CONSTITUENTS OF CRUDE COTTONSEED OIL

(Palmitin, Di-palmitin, and Phytosteroline)

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This paper reports further progress in the investigation assigned to the Oil, Fat and Wax Laboratory at the conference of the Basic Research Committee and the Department of Agriculture, September, 1921.

The work of 1925 was confined to investigating further the alcohol-soluble portion of the "settlings" which separate when crude cottonseed oil is permitted to stand. By "settlings" we mean those substances which precipitate from the clear crude oil and have no connection with meal or press foots. The settlings were separated from a 5-gallon sample of high-grade crude oil, which had been standing in the laboratory for about 3 years, and were washed with acetone until free from oil. The extracted settlings freed from moisture and solvent weighed 485 grams. Half of the product, which had been ground to a powder, was repeatedly boiled with 500 cc. portions of absolute alcohol. After each treatment the hot alcoholic solution was filtered by suction and the insoluble residue was washed with small quantities of hot alcohol. Boiling with five 500 cc. portions of alcohol was sufficient to extract the soluble constituents. The combined alcohol extracts and washings, which amounted to about 3 liters, were concentrated by distillation to about one-half the original volume. After this solution had stood for several days in a cool place, the large quantity of almost white crystals was removed by filtration and washed with several small portions of cold alcohol. The filtrate was concentrated to about 800 cc. and set aside for further investigation.

The crystals were agitated with 1 liter of absolute alcohol, slowly heated to about 60° C., and filtered to remove a small quantity of dark brown residue. Then the yellow solution was decolorized by boiling with norite. The filtered solution, on cooling, deposited a bulky white mass composed entirely of minute slender prisms. After filtering, washing, and drying in a vacuum desiccator, the substance was found to melt at 63.5 to 64° C. Recrystallization did not change the melting point. Several grams of the crystals were dissolved in ether and shaken with an N/5 aqueous solution of potassium hydroxide. When the layers had separated, the alkali solution was removed and examined for fatty acids, but none was detected. A larger portion was saponified. After suitable treatment about 2 cc. of glycerine was separated and identified. The free fatty acids obtained from the saponified glyceride were subjected to fractional crystallization from 80 per cent alcohol. No acid other than palmitic was detected. The saponification value of this glyceride was found to be (*a*) 208.6, (*b*) 208.4. The calculated value for palmitin is 208.6. The yield of palmitin was 60 grams, or 24.7 per cent of the settlings.

[1] Previous Reports: The Cotton Oil Press 6 (No. 4), 33; 7 (No. 2), 35; 7 (No. 5), 29. THIS JOURNAL 1, 30; 2, 101.

The alcohol solutions from which the palmitin had been recrystallized were combined and concentrated to a volume of about 200 cc. On standing, a small quantity of another glyceride, which melted at 68° C., was obtained. Recrystallization of the glyceride from absolute alcohol raised the melting point to 70° C. It crystallizes from alcohol in radiating clusters of minute needlelike prisms, which appear to belong to the orthorhombic system. A microscopic examination showed that all of the crystals were identical. The saponification value was 196.7. Calculated for di-palmitin, it is 197.4. No acid other than palmitic could be detected. This glyceride agrees with the di-palmitin synthesized by Ad. von Grün (Berichte, 1905, 38, 2285).

In all, 2.6 grams of this glyceride, corresponding to 1.07 per cent of the settlings, was obtained. The di-palmitin was presumably formed by the hydrolysis of a very small quantity of a triglyceride. The presence of this and perhaps other diglycerides may account for the acetyl values obtained with cottonseed and many other oils from which it has not been possible to isolate any hydroxy-acids such as are found, for example, in castor oil.

Although it had stood for about 3 years in the laboratory, the oil from which the settlings were obtained was free from rancidity and generally in excellent condition. Its acidity as oleic acid was about 2 per cent at the time the settlings were removed.

In so far as known, this is the first instance in which pure glycerides, palmitin and di-palmitin, have been isolated and identified from cottonseed oil. In 1919, David Wesson (The Cotton Oil Press 2 (No. 7), 34) obtained by fractional crystallization of cottonseed oil stearine, a glyceride melting at 54.9 which contained 91.5 per cent of palmitin.

Phytosteroline. The original alcohol solution obtained by extracting the settlings, and from which the glycerides already described had been separated, was further concentrated to a volume of about 400 cc. and allowed to stand for several days. The small quantity of aggregates of minute brown crystals, which had separated, was removed by filtration. Repeated recrystallization from alcohol finally gave a white granular powder. After each crystallization the substance became much less soluble in alcohol, although from the first it was not readily soluble. The purified substance when heated gradually turns brown from 250° and melts with decomposition at about 275° to 276° C. It gave an intense reaction for sterol when tested in the usual manner with acetic anhydride and sulphuric acid. A combustion made by R. M. Hann gave the following.

Substance, 0.1278 gram gave 0.3382 gram of CO_2 and 0.1156 gram of H_2O.

Found, carbon 72.16 per cent, hydrogen 10.12 per cent.

Calculated for $C_{27}H_{45}O.C_6H_{11}O_5$, carbon 72.20 per cent, hydrogen 10.27 per cent.

About 0.15 gram was acetylated by boiling it for a half hour with 10 cc. of acetic anhydride and 0.2 gram of fused sodium acetate. When cool, 30 cc. of water was added and the mixture was allowed to stand over night. The precipitate was filtered on a hardened filter paper, washed thoroughly, and dried at about 80° C. Crystallization from 95 per cent alcohol gave flat prisms, with a pearly luster, melting at 166° to 167° C.

This substance agrees in all its properties as well as in composition with the phytosteroline from other sources, previously described by F. B. Power and A. H. Salway (J. Chem. Soc., 1913, 103, 399). The total quantity of phytosteroline isolated amounted to about 0.1 per cent of the settlings.

A similar substance has been isolated and identified from the benzene extract of cotton lint by R. G. Fargher and M. E. Probert (J. Textile Inst., 1923, 14, T 49).

Several very small quantities of a high melting substance which had been obtained in previous years in our study of the constituents of the settlings from different lots of crude cottonseed oil gave the sterol test. These were combined and acetylated. The purified acetyl derivative melted at 166° to 167° C., showing that the original compound was the same phytosteroline as that just described.

Not being an emulsifying agent, phytosteroline plays no part in the retention of neutral oil by the soap stock or foots which results when the oil is refined by the caustic soda method. On the other hand, the mucilage, phosphatides, and resin reported in previous papers as occurring in crude oil are characteristic emulsifying agents.

Summary

The alcohol-soluble portion of the settlings which separate from clear crude cottonseed oil has been studied further. Although the oil had stood in the laboratory for about 3 years before the settlings were removed for the investigation, it was free from rancidity and in excellent condition.

Palmitin, amounting to about 25 per cent, and di-palmitin, amounting to about 1 per cent of the total settlings, were isolated and identified. About 0.1 per cent of a phytosteroline, which gave an acetyl derivative melting at 166° to 167° C., was obtained.

It appears from the results of this investigation that the dipalmitin, which was probably produced by the hydrolysis of a very small quantity of a tri-glyceride, accounts in part for the acetyl value obtained with cottonseed oil. As no investigation has succeeded in showing the presence of any hydroxy acids in cottonseed, or for that matter in many other oils giving a comparatively small acetyl value, the values noted may be largely due to the presence of small quantities of various di-glycerides.

CONTRIBUTION FROM THE OIL, FAT AND WAX LABORATORY,
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