40	21	31	71	54	68	54
SM	30	43	69	41	23	32	22
PS	-	43	-	29	20	28	18

PC - Phosphatidylcholine

PI - Phosphatidylinositol PE - Phosphatidylethanolamine

PA - Phosphatidic acids

PS - Phosphatidylserine SM - Sphingomyelin

LPC - Lysophosphatidylcholine

LPE - Lysophosphatidylethanolamine

Table 5
Tocopherol composition of the oils, g.kg⁻¹

Tobacco species	Barley 21	Barley 1000	Barley 1317	Virgin 330	Virgin 454	Virgin 514	Koker 254
Tocopherols							
α- tocopherol	981	835	640	169	212	189	231
α- tocotrienol	-	30	66	169	156	199	140
β- tocopherol	8	60	82	28	93	81	29
γ- tocopherol	9	43	111	351	335	336	346
δ- tocopherol	2	32	101	283	204	195	254

(Table 4). Phosphatidylcholine (247-405g.kg⁻¹) was found to be the main constituent followed by phosphatidylinositol (215-276g.kg⁻¹) and phosphatidylethanolamine (143-320g.kg⁻¹). The other components were identified in negligible quantities. These pictures were closed to the composition of other vegetable oils as sunflower and safflower (FAO/WHO Codex-Stan., 1999).

Tocopherol composition of the oils is presented in Table 5. α-tocopherol (640-981g.kg⁻¹) predominated in *Barley* species tobacco oils. All of tocopherol derivatives α-, β-, γ- and δ- were identified in relatively closed percentages in the Virgin and Koker 254 seed oils. Unsaturated α-tocotrienol was found to be in those seed oils too. Although tobacco seed oil is non-edible oil, it can be utilized for bio-diesel pro-

duction as a new renewable alternative engine fuel. The addition of tobacco seed oil methyl esters to the diesel fuel reduced CO and SO₂ emissions (Usta, 2005). 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.

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