Analysis and Fatty Acid Composition of Tobacco Seed Oils

R. W. RIEMENSCHNEIDER, R. M. SPECK, and E. G. BEINHART
Eastern Regional Research Laboratory,[1] Philadelphia, Pennsylvania

PRODUCTION of tobacco seed in the United States at present is essentially limited to that required for growing leaf tobacco. Because of cultural practices required for the production of special qualities in the tobacco leaf, the seed heads of some types of tobacco are removed before they mature. With certain types, however, such as the flue-cured, cigar wrapper, and Turkish types, considerable quantities of surplus seed could be harvested. A satisfactory outlet or market for the seed would have to be found, of course, to provide an incentive.

Flue-cured tobacco is grown on the largest acreage of any of our domestic types; the average annual acreage for the ten-year period 1933 to 1942 was 875,760 acres. Under present cultural practices the terminal flower or seed head is removed when it first appears. From a sucker growth which then develops, a number of seed heads form that produce from one-fourth to one ounce of seed per plant, or a yield ranging from 80 to 320 pounds of seed an acre. About 10,000 acres annually are planted for the production of shade-grown cigar wrapper tobacco. Some modification in the agronomic practices would have to be introduced to harvest the sucker seed from this crop.

In the production of most Turkish varieties of tobacco the seed heads are allowed to remain on the plant. The leaves are removed as they reach the proper stage of development, thus leaving essentially only the stalks and seed heads. The cultural practice employed for this type offers the best possibilities for harvesting appreciable quantities of seeds. Production of Turkish tobacco in this country, however, is still in the experimental stage although increasing interest is being shown in this type.

Mention might also be made of the possibilities of tobacco seed production from the species, *Nicotiana rustica*. Experiments have been made in recent years to establish this species in this country as a source of nicotine. Under proper conditions some varieties of *rustica* produce heavy yields of seed—800 to 1,200 pounds per acre. Unfortunately, however, the yield of nicotine is affected if the seed heads are allowed to develop. With complete topping of the seed heads and suckering 140 to 160 pounds of nicotine have been obtained per acre. The Brasilia variety of *rustica* in the fog areas of the west coast has produced, without topping or suckering, 100 pounds of nicotine and 1,000 pounds of seed per acre. Provided a substantial income from the seed could be realized, the growing of this species of tobacco as a source of nicotine and oil might be a profitable venture.

Comparatively little work has been done on the composition of tobacco seed oil, particularly from varieties grown in this country. Most of the published work has been concerned with the chemical constants and characteristics of the oil. The oil content of the seed has been reported as ranging from 30 to 43%. It is generally classified as semi-drying although it has also been described (1) as a rapidly drying oil, better than linseed oil and useful in varnishes. The oil has also been considered edible, particularly after refining (2), (3). A number of publications (4), (5), (6), (7), (8), (3), (9), dealing with the fatty acid composition differ widely as to the percentages of these acids. The linoleic acid content has been reported to be from 22.1% (4) to as high as 70.4% (6). There is agreement generally, however, that the principal fatty acids are linoleic, oleic, palmitic, and stearic acids although several have claimed that palmitic is the only saturated acid present. The presence of fatty acids more highly unsaturated than linoleic acid has not been reported. Nearly all the work reported on the composition of the oil was carried out prior to the improvements in the thiocyanometric method and prior to the development of the spectrophotometric method for the determination of polyunsaturated acids. In view of lack of agreement of published data and the scarcity of information on variation of domestically produced tobacco seed oil with variety and location, it was decided to determine the composition of this oil by modern methods.

The oils extracted from seed of various types and varieties of tobacco grown in different sections of the United States were remarkably uniform in fatty acid composition. Of principal interest was the unusually high linoleic acid content. The linoleic acid content of the 12 tobacco seed oils analyzed ranged from 71.7 to 77.3% of the total fatty acids. This oil, therefore, is one of the best potential sources of pure linoleic acid. The principal industrial application suggested by its composition would be in special-grade varnishes and alkyd-type resins.

Experimental

Starting material: Air-cleaned tobacco seeds of first quality from a number of different types and varieties of tobacco, grown in different sections of the United States, were used as a source of the oils.

Extraction of the oil: For the analytical determination of oil content, reported in Table I, the official solvent extraction procedure of the American Oil Chemists' Society was employed. Five-gram samples of seed, crushed by means of mortar and pestle, were extracted with anhydrous ethyl ether.

Larger samples of oil for composition studies were obtained by extracting seed, which had been crushed in a hammer mill, with successive portions of anhydrous ethyl ether at room temperature. This extraction was done by shaking the sample with ether in a flask and filtering off the extract. The solvent was

[1] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.

removed by distillation, the last traces by warming under high vacuum. The yield of oil by this procedure was about 1% lower than by the analytical method.

Composition of fatty acids: The fatty acid distribution of the various oils was calculated from the iodine and thiocyanogen values. The standard Wijs method was used to determine the iodine values. The thiocyanogen values were determined with 0.1 N solutions of thiocyanogen at 21° C., a 24-hour absorption period being used as described in previous work (10). The equations for calculating composition from the analyses of the oils are given below, along with the equations used when the analyses were made on methyl esters or fatty acids:

Equations for Calculating Composition from Analyses on Glycerides, Methyl Esters and Fatty Acids

Only oleic, linoleic, and saturated acids present (as glycerides, methyl esters, or acids)
Iodine Values (I. V.) by standard Wijs method
Thiocyanogen Values (T. V.), with 0.1 N solution, at 20-21° C., for 24-hour absorption period.

Type of Fatty Material on Which the I. V. and T. V. Were Determined

	Glycerides (Oils or Fats)	Methyl Esters	Fatty Acids
% Oleic.....	2.439 T. V.— 1.264 I. V.	2.450 T. V.— 1.271 I. V.	2.338 T. V.— 1.213 I. V.
% Linoleic.....	1.205 I. V.— 1.211 T. V.	1.211 I. V.— 1.216 T. V.	1.155 I. V.— 1.161 T. V.
% Saturated...	100—(% oleic + % linoleic)	100—(% oleic + % linoleic)	100—(% oleic + % linoleic)

The analytical data and calculated compositions of the oils (Table 1) show that they were remarkably uniform in composition regardless of the type and variety of the seeds or the location where they were grown.

A MORE complete investigation of the fat acid constituents was carried out on the oil from one sample of tobacco seed (T9) which was available in sufficiently large quantity. The methyl esters of the total mixed fatty acids prepared by methanolysis of the oil were fractionally distilled *in vacuo* through a 30-inch Vigreux column. Two principal fractions and a small residue were obtained. The distilled fractions were further separated by low-temperature crystallization. By crystallization from acetone (10:1) at —38° C., methyl palmitate (P-1, Table 2) was isolated from the first distilled fraction, and methyl stearate (P-2, Table 2) from the second. A second crystallization was necessary to obtain these saturated esters essentially pure.

The filtrates combined after the removal of the saturated esters and cooled to —65° C. yielded impure methyl oleate as a precipitate. Several additional crystallizations of this material were required to obtain methyl oleate in sufficient purity for identification.

The final filtrate material (F-1, Table 2) after removal of the solvent was saponified, and the fat acids were recovered. A 15-gram portion of these was brominated in ethyl ether at 0° C., yielding 14.9 grams of tetrabromostearic acid, m.p., 115-0-115.5° C. No evidence of a higher polybromide was obtained. This yield corresponds to a tetrabromide number of 99.3, a value which is about as high as ordinarily obtained with pure linoleic acid. Its iodine value, 172.0, and thiocyanogen value, 93.5, however, showed that this material was 90.1% linoleic acid. Spectrophotometric analysis gave a value of 93.7% linoleic acid.

The residue (14.0 grams) from the distillation of the mixed methyl esters contained 30.3% unsaponifiable material, essentially all that was present in the original oil (1.42%). The fatty acids from this fraction were brominated in ethyl ether at 0° C. Only tetrabromostearic acid was obtained as a solid or crystalline bromide. Stearic acid was also isolated from this brominated mixture.

Calculations of fat acid distribution were made from the analysis of the distilled methyl ester fractions and also from the analysis of the fractions obtained by the crystallization of the methyl esters. The results are summarized in Table 2.

The fatty acid compositions calculated from the various analytical data are in good agreement.

Spectrophotometric analysis[2]*:* Two samples of tobacco seed oil, T9 and T16, and Fraction F-1 (Table 2) were analyzed spectrophotometrically for polyunsaturated constituents by a modification (11) of the method described by Mitchell, Kraybill and Zscheile (12) as extended by Beadle and Kraybill (13). The isomerization was carried out in KOH-ethylene gly-

[2] The authors acknowledge with thanks the contribution of the spectrophotometric analyses by Dr. B. A. Brice and Dr. M. L. Swain.

TABLE 1

Oil Content and Fatty Acid Composition of the Oils From Tobacco Seed of Different Types and Varieties

Sample No.	Type and Variety of Tobacco Seed	Where Grown	Year Grown	Oil Content %	Ref. Index 25° n—— D	Free Fatty Acids %	I. V. (Wijs)	T. V.*	Fatty Acid Composition		
									Oleic %	Linoleic %	Saturated
T1	Cigar, shade, resistant	Florida	1943	39.2	1.4738	1.27	141.0	78.3	12.7	75.1	12.2
T2	Cigar leaf, Pa., broadleaf	Pa.	1943	39.1	1.4742	0.81	141.9	78.5	12.0	76.0	12.0
T4	Cigar, shade, Cuban, R-9	Florida	1942	40.0	1.4738	1.74	139.5	78.5	15.1	73.1	11.8
T5	Flue-cured	N. C.	1941	40.8	1.4740	1.41	142.5	80.0	14.9	74.9	10.2
T9	Flue-cured	Georgia	1943	40.3	1.4740	1.24	142.6	80.4	15.9	74.5	9.6
T6	Turkish	N. C.	1940	43.4	1.4739	0.95	141.8	79.8	15.3	74.3	10.4
T10	Burley, Kentucky No. 16	Kentucky	1942	40.5	1.4741	0.43	144.2	79.7	12.1	77.3	10.6
T11	Burley, Kentucky No. 16	Kentucky	1943	40.7	1.4741	0.14	144.6	80.4	13.3	76.9	9.8
T3	*Nicotiana rustica* No. 34753	Pa.	1943	39.1	1.4739	1.46	141.6	80.5	17.2	73.2	9.6
T12	*Nicotiana rustica*	Calif.	1943	35.1	1.4743	0.26	145.9	81.6	14.5	77.0	8.5
T15	*Nicotiana rustica* No. 4385, Line 5.6	Maryland	1943	38.6	1.4738	2.34	140.7	80.8	19.2	71.7	9.1
T16	*Nicotiana rustica* Olson 68	Maryland	1943	35.3	1.4738	3.76	141.3	80.5	17.6	72.8	9.6

* The thiocyanogen values were determined with 0.1 N thiocyanogen solution at 21° C., 24-hour absorption time being used (10).

TABLE 2

Fatty Acid Composition of Tobacco Seed Oil, T9, Calculated From Analyses of Fractions Obtained by Distillation and Crystallization of the Methyl Esters

Fraction	Weight (a) gms.	I. V.	T. V.	Fatty Acid Composition			
				Linoleic gms.	Oleic gms.	Palmitic gms.	Stearic gms.
Distilled esters:							
D-1	57.3	94.5	53.9	28.0	6.9	22.4	.....
D-2	220.8	154.1	85.6	182.3	30.9	.....	7.6
D-3 (residue)	9.8(e)	123.5(b)	73.8(b)	5.6	2.2	.....	2.0
Total	287.9	.....	.....	215.9	40.0	22.4	9.6
Fatty acid composition	.....	.....	.....	75.0%	13.9%	7.8%	3.3%
Crystallization fractions:							
P-1 (from D-1)	21.9	0.0	0.0	.....	.....	21.9	.....
P-2 (from D-2)	7.3	0.0	0.0	.....	.....	.....	7.3
P-3 (from D-1, D-2 filtrates)	15.6	76.2	73.8	0.4	13.1	1.9	0.2
F-1 (filtrates from P-3)	233.3	172.0(c)	93.5(c)	210.3	23.0	.....	.....
Distillation residue (D-3)	9.8	123.5(b)	73.8(b)	5.6	2.2	.....	2.0
Total	287.9	.....	.....	216.3	38.3	23.8	9.5
Fatty acid composition	.....	.....	.....	75.1%	13.3%	8.3%	3.3%
Fatty acid composition from Table 1	.....	142.6	80.4	74.5%	15.9%	6.4%(d)	3.2%(d)

(a) Weights corrected for samples taken for analysis and for manipulative losses.
(b) Determined on fatty acids after removal of unsaponifiable material.
(c) Determined on fatty acids.
(d) Determined by analysis of the saturated acids obtained by Bertram oxidation.
(e) Weight corrected for 4.2 gm. of unsaponifiable material.

col. The averages of duplicate analyses are given below:

	T9 %	T16 %	F-1 % (Table 2)
Linoleic acid	77.5(a)	78.2(a)	93.7
Linolenic acid	0.18	0.38	0.37
Arachidonic acid	0.00	0.00	0.00
Conjugated diene acid	0.08	0.21	1.06
Conjugated triene acid	0.004	0.007	0.009
Conjugated tetraene acid	0.0003	0.000	0.0015

(a) The analyses were made on the oils, but the content of linoleic acid was calculated as percentage of the total fatty acids.

The percentages of linoleic acid determined for tobacco seed oils T9 and T16 by the spectrophotometric method are 3.0 and 5.4 units higher than those obtained by the thiocyanometric method.

Stability and tocopherol determinations: The stability of one sample (T9) of tobacco seed oil was determined by the active oxygen method (14). When using a peroxide value of 25 millimols per kilogram as the end of the induction period, the stability was found to be 20 hours, an unusually good stability for an oil of such high unsaturation.

This sample of oil contained 0.043 percent tocopherol, as determined by the Parker-McFarlane (15) modification of the Emmerie-Engle method.

Summary

THE fatty acid compositions of twelve samples of oil representing a number of different types and varieties of tobacco were determined by the thiocyanometric method. The samples were remarkably uniform in composition, containing on the average 75% linoleic, 15% oleic, and 10% saturated acids.

Spectrophotometric determination of the linoleic acid content of two samples of oil gave values 3.0 and 5.4% higher than those by the thiocyanometric method.

A more complete investigation of the fatty acid constituents of one sample of flue-cured tobacco seed oil was carried out by analysis of fractions obtained by distillation of the methyl esters and by low-temperature crystallization of the distilled ester fractions. The composition calculated from these analyses agreed well with that determined from analysis directly on the oil. The saturated acids consisted of palmitic and stearic acids, the proportions being about 7 and 3%, respectively, of the total fatty acids. Analysis of this sample of oil showed that it contained 0.043% of tocopherol.

From its composition, tobacco seed oil would seem to be particularly suitable for the manufacture of nonyellowing alkyds or for the preparation of technical linoleic acid.

LITERATURE CITED

(1) Cohen, N. H., Mededeel, Proefsta. Vorstandland. Tabak No. 14, 57 (1915).

(2) Morozov, I., and Grashin, A., Masloboino-Zhirovoe Delo, No. 11-12, 53 (1930).

(3) Kaufmann, H. P., Fette u. Seifen *48*, 193 (1941).

(4) Paris, G., Bot. tec. (R. ist. sci. sper. tabacco, Scafati) *17*, No. 1, 101 (1920).

(5) Pyatnitzkii, M. P., U.S.S.R. State Inst. Tobacco Inves. Bull. *61*, 20 (1929).

(6) Roberts, W. L., and Schuette, H. A., J. Am. Chem. Soc. *56*, 207 (1934).

(7) Cruz, A. O., and West, A. P., Phillipine J. Sci. *61*, 161 (1936).

(8) Salisbury, L. F., J. Biol. Chem. *117*, 21 (1937).

(9) Venkatarao, C., Narasingarao, M., and Venkateswarula, A., J. Ind. Chem. Soc. *20*, 374 (1943).

(10) Riemenschneider, R. W., Swift, C. E., and Sando, C. E., Oil and Soap *18*, 203 (1941).

(11) Brice, B. A., and Swain, M. L., paper presented at the Meeting of the Optical Society of America, New York, October 20-21, 1944.

(12) Mitchell, J. H., Jr., Kraybill, H. R., and Zscheile, F. P., Ind. Eng. Chem., Anal. Ed., *15*, 1 (1943).

(13) Beadle, B. W., and Kraybill, H. R., J. Am. Chem. Soc. *66*, 1232 (1944).

(14) King, A. E., Roschen, H. L., and Irwin, W. H., Oil and Soap *9*, 89 (1932).

(15) Parker, W. E., and McFarlane, W. D., Can. J. Research *18B*, 405 (1940).