

[CONTRIBUTION FROM THE LABORATORY OF FOODS AND SANITATION, UNIVERSITY OF WISCONSIN]

The Characteristics and Composition of Wisconsin-Grown Tobacco Seed Oil

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The literature of tobacco seed oil investigations divides itself into two fields of interest. One deals with the presence of nicotine in this oil¹ and the other centers around the characteristics and composition² thereof. The question suggested by the former, which is the older, has apparently been settled in the negative. The latter did not come into being until soon after the turn of the present century. It records, without exception, data relevant only to foreign-grown oils. A uniformity in description of color is lacking therein, for this property has been recorded as being either some shade of yellow^{1b,2a,f,g,j} or dark brown,^{2c} and even dark green.^{2e} Furthermore, the reported percentage composition^{2a,f,h} with respect to its acids does not agree even as to the order of magnitude thereof; at least one set of data^{2a} is clearly contradictory in that the oil in question is said to contain 25% oleic acid and 52% palmitic acid, yet its iodine number is reported to be 118.6; besides which, none of these analyses is as comprehensive as the newer technique in this field now admits of.

There is an obvious need for a re-investigation of the subject not only for reasons which naturally

follow from this review of the earlier work, but also because of the desirability of comparing the characteristics of American-grown oils with those already recorded in the literature. The data which are communicated herein pertain to oils recovered from seeds grown in the tobacco-producing areas of the State of Wisconsin.

I. Preparation of Materials³

A preliminary survey of five oils (Table I) grown in different localities of the tobacco-producing areas of this state made it apparent that significant differences in composition would probably not be found even though there might be qualitative differences in the condition of the seeds from which they have been recovered. Two of the groups in question (A, B) consisted of well filled seed possessing a uniform brown color; a third, (C), separated from the by-products resulting from the cleaning of one of the preceding lot, was composed of unevenly pigmented, shriveled seeds of low density; another group, (D), apparently the product of careless threshing, contained many broken seeds; finally another (E) was composed of seed at least twenty-seven years old.

The comprehensive examination originally planned for the entire group of five was, therefore, narrowed to two oils, each the product of a different mode of recovery, *viz.*: (1) petroleic ether extraction following a preliminary crushing of the seed because of its almost impervious seed coat and (2) expulsion, in approximately 80% efficiency, by means of an hydraulic press. These oils are hereinafter referred to as extracted and expressed, respectively. They resembled virgin olive oil in color. The first, which had been recovered from an unknown strain of seed, possessed a faintly bitter, though not unpleasant, taste. This bitterness was not, however, apparent in the second oil, one obtained from seed designated as "Havana 142."

(3) Acknowledgment is gratefully made to Professor James J. Johnson of the Wisconsin Agricultural Experiment Station who furnished the tobacco seeds which were used in this investigation and to Mr. C. S. Ladd, Commissioner and Chemist, State Regulatory Department, Bismarck, North Dakota, for placing the facilities of his laboratory at the disposal of W. L. Roberts for carrying out part of the experimental work incidental to this paper.

TABLE I

PRELIMINARY SURVEY OF TOBACCO SEED OILS

Characteristic	Oil A	Oil B	Oil C	Oil D	Oil E
Specific gravity 20°/20°	0.9240	0.9235	0.9244	0.9260	0.9240
Index of refraction 20°	1.4763	1.4757	1.4759	1.4762	1.4755
Iodine number (Wijs)	146.7	145.4	145.5	143.6	142.7
Saponification number	189.8	190.4	190.7	189.1	189.8
Insoluble acids, %	95.4	95.8	95.8	95.9	95.8
Soluble acids, %	0.05	0.03	0.07	0.22	0.03
Acidity (% oleic)	1.38	0.77	3.51	9.85 ^a	6.52 ^b

^a This oil was rancid. Its high acidity is probably due to exposure to the air because of the damaged seed coat.

^b Probably due to the age of the seed.

(1) (a) Buchner, *Repert. Pharm.*, **32**, 361 (1829); (b) Brandl, *Vierteljahresschr. prakt. Pharm.*, **13**, 161 (1864).

(2) (a) Ampola and Scurti, *Gazz. chim. ital.*, **34**, II, 315 (1904); (b) Cohen, *Indische Mercur*, **38**, 884 (1915), see also *Chem. Abstracts*, **10**, 1105; (c) Dubovitz, *Seifenfabr.*, **37**, 310 (1917); (d) Preisseecker and Brezina, *Fachl. Mitt. Oesterr. Tabakregie*, **1917**, No. **4**, 97, through *Chem. Zentr.*, **1918**, II, 1042; (e) Klimont, *Pharm. Post*, **51**, 561 (1918); (f) Paris, *Boll. tecnico. ist. sci. sper. tabacco*, **17**, 101 (1920); (g) Voerman, *Chem. Weekblad*, **22**, 61 (1925); (h) Pyatnitskii, U. S. S. R. State Inst. Invest. *Bull.*, **61**, (1929), through *Chem. Abstracts*, **24**, 4648; (i) Kandilis and Karnis, *Praktika (Akad. Athenon)*, **4**, 475 (1929), through *C. A.*, **25**, 3188 (1931); (j) Morozov and Grashin, *Masloboino-Zhirnoe Delo*, **1930**, No. **11-13**, 53, through *C. A.*, **26**, 4193 (1932); (k) Belyaev, *ibid.*, **1932**, No. **3**, 47, through *C. A.*, **27**, 2594; (l) Kraft, *ibid.*, No. **6**, 55, through *Chem. Zentr.*, **1933**, I, 3816.

The seeds themselves were found to have an oil content which lies within the range reported by others (31 to 41.8%).^{1,2b,c,f,i,j,k} Their proximate composition follows (Table II).

TABLE II
COMPOSITION OF TOBACCO SEED

Constituent	Strain unknown, "Havana 142,"	
	%	%
Moisture	4.26	3.61
Ether extract	38.90	38.54
Crude protein	24.29	24.82
Crude fiber	18.92	19.02
Ash	3.66	3.83
Soluble ash	1.45	1.47
Insoluble ash	2.21	2.35
Alkalinity of soluble ash ^a	9.4	9.3
Alkalinity of insoluble ash ^a	25.2	28.4
Nitrogen-free extract	9.97	10.18

^a Cc. 0.1 N acid per gram of seed.

II. Analysis of Oils

(a) **Chemical and Physical Characteristics.**—The more important chemical and physical characteristics (Table III) of both oils were determined by well-established procedures.

Of technical interest are the flash and fire points (Cleveland open cup) and the viscosity (Saybolt). Except for the flash point of the expressed oil, which was 610°F. (321°), the values observed were alike in both cases. They are, in the order named above, 605°F. (318°), 665°F. (352°), and 155 sec. at 100°F. (38°), 96 sec. at 130°F. (54°), 50 sec. at 210°F. (99°).

TABLE III
CHEMICAL AND PHYSICAL CHARACTERISTICS OF TOBACCO SEED OIL

	Extracted	Expressed
Specific gravity, 20°/20°	0.9240	0.9235
Index of refraction, 20°	1.4763	1.4757
Titer test	18.1	18.2
Solidifying point, °C.	-16.0	-14.0
Cloud point, °C.	-7.0	-7.0
Iodine number (Wijs)	146.7	145.4
Thiocyanogen number	84.7	84.0
Saponification number	189.8	190.4
Reichert-Meißl number	0.26	0.28
Polenske number	0.13	0.11
Hydroxyl number	8.7	5.3
Acid number	2.8	1.5
Soluble acids (per cent. as butyric)	0.05	0.03
Insoluble acids (Hehner number)	94.2	94.6
Mean molecular weight of same	278.9	278.8
Saturated fatty acids (per cent.) corr.	7.7	8.0
Mean molecular weight of same	266.2	266.5
Unsaturated fatty acids (per cent.) corr. ^a	86.5	86.7
Mean molecular weight of same	279.6	279.9
Iodine number of same	164.7	163.5
Unsaponifiable matter (per cent.)	1.25	1.24
Iodine number of same	77.0	77.4

^a Corrected also for unsaponifiable matter.

(b) **Saturated Acids.**—The low polenske number indicated that myristic acid, if present, would be found only in traces, and that others of lower molecular weight are absent. The almost negligible value for the soluble fatty acids, together with the low Reichert-Meißl number eliminated the others of this group. Negative Renard⁴

(4) Renard, *Compt. rend.*, **73**, 1330 (1871).

and Bellier tests⁵ contraindicated the presence of arachidic acid and its higher homologs. The search was, therefore, narrowed to stearic and palmitic acids.

A 20-g. portion of the saturated acids, prepared by the conventional lead salt-ether method, was separated into ten approximately equal fractions with the aid of magnesium acetate.⁶ The melting points and mean molecular weights of the acids isolated from them showed that the first fraction consisted of practically pure stearic acid, that the last three were palmitic acid of a very high degree of purity, and that the intermediate fractions were mixtures of the two. Systematic fractional recrystallizations from hydro-alcoholic solution eventually yielded palmitic acid (m. p. 62.5–63.0°, m. p. of the *p*-toluidide 95–96°) and stearic acid (m. p. 68–69°, m. p. of the *p*-toluidide 97–98°). Qualitatively it was apparent that the palmitic acid predominated over the stearic acid. Such proved to be the case when calculations (Table IV), based on the mean molecular weight of the saturated fatty acids and the molecular weights of the component acids, were made, and, by way of confirmation, the stearic acid content of the saturated acid fraction was directly determined by the method of Hehner and Mitchell.⁷ The satisfactory agreement in the results obtained by these two methods of approach lends additional proof for the presence of the acids in question in this fraction and confirms the applicability of the latter method to the analysis of this particular mixture.

TABLE IV
PERCENTAGE COMPOSITION OF THE SATURATED ACID FRACTION

Acid	Extracted oil		Expressed oil	
	Obs.	Calcd.	Obs.	Calcd.
Palmitic	39.0	37.9	39.5	38.9
Stearic	61.0	62.1	60.5	61.1

(c) **Unsaturated Acids.**—No hexabromide derivative of linolenic acid was obtained on brominating⁸ a 10-g. portion of the unsaturated acids at -10°. It is true that a voluminous precipitate was obtained at this temperature but analysis proved it to be the tetrabromo derivative of linoleic acid. It had the same composition as that fraction which was insoluble in cold petroleum ether (m. p. 113–114°; Br theoretical 53.3%, observed 53.6%). Its precipitation at this point was apparently due to unexpected solubility conditions in cold ethyl ether which had been created because of its abundance. The petroleic ether-soluble fraction (Br 48.7%), which also was very large, consisted of a mixture of the di- and tetrabromo derivatives of oleic and linoleic acids, respectively. Its abundance suggested the thought that there are involved here the two isomeric forms of the latter.

Confirmation of the foregoing conclusions was subsequently obtained when identification of these acids was attempted by means of their permanganate oxidation products. No hexahydroxystearic acid was detected but a large quantity of the tetrahydroxy derivative (mol. wt. 348.6, OH/COOH₄) and a relatively smaller amount of the corresponding dihydroxy compound (mol. wt. 316,

(5) Bellier, *Ann. chim. anal.*, **4**, 4 (1899).

(6) Heintz, *J. prakt. Chem.*, **66**, 1 (1855).

(7) Hehner and Mitchell, *Analyst*, **21**, 316 (1886).

(8) Bibener and Muggenthaler, *Farben-Ztg.*, **18**, 131 (1912).

OH/COOH₂) was obtained. From the former there was eventually recovered by replicated crystallizations a small fraction melting at 166°, whereas the bulk of it melted at 158–159°.

It is well known that the common form of sativic acid (tetrahydroxystearic acid) melts at 174°. Some uncertainty apparently surrounds the melting point of its isomer for this value has been variously reported as lying between 152 and 165°. The fact that the former melting point was not observed throughout this investigation, although molecular weight and melting point of the petroleic ether-insoluble tetrabromide indicates the contrary, makes it evident that the material under analysis was, as was predicted, a mixture of the two sativic acids. It appears, too, that they are present in this oil in eutectic mixture and that the uncertain melting points found in the past by various investigators are in reality those of mixtures of this type rather than those of pure compounds. Inasmuch as purification with ethyl ether and with benzene, and repeated crystallization, in turn, from hot water and anhydrous ethyl alcohol failed appreciably to raise the melting point, it is evident that some other mode of separation is necessary.

The relative proportions of oleic and linoleic acids as calculated from the iodine numbers of the unsaturated acids and the bromine content of the several fractions which were obtained in the Eibener-Muggenthaler⁸ mode of separation gave the percentage composition of this fraction shown in Table V.

TABLE V

Acid	PERCENTAGE COMPOSITION OF THE UNSATURATED ACID FRACTION			
	Extracted oil		Expressed oil	
	Obs. ^a	Calcd. ^b	Obs. ^a	Calcd. ^b
Oleic	18.9	18.0	20.4	19.4
Linoleic	81.1	82.0	79.6	80.6

^a From bromine addition products. ^b From iodine numbers of unsaturated acids.

Besides the case previously criticized,^{2a} there is a record^{2b} the substance of which is that this oil was found to contain 50 and 20%, respectively, of oleic and linoleic acids, whereas palmitic acid was present to the extent of 22.8%. No stearic acid was found. On the other hand, the data of Pyatnitzkii,^{2b} with one exception, approximate the order of magnitude of the observed percentage composition of the Wisconsin oils, to wit: oleic acid, 21.7%; linoleic acid, 60.0%; palmitic acid, 9.6%. The presence, if any, of stearic acid was not accounted for.

(d) **Unsaponifiable Matter.**—From the unsaponifiable matter, which appeared to be homogeneous, there was

isolated a well crystallized phytosterol (m. p. 135–137°; m. p. of acetate 125.5–127°), apparently sitosterol.

TABLE VI

COMPARISON OF THE CHARACTERISTICS OF TOBACCO SEED OILS

Constant	Foreign-grown oils	Wisconsin-grown oils
Specific gravity	0.9175–0.9440	0.9235–0.9260
Refractive index	1.4735–1.4800	1.4755–1.4763
Solidifying point, °C.	–12° to –25°	–14° to –16°
Titer test, °C.	18	18.1–18.2
Iodine number	117.8–151.6	142.7–146.7
Saponification number	186.8–200.7	189.1–190.7
Hehner number	94.7–96.3	95.4–95.9
Reichert–Meissl number	0.30–2.03	0.26–0.28
Polenske number	0.15–0.30	0.11–0.13
Unsaponifiable matter	1.20–2.98	1.24–1.25

III. Summary

That it forms a film on exposure to air is perhaps the earliest characterization^{1b} which tobacco seed oil has received. As a result, practically all investigators since that time have classified it as a drying oil. If this classification be a valid one, then this oil is unusual in that it contains none of those highly unsaturated acids usually associated with products of this type. Its predominant unsaturated acid is linoleic acid which apparently is present here in two isomeric forms. In far lesser amount is found oleic acid.

The average percentage composition of these oils has been found to be as follows: palmitic acid, 3.1; stearic acid, 4.8; oleic acid, 16.2; linoleic acid, 70.4; unsaponifiable matter, 1.25. In so far as comparable data² are available, it appears that the physical and chemical constants of Wisconsin-grown oils fall within the limits of those reported by others² (Table VI), but not so the chemical composition. In view of the similarity of the two groups of data it seems a bit strange that the latter condition should exist. It is perhaps explainable on the ground that the percentage compositions reported by several others^{2a,f} represent mere approximations.

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