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A COMPARATIVE ANALYSIS OF THE FATTY ACIDS OF *YAKA*, *PRILEP* AND *OTLJA* TOBACCOS

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Abstract. *Content and composition of total and free fatty acids of the air dried leaves of the Serbian selected tobaccos type Prilep, Yaka and Otlja were investigated by GC. In all three tobacco types palmitic acid was the major component: 47.1% of the total acids and 17.0% of the free acids for Prilep, 43.9% of the total acids and 16.0% of the free acids for Yaka and 34.2% of the total acids and 5.0% of the free acids for Otlja tobacco. The U/S values for free and total fatty acids, as well as the content of free fatty acids for the semioriental tobacco Otlja, were almost twice higher than in oriental Yaka and Prilep making them a good marker characteristic distinguishing the two tobacco types.*

Key words: *Nicotiana tabacum, oriental and semioriental tobaccos, fatty acids, GC*

INTRODUCTION

There are more than sixty species of the genus *Nicotiana L.* but only *N. tabaccum* ("tobacco") and *N. rustica* ("makhorka") are cultivated commercially as smoking materials. The oriental tobaccos type *Prilep* and *Yaka* and semi-oriental tobacco type *Otlja* (characteristic for the Balkan Peninsula) are known for their characteristic strong aroma and pleasant sweet smoking taste. The analysis of the chemical composition and antimicrobial activity of the essential oil and CO_2-extracts of *Otlja* [1], *Yaka* [2] and *Prilep* [3] as well as chemical composition of the alkanes [4], ether and ethyl acetate extracts [5] was previously conducted by our research group but to best of our knowledge nothing is known about the content and composition of fatty acids of these tobaccos. Fatty acids contribute significantly to the flavor and aroma of cigarette smoke [6-12]. The saturated higher fatty acids are known for their smoothing effects upon smoke. Unsaturated acids, linolenic and linolenic, however, add harshness to smoke.

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The transfer rate of nonvolatile fatty acids from tobacco into the smoke was found to vary between 16-34%. Bioassays have demonstrated a statistically significant tumor promoting activity for the acidic fractions of cigarette smoke condensate. So, investigation of content and composition of fatty acids is of interest not only to tobacco chemist but may have even broader implication because of the possible role that nonvolatile fatty acids play in the tumor promoting activity of the tobacco smoke condensate.

Carruthers and Johnstone were among the first to analyze tobacco for free nonvolatile fatty acids [13]. They reported in flue-cured British cigarette tobacco the presence of all fatty acids between C_{10}-C_{26} with palmitic acid as the major saturated and linolenic acid as the major unsaturated component. Mold and his co-workers explored in detail the qualitative composition of free and combined higher fatty acids in Bright tobacco [14]. A higher concentration of free nonvolatile fatty acids in flue-cured and sun-cured tobacco than in air-cured tobaccos was also noticed [15].

EXPERIMENTAL

Leaves of the examined tobaccos (registered in the Federal Institute for genetic resource of plants and animals with following codes: *Otlja* MD-159-78, *Yaka* ALH 98 and *Prilep* Dinovka) were collected from an experimental field of the Institute "Selekcija"-Aleksinac (Serbia). The leaves were air dried arranged in strings (the moisture content was for *Prilep* 7.2%, *Yaka* 7.3% and *Otlja* 7.6%) and aged in a dark and cool place for 12 months. Voucher specimens have been deposited in the Herbarium of the Institute "Selekcija"- Aleksinac. All solvents employed were of analytical grade quality (obtained from Merck, Germany) and were redistilled before use.

The dried plant material (100 g) was extracted three times with 500 cm^3 $CHCl_3$-MeOH (2:1 v/v) in the duration of 24 hours at room temperature. The extracts were combined and the solvent mixture was evaporated under reduced pressure. The extraction of *Otlja* gave 14.6%, *Yaka* 20.8% and *Prilep* 20.1% of extractable material.

Isolation and esterification of free and total fatty acids were performed according to known previously published procedures [15, 16]

The methyl esters of fatty acids were analyzed on Agilent 6850 Gas Chromatograph, equipped with HP-1 Methyl Siloxane (30.0 × 320 μm × 0.25 μm) capillary column and FID detector. The operating conditions were: oven temperature program: isothermal 150^0C (4 min) then 150-250^0C (4^0C/min) and isothermal 250^0C (2 min); an injector and detector temperature 250^0C, carrier gas was H_2 (1.8 ml/min).

The identification was carried out by co-injection of authentic compounds and retention times. Percentage area values were obtained electronically from the GC-FID response without the use of an internal standard or correction factors.

RESULTS AND DISCUSSION

The amount of total acids was similar in all three examined tobacco types but free acids were present in a much higher amount in semi-oriental tobacco *Otlja*. The quantity of free fatty acids isolated from *Yaka* and *Prilep* was similar to those found by Arrendale et al. [17]. The total and free fatty acid composition in dried leaves of *Otlja*, *Yaka* and *Prilep* is presented in the Table.

Palmitic acid (total and free) was the most abundant component in all three types but less abundant in semi-oriental *Otlja* than in the oriental tobaccos. The content of total stearic acid was almost equal in all three tobaccos but the amount of free stearic acid in *Otlja* was less than two times of the amount found in *Prilep* and *Yaka*.

Both examined types, oriental and semi-oriental, had lower content of linoleic and linolenic acid than bland tobaccos (excluding *Burley*) [17].

Table 1. Distribution and abundance of tobaccos' fatty acids (%)

	Otlja		*Yaka*		*Prilep*	
Fatty acids	total	free	total	free	total	free
$C_{14:0}$	1.7	0.1	2.1	0.5	2.2	0.5
$C_{15:0}$	4.6	0.2	4.3	0.9	1.3	/
$C_{16:0}$	34.2	5.0	43.9	16.0	47.1	17.0
$C_{17:0}$	1.3	0.2	2.3	0.7	2.1	0.6
$C_{18:3} + C_{18:2}$	36.0	4.6	27.7	9.0	24.8	8.2
$C_{18:1}$	10.2	1.4	5.1	1.2	5.7	1.3
$C_{18:0}$	9.0	1.4	11.2	3.9	12.1	4.0
$C_{20:0}$	3.0	0.3	3.3	1.2	4.8	1.9
YIELD ($W_{ACIDS}/W_{DRIED\ LEAVES}$)	2.8	1.8	2.4	1.0	2.7	0.9
U/S (UNSATURATED/SATURATED)	0.9	0.8	0.5	0.4	0.4	0.4

/, not detected

The two oriental tobaccos (*Yaka* and *Prilep*) had similar U/S values for total and free fatty acids while *Otlja* had almost two times higher U/S values (Table).

The noticed differences in fatty acid composition of oriental, semi-oriental and bland tobaccos could be besides all other factors responsible for the traditionally established difference in the smoking experience of these three tobacco types.

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UPOREDNA ANALIZA MASNIH KISELINA DUVANA TIPA *JAKA*, *PRILEP* I *OTLJA*

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Određen je kvalitativni i kvantitativni sastav ukupnih i slobodnih masnih kiselina duvana tipa Jaka, Prilep i Otlja GC-metodom. Glavna komponenta sva tri tipa duvana je palmitinska kiselina: 47.1% ukupna i 17.0% slobodna kod Prilepa, 43.9% ukupna i 16.0% slobodna kod Jake i 34.2% ukupna i 5.0% slobodna kod Otlje. Količina ukupnih masnih kiselina je slična kod sva tri ispitivana tipa duvana, međutim količina slobodnih masnih kiselina, kao i U/S vrednosti kako za slobodne, tako i za ukupne masne kiseline, su dvostruko veće kod poluorijentalnog tipa duvana, Otlje, nego kod orijentalnih tipova Jake i Prilepa. Dobijeni rezultati ukazuju na mogućnost korišćenja količine slobodnih masnih kiselina i U/S vrednosti za razlikovanje Otlje od Jake i Prilepa. S obzirom na promotorno dejstvo kisele frakcije kondenzata duvanskog dima u nastajanju tumora, pogodniji su Jaka i Prilep od Otlje za komponovanje cigaretnih mešavina zbog manjeg sadržaja slobodnih masnih kiselina.