Chemical Composition of Tobacco Seeds (*Nicotiana tabacum* L.)

N. Frega[a,*], F. Bocci[a], L.S. Conte[b] and F. Testa[a]
[a]Istituto di Microbiologia Agraria e Tecnica, Unviersitá di Firenze, Piazzale delle Cascine, 27 - 50144 Firenze, Italy and
[b]Ispettorato Prevenzione e Repressione delle Frodi Agroalimentari, Via S. Giacomo, 7 - 40126 Bologna, Italy

Chemical characterization of tobacco seeds is supposed to be an interesting tool in order to extend the knowledge on alternative products of this crop which is of great economic interest. This paper describes chemical composition of meals, fat, ash, protein, fiber and nitrogen-free-extract; furthermore, particular emphasis is given to the composition of lipid fraction, with a complete characterization of triglycerides, fatty acids, and unsaponifiable matter. Trilinolein and palmitodilinolein are the main triglycerides, while linoleic is the main fatty acid. Cholesterol, as in other *Solanaceae*, is present in the sterol fraction. Cycloartenol is the main component of the triterpenic alcohols fraction. Amino acids and glucides composition is also reported.

KEY WORDS: Amino acids, chemical analysis, fats, fatty acids, methylsterols, *Nicotiana tabacum* L., oligosaccharides, proteins, sterols, therpenic alcohols.

A number of papers dealing with chemical composition of tobacco leaves, grown both for smoking purposes (1-3) and as a protein source (4-6), are available.

Interest in this kind of study is understandable considering the economic importance of smoking tobacco cultivation, which is the basis of the agricultural economy of several countries. On the other hand, only a few papers have been published concerning the chemical composition of tobacco seeds. We consider this kind of research to be of some importance, both for the need to find new uses for this important crop, as the number of smokers in the developed countries steadily decreases, and for its usefulness to characterize seeds, with the intention of preserving seed purity.

The aim of this paper is to widen knowledge of the composition of the seeds and to apply the latest analytical techniques to supply the most up-to-date contribution.

EXPERIMENTAL

The seeds of three different varieties—Bright Italia, Kentucky 104, and Bright V—of *Nicotiana tabacum* L., collected at ripening, were oven-dried at 40°C for 4 hr in order to eliminate most of the moisture. Seeds were then ground in a Jenkl-Kenkel water cooled mill (T≤ 30°C). Moisture (residue) and ash content were dosed as described in the *Italian Official Methods of Analysis of Cereals* (7). Crude fat content was determined gravimetrically, after extraction with n-hexane in a Soxhtec System apparatus (Tecator, Hoganas, Sweden) while the raw fiber content was determined with a semi-automatic Fibertec apparatus (Tecator), which is based upon the Weende method. The raw protein content was dosed with a Gerhardt Vapodest Instrument. The nitrogen-free-extract was calculated by subtraction, at 100.

The amino acid profile was determined after hydrolysis with 6 M HCl solution carried out in an inert atmosphere for 21 hr at 127°C, as described by Zumwalt *et al.* (8). Free amino acids thus obtained were then analyzed by means of a Carlo Erba 3A30 Amino Acids Analyzer (Carlo Erba, Rodano, Milano, Italy).

The composition of the glucidic fraction was determined by extraction of glucides from defatted meal with methyl alcohol, as described by Mariani *et al* (9); in contrast to what is described in this procedure, however, in order to obtain oxime derivatives, the glucides were treated with Stox solution (Pierce Chemical Co., oudbeijerland, Netherlands), which contains hydroxylamine and β-phenil-D-gluco-piranoside as internal standards. Reaction with hydroxylamine was carried out for 30 min at 70°C in a closed screw-cap tube. After cooling, silanyzation was applied, according to Sweeley *et al.* (10). Gas-liquid chromatographic (GLC) analysis was carried out by a Carlo Erba 4200 gas chromatograph, in the analytical conditions reported in Table 1.

The lipid fraction was extracted with n-hexane for 8 hr in a Soxhlet-type extractor. Hexane was evaporated by distillation at reduced pressure (15 Torr) in a rotary evaporator, with temperature lower than 40°C. Glyceride composition of the oil was obtained by GLC with a Carlo Erba Mega 5160 apparatus, connected to a Mega 2 integrator, in the analytical conditions reported in Table 1. Fat was then saponified according to NGD (Norme Grassi e Derivati) method (11), and fatty acids were converted to methyl esters by reaction with an ethereal solution of diazomethane (CH_2N_2 (12) and analyzed using a Carlo Erba Series Mega 5160 gas chromatograph with analytical conditions reported in Table 1. The unsaponifiable fraction, obtained by the NGD method C-12 (13), was treated with CH_2N_2 in order to transform free fatty acids, which in the thin-layer chromatography (TLC) that was to follow would have interfered with the sterols band, into methyl esters, whose R_f is higher than the sterols one (14).

TLC fractionation of unsaponifiable matter was realized with silica-gel G plate (Stratochrom SI Carlo Erba), using n-hexane/diethyl ether 60:40 (v/v) as eluent. Plates were sprayed with 0.2% ethanolic solution of 2,7′-dichlorofluorescein (sodium salt), and bands were scraped off from the plate and twice extracted with ethyl ether. Unsaponifiable bands were analyzed with the GLC apparatus described above. Experimental conditions are reported in Table 1 (15). All determinations were repeated twice; data reported in this paper are the average of two performances of each test.

RESULTS AND DISCUSSION

The percentage composition of meals obtained from tobacco seeds, reported in Table 2, shows fat, raw protein

*To whom correspondence should be addressed.

TABLE 1

Experimental Conditions for Gas Chromatographic Determinations

Analyte	Triglycerides	Fatty acids methyl esters	(Triterpene alchohols, methyl sterols, sterols)[a]	Carbohydrates
References			(13)	(9)
Stationary phase	TAP	SP 2330	SE 52	RTX 1
Internal diameter (mm)	0.25	0.32	0.32	0.32
Length (m)	25	60	25	10
Film thickness (μ)	0.1	0.20	0.15	0.15
Injection technique	split	split	split	split
Carrier gas flow rate (mL/min)	1	0.6	1	1..7
Split ratio	1/60	1/60	1/80	1/80
Carrier gas	He	He	He	He
Oven temperature (°C)	340-360	130-210	270	180-340
Temperature rate	1°C/min	isot. 6 min; 3°C/min	isothermal	8°C/min
Detector temperature (°C)	400	260	320	320
Injector temperature (°C)	380	260	320	320
Detector	FID[b]	FID	FID	FID

[a]Components analyzed as trimethylsilyl (TMS) derivatives.
[b]FID, flame ionization detector.

TABLE 2

Composition of Tobacco Seed Meal Compared to Other Oil-Bearing Seeds (% dry wt)

	Moisture	Raw proteins	Lipids	Unsaponifiable oil fraction	Fiber	Ash	Nitrogen-free extract
Kentucky 104	5.1	25.0	48.0	1.2	19.9	3.2	2.7
Bright Italia	5.1	25.3	47.8	1.5	21.2	3.6	0.6
Bright V	5.3	25.9	47.2	1.5	21.0	3.2	1.2
Sunflower (16)	5-6	20-30	30-50	0.8-1	11-22	3-6	8-20
Rapeseed (17)	—	—	—	—	15.1	—	—

TABLE 3

Amino Acid Composition of Tobacco Seed Meals (mg aa/100 mg protein)

	Kentucky 104	Bright Italia	Bright V
Aspartic acid	9.8	9.8	9.5
Threonine	3.1	3.0	3.0
Serine	2.5	2.4	2.4
Glutamic acid	22.4	22.4	22.1
Proline	3.0	3.3	3.2
Glycine	5.0	4.9	5.0
Alanine	4.4	4.2	4.3
Cysteine	0.5	0.4	0.7
Valine	5.6	5.6	5.7
Methionine	1.2	1.3	1.4
Isoleucine	4.5	4.6	4.5
Leucine	7.0	6.9	7.0
Tyrosine	2.6	2.8	2.6
Phenylalanine	4.0	4.0	3.8
Histidine	2.4	2.3	2.3
Lysine	2.4	2.4	2.5
Arginine	12.7	12.9	12.6
Ammonia	2.6	2.8	2.9

and ash content similar to those of meals obtained from other common oil-bearing seeds (e.g. sunflower) (16), while the nitrogen-free-extracts content is definitely lower; the high fiber content makes these meals similar to those obtained from other crops with little dimension seeds (e.g. rapeseed) (17); generally this fact is explained with a hull/seed ratio in favor of the former.

Table 3 shows the amino acid composition of the three samples. It is well-known (18) that the acid hydrolysis does not permit the determination of thryptophan which is completely destroyed, while cysteine and methionine results are not quantitative; glutamine and asparagine are converted to the corresponding acids by deamination due to acid action. The other amino acids are essentially represented by glutamic acid and, in decreasing amounts, by arginine, aspartic acid and leucine. Comparison with the Food and Agricultural Organization (FAO) amino acids reference pattern (19) shows that leucine, phenylalanine, lysine and the sulphonated amino acids are low in content, while the other amino acids show values close to the optimum; anyway, low content of some amino acids, lysine in particular, is a common problem in many vegetable meals (e.g. sunflower, safflower etc.).

Contents of carbohydrates (Table 4) are not in complete agreement with data of Kuo *et al.* (20). The carbohydrates of the tobacco seeds are, according to our results, basically represented by sucrose, at about 1.5–2.5% (w/w) of defatted meal. The high variability of stachiose content is not easy to interpret, since we do not know the "historical background" of our samples: it is known that this oligosaccharide may derive from stress phenomena (pH or temperature) (21).

Composition of triglycerides is reported in Table 5, while a typical high resolution gas chromatographic

(HRGC) trace is reproduced in Figure 1. Trilinolein (LLL) and palmitodilinolein (PLL) are the main components in the three samples; this uniformity is confirmed by the fatty acid compositions reported in Table 6: linoleic is the main fatty acid, while palmitic together with oleic accounts for about 20% of the total fatty acids. The kind of GLC phase used enables us to separate $C_{18:1\Delta 11}$ from $C_{18:1\Delta 9}$ as well. These fatty acid compositions resemble the ones of grape seed oil (22) and "traditional" safflower oil (23), while differences are to be noted in the composition of the sterol fraction, reported in Table 7. Sterols were identified by comparison of relative retention time (RRT) with the ones reported in the literature (15,24,25) and with the ones of lipidic extract of known composition; furthermore, the characteristic modification of retention times on two phases of different polarity (SE 52 and OV 17) was considered.

A peculiar characteristic of the lipid fraction extracted from tobacco seeds is the presence of high amounts of cholesterol, which is typical of *Solanaceae* as reported in Table 7 (15,27), while β-sitosterol, even if it is the main component, is present in lower amounts than in other vegetable lipids (15), with the exception of oils extracted

TABLE 4

Carbohydrates Content of Tobacco Seeds Defatted Meals (g/kg)

Cultivar	Kentucky 104	Bright Italia	Bright V
Identification			
Fructose	0.11	0.13	0.20
Glucose	0.12	0.10	0.30
Sucrose	16.80	16.70	23.70
Raffinose	1.65	.147	2.29
Stachiose	3.30	1.33	0.40
Total carbohydrate	21.98	19.73	26.90

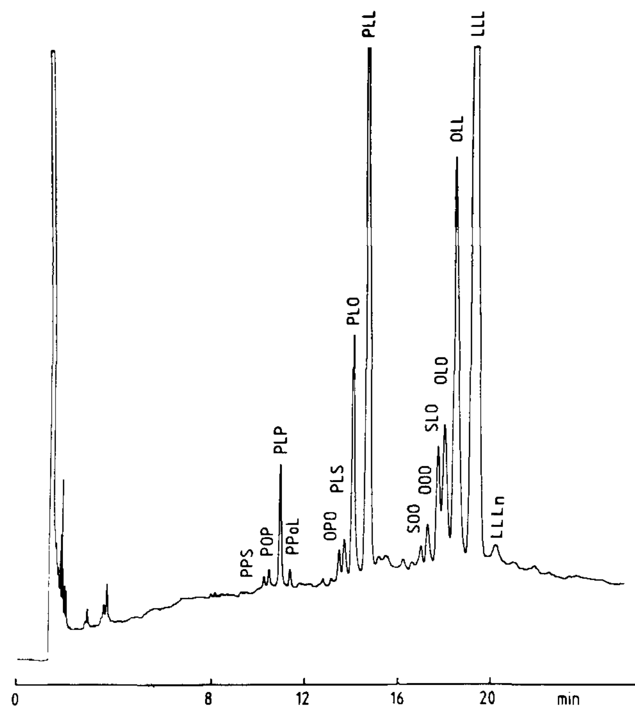

FIG. 1. Gas chromatographic trace of the triglycerides of oil extracted from seeds of Bright V cultivar. P = palmitic acid; S = stearic acid, O = oleic acid, L = linoleic acid, Ln = linolenic acid.

TABLE 5

Triglyceride Composition[a] of the Untreated Oil

RRT[b]	Identification[c]	Kentucky 104	Bright Italia	Bright V
0.63	PPS	0.1	0.1	0.1
0.64	PPO	0.4	0.4	0.4
0.67	PLP	3.2	2.9	3.0
0.70	PPoL	0.3	0.2	0.6
0.83	POO	1.1	0.7	0.7
0.84	PLS	1.3	1.2	1.2
0.87	PLO	7.2	6.6	6.7
0.91	PLL	25.0	24.0	22.9
1.00	SOO	0.5	0.2	0.3
1.07	OOO	1.4	0.9	1.2
1.10	SLO	3.2	2.4	3.7
1.12	OLO	5.3	5.3	5.2
1.15	OLL	15.0	15.0	16.1
1.20	LLL	36.0	40.1	37.9

[a]Calculated on the basis of the relative HRGC areas.
[b]Referred to SSO = 1.00 in the analytical conditions described in Table 1.
[c]Tentative identification on the basis of comparison with chromatographic behavior of available standards.

TABLE 6

Fatty Acids Composition[a] of Oil Obtained from Tobacco Seeds Compared with Those of Other Oil-Bearing Seeds

	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{18:0}$	$C_{18:1\Delta 9}$	$C_{18:1\Delta 11}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$C_{22:0}$
Kentucky 104	9.5	0.1	0.1	2.8	10.6	0.6	74.9	1.1	0.2	0.1
Bright Italia	9.2	0.1	0.1	2.5	9.5	0.8	76.1	1.4	0.2	0.1
Bright V	8.9	0.1	0.1	2.6	11.1	0.7	75.1	1.1	0.2	0.1
Grape seed (22)	7.7	0.2	—	3.8	14.0	0.6	73.1	0.4	0.2	—
Safflower (23)	7.5	—	—	2.8	12.0	0.8	76.1	—	—	—

[a]% Calculated with respect to total acids, on the basis of the HRGC areas.

TABLE 7

Composition[a] of the Sterol Fraction (as TMS), Compared to Those of Other Oil-Bearing Seeds

RRT[b]	Identification[c]	Kentucky 104	Bright Italia	Bright V	Safflower (23)	Grape seed (22)	Tomato (15)
0.62	Unknown	Tr	Tr	0.4	—	—	—
0.67	Cholesterol	8.6	7.3	9.8	Tr	0.3	20.6
0.69	Cholestanol	0.4	0.2	Tr	Tr	Tr	1.0
0.74	Brassicasterol	1.1	0.1	1.0	—	—	1.1
0.77	24-methylencholesterol	0.5	0.9	0.4	1.3	—	0.9
0.78	Unknown	Tr	0.5	0.7	0.1	—	—
0.83	Campesterol	13.0	13.4	13.6	13.6	9.5	4.8
0.84	Campestanol	0.3	0.3	Tr	—	—	—
0.88	Stigmasterol	9.3	10.2	9.6	5.5	11.3	9.6
0.90	Stigmastenol	0.6	0.6	0.6	—	—	—
0.92	Δ^7-campestanol	1.5	0.9	1.9	4.2	—	—
0.98	Clerosterol	0.7	0.8	1.3	—	—	0.5
1.00	β-sitosterol	39.5	43.8	39.5	48.9	75.0	49.8
1.03	Δ^5-avenasterol	22.7	19.3	19.2	4.0	1.7	10.2
1.06	Unknown	Tr	0.2	0.3	—	—	—
1.08	$\Delta^{3,2}$-stigmastadienol	1.0	0.8	0.8	—	—	—
1.11	Δ^7-stigmastenol	0.1	0.1	0.2	18.5	1.4	1.0
1.16	Δ^7-avenasterol	0.7	0.6	0.7	2.1	0.8	0.5

[a]Calculated on the basis of the HRGC areas.

[b]Referred to β-sitosterol (TMS) = 1.00 in the analytical conditions described in Table 1.

[c]Tentative identification on the basis of data reported in literature (15,24-26).

Note: cholesterol = Δ^5-colesten-3β-ol; cholestanol = 5α-cholestan-3β-ol; brassicasterol = [24S]-24-methyl-$\Delta^{5,22}$-cholestadien-3β-ol; 24-methylencholesterol = 24-methylen-$\Delta^{5,24}$-cholestadien-3β-ol; campesterol = [24R]-24-methyl-Δ^5-cholesten-3β-ol; campestanol = [24R]-24-methyl-cholestan-3β-ol; stigmasterol = [24S]-24-ethyl-$\Delta^{5,22}$-choelstadien-3β-ol; stigmastanol = [24S]-24-ethyl-cholestan-3β-ol; Δ^7-campesterol = [24R]-24-methyl-Δ^7-cholesten-3β-ol; clerosterol = [24S]-24-ethyl-$\Delta^{5,25}$-cholestadien-3β-ol; β-sitosterol = [24R]-24-ethyl-Δ^5-cholesten-3β-ol; Δ^5-avenasterol = [24Z]-24-ethyliden-Δ^5-cholesten-3β-ol; $\Delta^{5,24}$-stigmastadienol = [24R,S]-24-ethyl-$\Delta^{5,24}$-cholestadien-3β-ol; Δ^7-stigmastenol = [24R,S]-24-ethyl-Δ^7-cholesten-3β-ol; Δ^7-avenasterol = [24Z]-24-ethyliden-Δ^7-cholesten-3β-ol.

TABLE 8

Composition[a] of the Triterpene Alcohols Fraction

RRT[b]	Identification[c]	Kentucky 104	Bright Italia	Bright V
0.79	Unknown	0.1	0.1	0.1
0.82	Unknown	2.4	0.7	1.6
0.86	Unknown	0.4	0.5	0.4
0.88	Unknown	3.7	0.7	2.8
0.92	Unknown	0.2	0.5	0.5
0.98	Unknown	0.3	0.3	0.4
0.99	Unknown	1.9	—	1.8
1.00	Unknown	1.5	1.9	1.6
1.01	β-amirin	3.9	5.4	4.7
1.06	Unknown	2.3	2.4	2.8
1.12	Cycloartenol	76.8	82.2	75.0
1.18	Unknown	0.7	1.0	1.0
1.21	Unknown	2.9	1.8	3.7
1.24	24-methylenecycloartanol	2.9	2.5	3.5

[a]Calculated on the basis of the HRGC areas.

[b]Referred to β-sitosterol (TMS) = 1.00 in the analytical conditions described in Table 1.

[c]Tentative identification on the basis of data reported in literature (15, 29, 30).

Note: β-amirin = 5α-olean-12-en-3β-ol; cycloartenol = 9β,19-cyclo-5α-lanost-24-en-3β-ol; 24-methylene-cycloartanol = 24 methylene-9β,19-cyclo-5α-lanostan-3β-ol.

from flaxseed, tomato and soybean (cv. azuchi) seeds. Another component of this fraction which is present in high amounts is Δ^5-avenasterol (20%); it is characteristic of the lipid fraction extracted from oat, azuchi and coconut (15). Δ^7-Stigmastenol, on the other hand, is present in minimal quantity, even if tobacco seed oil contains large amounts of linoleic acid, a characteristic which is often accompanied by high levels of this sterol (more than 10%) as is the case, for example, in the safflower and sunflower oils (23,28).

Table 8 summarizes the composition of the triterpenic alcohols fraction: besides small quantities of β-amirin and 24-methylenecycloarthanol, high percentages of cycloartenol were detected in our samples. Table 9 shows the composition of the methyl sterols identified on the basis of the comparison of relative retention times (RRT) with those reported in the literature (15,29,30) for lipid

TABLE 9

Composition[a] of the Methyl Sterols Fraction (TMS)

RRT[b]	Identification[c]	Kentucky 104	Bright Italia	Bright V
0.80	Unknown	3.6	3.7	2.1
0.83	Unknown	2.9	2.0	2.6
0.88	Unknown	11.1	12.1	8.5
0.90	Unknown	13.5	11.6	11.2
1.01	Obtusifoliol	3.7	4.0	4.0
1.04	Unknown	2.4	3.3	3.2
1.07	Unknown	3.9	3.8	6.2
1.11	Cycloeucalenol	11.1	11.8	10.5
1.13	Gramisterol	Tr	Tr	Tr
1.30	Unknown	2.8	3.3	3.5
1.35	Isocitrostadienol	1.3	1.7	1.5
1.41	Citrostadienol	43.0	41.1	45.6
1.45	Unknown	0.7	1.6	1.0

[a]Calculated on the basis of the HRGC areas.

[b]Referred to β-sitosterol (TMS) = 1.00 in the analytical conditions described in Table 1.

[c]Tentative identification on the basis of data reported in literature (15, 29).

Note: obtusifoliol = $4\alpha,14\alpha$-dimethyl-24-methylene-5α-cholest-8-en-3β-ol; cycloeucalenol = $4\alpha,19\alpha$-dimethyl-9β,19 cyclo-24-methylene-5α-cholestan-3β-ol; gramisterol = 4α-methyl-24-methylene-5α-cholest-7-en-3β-ol; citrostadienol = 4α-methyl-(24Z)-24-ethylidene-5α-colest-7-en-3β-ol.

substrates of known composition. The most common component is citrostadienol, as is the case with the oil extracted from the grape seed and cotton (15). Methyl sterols fraction includes, besides this compound, eight other components, some of which are present in substantial amounts; identification is presently being completed and will be the subject of a forthcoming paper—the research will also include a study on tocopherol and hydrocarbon fractions.

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