THE COMPOSITION OF CRUDE COTTONSEED OIL; A SUMMARY

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Cottonseed oil is the most important edible oil produced in the United States and in recent years the percentage of the output used for alimentary purposes has been increasing. The leaders in the cottonseed oil industry realize the importance of a close acquaintance with the raw material, i.e., the character and composition of the crude oil, because this familiarity will help to increase the efficiency of the present processes and aid in developing new ones. Several associations and societies have requested that the composition of the crude oil be studied by the Department of Agriculture. This paper summarizes the results so far obtained from the investigations conducted in the Oil, Fat and Wax Laboratory.

Physical and Chemical Characteristics

In Table 1 are tabulated the variations and averages of the results obtained by the analyses of 41 authentic commercial samples of oil.* The samples were collected at the mills in nine southern states during the pressing season 1919-20 and represent the current production of hot pressed oil. The acidity test was made on the crude oil. For the other determinations, oils refined according to the official methods of the American Oil Chemists Society and the Interstate Cottonseed Crushers' Association were used.

TABLE 1. ANALYSES OF AUTHENTIC AMERICAN COTTONSEED OILS
1919-1920

		Acidity Oleic Acid Per Cent	Refining Loss Per Cent	Titer	Refractive Index at 20° C.	Specific Gravity 25°/25° C.
Average		1.9	8.7	34.4	1.4695	0.9173
Usual Variations	High	3.0	10.8	35.0	1.4700	0.9174
	Low	0.9	5.3	33.8	1.4690	0.9171
Extremes	High	9.2	29.2	35.6	1.4720	0.9181
	Low	0.6	3.6	32.2	1.4768	0.9168

		Iodine No. (Hanus)	Saponification Value	Saturated Acids Corrected Per Cent	Unsaturated Acids Corrected Per Cent	Iodine No. (Hanus) of Unsaturated Acids
Average		107.4	194.7	23.5	71.5	146.3
Usual Variations	High	109.0	195.7	24.2	72.3	147.0
	Low	106.0	193.9	22.9	70.6	145.0
Extremes	High	111.2	195.7	25.3	73.9	148.0
	Low	103.0	193.9	20.9	69.7	144.2

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* For detailed results see Cotton Oil Press 4: 3, 85 (July, 1920; and 4: 6, 48 (October, 1920).

Neutral Oil

The most important constituent of crude oil is, of course, the neutral oil. It is possible to determine the amount present by the David Wesson method,* which, briefly, is as follows:

A weighed sample of crude cottonseed oil (about 10 g.) dissolved in petroleum ether is shaken with 10 cc. of a 14 per cent potassium hydroxide aqueous solution. Then 25 cc. of 50 per cent alcohol is added and the agitation repeated. By this treatment the free fatty acids are neutralized and dissolved in the alcoholic alkaline solution, the dark coloring matter is extracted, and very small quantities of certain fatty acid compounds other than fatty acid triglycerides are saponified. The mixture is allowed to stand until it separates into two layers, an upper layer consisting of a petroleum-ether solution of neutral (refined) oil and a lower layer containing the potassium salts of the fatty acids, the coloring matter, and other impurities. The two layers are separated. The solvent is distilled off and the residue, which consists of neutral oil, is weighed. The oil thus obtained has about the same color as that obtained by the official refining loss test. The refining loss (Wesson method) or the percentage of concomitants other than neutral oil in the crude oil is obtained by substracting the percentage of neutral oil from one hundred.

TABLE 2. ANALYSES OF CRUDE COTTONSEED OIL

Sample	Neutral Oil Per Cent	Refining Loss (Wesson Method) Per Cent	Fatty Acids in Wesson Refining Loss Per Cent	Free Fatty Acids (Calculated as Oleic Acid) Per Cent	Refining Loss (Official Method) Per Cent
1	98.07	1.93	1.41	0.65	6.69
2	97.82	2.18	1.99	1.42	12.00
3	96.80	3.20	2.23	1.57	10.40
4	96.28	3.72	2.96	2.05	8.60
5	95.98	4.02	2.98	2.19	8.20
6	94.05	5.95	4.99	3.79	18.56
7	87.42	12.58	11.43	9.18	29.60

To determine the total fatty acids and the small amounts of other acids neutralized by the potassium hydroxide in the alcoholic alkali solution, the alcohol is evaporated, about 100 cc. of water is added, and the fatty acids are precipitated with hydrochloric acid. The mixture is then heated until the fatty acids collect on top of the solution and cooled until they solidify, when a separation is made by filtration. After being washed with water and dried, the acids are dissolved off the filter with petroleum ether and weighed after distilling off the solvent. This determination is entitled "fatty acids in refining loss."

Table 2 gives some results of analyses of crude oil by the foregoing

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* Detailed procedure for making this determination and several others was published in The Cotton Oil Press 6: 4, 33 (August, 1922).

methods. For comparison the percentage of free fatty acids and the refining loss by the official method are included.

The Wesson procedure does not decompose fatty acid triglycerides. When applied to dry neutral oils, 100 per cent recovery of neutral oil was obtained and when applied to commercially refined oils a refining loss amounting to 0.20-0.22 per cent resulted. This is equal to the sum of the percentages of moisture (0.14-0.16) and acidity (0.04-0.06).

The official refining loss test, used by the cottonseed oil trade to evaluate crude oil, simulates the commercial refining process (alkali method) and determines more or less satisfactorily the commercial refining loss and how much refined oil can be obtained from the crude. The percentages of refined oil obtained by this test are always considerably less than the percentages of neutral oil determined by the Wesson method, because the emulsion formed at first when refining by the official method does not break as completely with some oils as with others and different quantities of neutral oil are carired down by the "soap stocks." For the same reason the commercial refining loss is always greater than the refining loss determined by the Wesson method. The two do not run parallel and the commercial loss is not proportional to the free fatty acids in the crude. The percentage of neutral oil represents the goal for which the refiner is to strive.

Composition of Neutral Oil

The approximate chemical composition of neutral oil is given in Table 3. These results were determined on an authentic sample of cottonseed oil (J. Am. Chem. Soc. **42**, 1197 (1920). The procedure used was briefly as follows:

The saturated and unsaturated acids were separated by the lead-salt-ether method. The saturated acid fraction was esterified with methyl alcohol. The methyl esters were distilled fractionally under diminished pressure in such a way that the fractions did not contain more than two esters, and the fractions were weighed. The fatty acids were liberated from the various fractions, separated by fractional crystallization, and identified by elementary analyses and melting point determinations. The percentage composition of each fraction was calculated from the mean molecular weight of the fraction as determined and the theoretical molecular weights of the two esters present. From these data the com-

TABLE 3. COMPOSITION OF NEUTRAL (REFINED) COTTONSEED OIL

		Per Cent
Glycerides of	Myristic acid	0.3
	Palmitic acid	20.0
	Stearic acid	2.0
	Arachidic acid	0.6
	Oleic acid	35.2
	Linolic acid	41.7

position of the main saturated acids fraction was calculated. The bromine addition derivatives of the unsaturated acids were made. No hexabromide, which is insoluble in ether, was found, proving the absence of the linolenic acid. The ether was removed by distillation and the residue dissolved by boiling with petroleum ether. The linolic tetrabromide isomer, which is insoluble in cold petroleum ether, was crystallized out in the ice box and weighed. The petroleum ether solution, which now contained the soluble linolic tetrabromide isomer and the oleic dibromide, was evaporated to dryness and weighed. The bromide content of the residue was determined and the proportion of tetrabromide and dibromide calculated. The percentages of linolic and oleic acids in the main unsaturated acids fraction were calculated from these data.

Palmitin and Di-Palmitin

Some interesting results which have a relation to the composition of the neutral oil were obtained by examining the "settlings" from a five-gallon sample of high-grade crude oil that had been standing in the laboratory for about three years (J. Oil and Fat Ind. **3**, 5, 153 (May 1926)). The acidity of the oil after this length of time was only 2 per cent, as oleic acid, and it was free from rancidity.

As much as possible of the oil was drained from the "settlings," after which the deposit was washed with cold acetone until free from oil. A 240 g. portion of the residue was repeatedly boiled with hot absolute alcohol until all of the alcoholic-soluble constituents had been extracted. The combined alcoholic extracts, amounting to about three liters, were concentrated to about half the original volume and allowed to remain in a cool place for several days. A crystalline almost white precipitate separated. This was removed by filtration, washed with several small quantities of cold alcohol, and then dissolved in one liter of warm alcohol. The yellow solution was decolorized with norite. On cooling, a bulky white mass composed of minute slender prisms was deposited. After filtering, washing, and drying, the melting point was 63.5°-64.0°C. and recrystallization did not change it. The crystals belonged either to the monoclinic or the triclinic system, but it was not possible to determine which. No free fatty acids were present, but after saponification palmitic acid and glycerine were separated and identified. The saponification value of the product was 208.5. The theoretical saponification value for the triglyceride of palmitic acid is 208.6. These data identify the compound as palmitic-triglyceride. The yield of palmitin was 60 g., or 24.8 per cent of the "settlings."

The alcoholic solution from which the palmitin had been recrystallized was concentrated to a volume of about 200 cc. On standing, a small quantity of another glyceride crystallized out. This was identified as di-palmitin. It crystallized from alcohol in radiating clusters of minute needle-like prisms, which appeared to belong to the orthorhombic system. Its melting point was 70°C. and saponification value 196.7. All of its properties agree with those of the di-palmitin synthesized by Ad. von Grün (B. 38, 2285 (1905)). The yield of di-palmitin was 2.6 g., or 1.1 per cent of the "settlings."

So far as known this is the first instance in which the pure glycerides, palmitin and di-palmitin, have been isolated from cottonseed oil.

Mixed glycerides, no doubt, are the preponderant constituents of the neutral oil, but this work establishes the presence of a small quantity of palmitin. The presence of the di-palmitin is believed to be due to the hydrolysis of a small quantity of a triglyceride.

Acetyl Value

At this point it may be worth while to consider for a moment the acetyl value, which is variable and amounts in some cases to 18 or 20. The quantity of phytosterol (theoretical acetyl value 131.1) present is less than 1 per cent. Hence this compound is not responsible for more than 1 in the acetyl value. Cottonseed oil as been examined by different investigators for hydroxylated acids, but none has been detected. Also careful search has been made for free glycerine but none found. It is believed that the acetyl value of cottonseed oil is due chiefly to the presence of di- and mono-glycerides, which is caused by the hydrolysis of some of the triglycerides (Lewkowitsch, Chem. Technology and Analysis of Oils, Fats and Waxes, 6th ed., Vol. 1, p. 440). Therefore, if this assumption is correct, low acetyl values should accompany low acid values, and oils with greater acidities should give higher acetyl values. This, however, remains to be investigated.

Free Fatty Acids

Crude oil, even in its fresh state, contains a small but appreciable amount of free fatty acids and the quantity increases upon storage, especially if the oil has not been properly settled or filtered and contains some "foots" (meal). This is due to the action of fat hydrolyzing enzymes which are present in the seed. Fortunately the alkali refining process destroys or removes any enzymes not previously removed from the crude oil by the settling process or by filtration.

It has been considered that some fatty acids are split off from the glycerides more easily than others and that the free fatty acids of cottonseed oil consist only of oleic acid. For commercial trading it is customary to calculate and express the acidity as oleic acid.

The free fatty acids were separated from five samples of crude oil and examined (Cotton Oil Press 7, 2, 35 (June, 1923)). It is concluded from the data obtained that the free fatty acids of cottonseed oil are set free by hydrolysis in practically the same proportions as they occur as glycerides in the oil.

Minor Constituents

The crude oil contains a number of other concomitants in small quantities. Some are pigments, others are strong emulsifiers which exert a marked influence on the amount of neutral oil that is carried down by the "soap stock" in the commercial refining process. The average commercial refining loss is about 8 or 9 per cent unless it is an "off" year, and about 25 per cent of this loss consists of neutral oil that has been carried down, entrained in the "soap stock" owing to emulsification. Therefore some of these other constituents, although they occur in small amounts, are important factors in determining the size of the refiner's profits. The quantity of these substances is indicated by the difference between Column 3, refining loss (Wesson method), and Column 5, free fatty acids, in Table 2. Some of these constituents have been identified.

A convenient manner for obtaining a quantity of these constituents for examination is to use the precipitate which usually appears in crude oil that is a few weeks old. Of course, all press cake or meal is removed from the oil before the precipitate separates. This precipitate is at first very bulky but slowly settles out, sometimes as a fine powder, but usually as a thick gelatinous mass having a dark or almost black color. It is, no doubt, caused by the presence of the small quantity of moisture that is commonly present in the crude oil, as it is known that the addition of 2-3 per cent of water to the crude oil, agitating and warming the mixture, will cause a precipitation of these constituents. When this precipitate first appears in commercial crude oil it appears as fine meal, so that the shipper is sometimes unjustly accused of shipping oil contain meal.

This precipitate, a dark gummy mass, is washed with cold acetone until free from oil. This treatment changes it to a bright yellow powder, which for convenience will be referred to as the "acetone insoluble settlings." The acetone washings also are valuable for examination.

An analysis of the "acetone insoluble settlings" gave the following:

	Per Cent
Chlorides	0.00
Silica	0.60
Phosphorus pentoxide	6.14
Sulphates	0.00
Calcium oxide	0.26
Magnesium oxide	1.46
Potassium oxide	1.79
Sodium oxide	0.33
Ferric oxide	trace
Nitrogen	1.71

Test for starch and tannin were negative. Fehling's solution indicated the absence of reducing sugars, but 4.2 per cent of raffinose (R. T. Balch) and 1.74 per cent of pentosans (F. B. La Forge) were found. Arno Viehoever and Ruth Capen could not detect the presence of quercetin, isoquercetin, quercimeritrim, or gossypitem which are present in the cotton plant, but their observations indicated the presence of xanthopyll and a wax. Charles E. F. Gersdorff obtained the following results: Tests for unaltered cottonseed protein, as well as tests for acid and alkaline meta-proteins, were negative. All tests for proteoses and peptones were positive and indicated the presence of a considerable quantity of these substances. There is no known method for making quantitative determinations of them in a mixture of this character.

The "acetone insoluble settlings" is partially soluble in water, ether, chloroform, and absolute alcohol. The aqueous extract is neutral.

Resins

When the alcohol is evaporated from a portion of the alcoholic extract of the settlings a brown residue remains. Boiling this residue with alcoholic potash causes the solution to become dark red and to deposit a crystalline salt of resin acids, which readily dissolves upon adding water. Upon acidifying the solution with hydrochloric acid a characteristic light-brown precipitate of resin is obtained. The resin is soluble in alcohol and produces a strongly reddish-yellow solution. The addition of an alcoholic solution of cadmium chloride gives no precipitate, which shows the absence of phospholipins such as lecithin. An alcoholic solution of copper acetate causes a dark yellow flocculent precipitate to separate. If oil from which the settlings have been removed is agitated with 2-3 per cent of water and heated to about 50°C., more resin, together with other substances, is precipitated. Furthermore, alcohol will extract some resin from a water treated oil. The caustic soda refining process, however, removes practically all of the resin. It occurs in the glands that permeate the kernels, not in the hulls of the cotton seed. Oil expressed from prime seed contains less resin than "off" oil from stored seed that contained excessive moisture and therefore "heated" and deteriorated. The resin was found to have strong emulsifying power.

Inosite Phosphates

Inosite phosphates may be separated from both the "acetone insoluble settlings" and the aqueous extract which is obtained by heating the oil with a small quantity of warm water. They are moderate emulsifiers.

It is interesting to note that the principal phosphorus compounds found in the seed by R. J. Anderson (J. Biol. Chem. **13**, 311 (1912) and **17**, 141 (1914)) and J. B. Rather (J. Am. Chem. Soc. **39**, 777 (1917)) are inosite phosphates.

Phospholipins, Phytosterols and Phytosteroline

Part of the phosphorus occurs in compounds which are related to the vegetable lecithins. The phospholipins may be precipitated in the form of cadmium chloride compounds from methyl alcohol extracts of both the "acetone insoluble settlings" and the oil. They may be precipitated also from a concentrated alcoholic extract by the addition of acetone. Repeated extraction of the crude oil with methyl alcohol removes only a portion of the phospholipins, but the caustic soda refining process removes practically all of the phosphatides. The phospholipins, like lecithin have strong emulsifying powers. Up to the present time no method has been found by which it is possible to determine the quantity of either the phospholipins or the inosite phosphates in the oil or in the "settlings."

A large part of the unsaponifiable matter consists of sterols. R. J. Anderson and M. G. Moore (J. Am. Chem. Soc. **35,** 1944 (1923)), who have made a very thorough study of the phytosterols in cottonseed oil, report the presence of at least two phytosterols which differ in melting points and optical activities. The phytosterols are moderate emulsifiers.

This glucoside, a compound of glucose and phytosterol, has been obtained from the concentrated alcoholic extract of the "acetone insoluble settlings" and purified by repeated crystallization from alcohol. When heated it turns brown at about 250°C. and melts with decomposition at 275°-276°C. It gives an intense reaction for sterol when tested in the usual manner with acetic anhydrid and sulphuric acid. The acetyl derivitive melts at 166°-167°C. An elementary analysis gave the following results: Carbon 72.16 per cent; hydrogen, 10.12 per cent. The theoretical percentages for $C_{27}H_{45}O.C_6H_{11}O_5$ are carbon 72.20 and hydrogen 10.27. It agrees in its properties with the phytosteroline from other sources previously described by F. B. Power and A. H. Salway (J. Chem. Soc., **103,** 399 (1913)). It constitutes about 0.1 per cent of the "settlings" and is not an emulsifying agent. A somewhat similar substance has been isolated from the benzene extract of cotton lint by R. G. Fargher and M. E. Probert (J. Textile Inst., **14,** T 49 (1923)).

Mucilaginous Substances

The alcohol-insoluble portion of the "acetone insoluble settlings" is partially soluble in water and mucilaginous material has been found in this water solution. It has not been possible to make a quantitative determination because when extracting in paper thimbles with boiling water the pores of the thimbles soon become clogged and other procedures are not more satisfactory. This aqueous extract is neutral in reaction. When a 20 per cent solution of normal lead acetate is added in excess, a slightly colored lead compound is precipitated. The lead compound may be suspended in water decomposed with hydrogen sulphide, and the lead sulphide removed by filtration. Adding approximately 30 per cent of alcohol to the filtrate gives a white gelatinous precipitate of the mucilage. It is a strong emulsifier, but fortunately for the refiner it is present only in very small quantities.

Basic lead acetate added to the filtrate from the lead compound of the mucilage produces no precipitate, which shows the absence of water-soluble gums in the "settlings."

The Coloring Matter

Many attempts have been made to separate the pigments, such as chlorophyll carotin, etc., from the oil for the purpose of identification, but so far these attempts have failed. Apparently, the only feasible method for the identification of these substances in oils is by means of the spectrophotometer. The spectrophotometric examination of both animal and plant pigments has been in progress for a number of years and some definite results have been obtained. Considerable progress has been made by I. G. Priest (The Cotton Oil Press, **3**, 3, 86 (1919) & **3**, 9, 37 (1920)) and his associates in the use of this instrument for measuring the colors of refined cottonseed and other vegetable oils and some attention has been given to the character of the pigments present in these oils. The evidence already obtained indicates the presence of carotin, xanthopyll, and chlorophyll in cottonseed oil. Part of the dark red color of crude cottonseed oil is due to resin, as already described. The color of a crude oil is noticeably lightened after it has been heated with water which has been shown to remove a portion of the resin. On the other hand, the treatment with water removes little or none of the plant pigments dissolved in the oil.

Conclusion

The qualitative composition of crude cottonseed oil is expressed in the following diagram:

- Crude Cottonseed Oil ...
  - Glycerides of.....
    - Myristic acid
    - Palmitic acid
    - Stearic acid
    - Arachidic acid
    - Oleic acid
    - Linolic acid
  - Free Fatty Acids.
    - Same proportions as in glycerides
  - Minor Constituents
    - Proteoses
    - Peptones
    - Phytosterols
    - Phytosteroline
    - Inosite phosphates
    - Phospholipins
    - Resins
    - Mucilaginous substances
    - Carbohydrates
      - Raffinose
      - Pentosans
    - Pigments
      - Xanthophyll
      - Carotin
      - Chlorophyll

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