identified a vegetable mucilage in the crude oil and he cannot obtain any evidence of the presence of a true gum.

Some further work is being done with the resin present in the crude oil. Dr. Jamieson expects to be able to make an interesting report at the coming New Orleans Meeting.

A visit with Dr. Jones of the Protein Investigation Laboratory, elicited the information that Dr. Jones has been doing a lot of work on the proteins of cottonseed meal.

He finds them to be complete proteins from the nutritional point of view. A commercial process for separating them, however, has not yet been devised although he has prepared some very large samples in the laboratory.

Experiments have also been made on the effect of gossypol on digestion *in vitro*.

Some interesting experiments are being made on peanuts from which the oil has been removed by expression. Apparently the peanut kernel contains a food element which is necessary for the ultilization of the peanut protein in the nutrition of animals. This substance occurs in the aqueous extracts of the defatted kernels, and is present in its nutritional effects in small quantities. The nature of this factor has not yet been determined.

Committee: DAVID WESSON (So. Cotton Oil Co., 120 Broadway, N. Y. City), C. B. CLUFF, H. J. MORRISON, L. M. TOLMAN, E. R. BARROW, P. S. TILSON.

(A CONTRIBUTION FROM THE OIL, FAT AND WAX LABORATORY, BUREAU OF CHEMISTRY, U. S. DEPARTMENT OF AGRICULTURE)

CONSTITUENTS OF CRUDE COTTONSEED OIL

BY GEORGE S. JAMIESON AND WALTER F. BAUGHMAN

Further progress in the investigation of the constituents of crude cottonseed oil which was assigned to this laboratory at the conference of the Basic Research Committee and the U. S. Department of Agriculture, September, 1921,* is reported in this paper.

The previous report was devoted chiefly to the results of the examination of the acetone-insoluble portion of the settlings obtained from the oil when it is held several weeks before refining. An analysis for acids and bases of this portion of the settlings gave the following results:

	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

* Previous reports, *The Cotton Oil Press*, 6 (No. 4) 33, 7 (No. 2) 35, 7 (No. 5) 29.

Sodium oxide	0.33
Ferric oxide	trace
Total	10.58

It is believed that the larger part of the bases as well as the phosphorus in the oil are combined with organic compounds some of which, owing to their instability, decompose. As a result, some of the phosphorus and the bases in the settlings are in the form of inorganic phosphates and organic decomposition products.

The settlings were found to contain, besides the constituents previously reported, a phosphatide related to the so-called vegetable lecithins. The phosphatide was first detected in the acetone-insoluble portion of the settlings and later it was found also in the acetone-soluble portion. The addition of a saturated alcoholic solution of cadmium chloride to the alcoholic solution of the settlings gave a light colored precipitate of a compound of cadmium chloride with the phosphatide. After the solution had stood for a day, it was filtered, and the precipitate was washed with alcohol and dried at 120°C. In contrast to the corresponding cadmium chloride compounds of the lecithins obtained from eggs and soy beans, this compound was soluble in ether, in which respect it is similar to the phosphatide cephalin of animal origin. The preparations from the acetone soluble and insoluble portions melted with decomposition at about 194° to 196°C. They contained 15.36% of cadmium and 3.91% of phosphorus (P). The cadmium chloride compound of soy bean lecithin prepared in this laboratory was found to contain 14.7% of cadmium. Gliken's Chemistry of the Fats, Lipoids, and Waxes, vol. 1, p. 420, reports an analysis of the cadmium chloride of a lecithin from animal sources showing that it contained 13.92% of cadmium and 3.09% of phosphorus (P).

After the discovery of the phosphatide in the settlings, an attempt was made to extract it from the crude oil by alcohol. It was hoped that a quantitative extraction might be obtained in view of the known fact that phosphatides are much more soluble in alcohol than in the oil. Methyl alcohol was selected as the solvent because cottonseed oil is much less soluble in it than in ethyl alcohol. The first attempt to extract this phosphatide was made by thoroughly shaking a liter of the oil with 3 successive 1500cc. portions of 99.5% methyl alcohol. The alcoholic extracts were combined and concentrated to a volume of about 300 cc. and allowed to stand at about 12°C. for 3 days. The alcoholic solution was readily decanted from the small quantity of oil which had separated. Cadmium chloride gave a precipitate identical with that obtained from an alcoholic solution of the settlings. An analysis of the untreated crude oil showed that it contained 0.035% of phosphorus (P), while the oil which had been extracted with alcohol contained 0.031% which indicated that the alcohol had removed only 11% of the total phosphorus.

Another portion of the crude oil was extracted nine times, using about the same weight of methyl alcohol for each treatment. In this experiment the oil was not as in the first case simply mixed with the methyl alcohol by shaking, but was rapidly stirred by mechanical means for an hour in each case besides being heated to about 30°C. after the addition of the alcohol. An analysis of the treated oil showed, as nearly as could be determined, that this exhaustive extraction with methyl alcohol had removed only about 15% of the phosphorus in the oil.

The extracted oil was freed from the small quantity of methyl alcohol which it contained and agitated with about 3% of water. During the agitation the mixture was heated to about 55°C. The oil was separated from the resulting precipitate and an extract, with methyl alcohol, of the acetone-insoluble portion of the precipitate gave the characteristic precipitate with an alcoholic solution of cadmium chloride identical with that obtained from the alcoholic extract of the oil. This showed that it was not possible to extract all of this phosphatide from the oil by methyl alcohol. It further indicates that a portion of this phosphatide exists in the oil in chemical combination which on the other hand is at least partially decomposed and removed from the oil by the water-heat treatment.

An experiment was made to determine the percentage of phosphorus removed when the oil is treated with water at room temperatures. While 500 g. of the oil were being rapidly stirred, about 3% of water was slowly added. The mixture was stirred for about an hour with the paddle of the laboratory refining apparatus revolving at 150 revolutions per minute; then it was allowed to stand for about 15 hours. The separated oil was decanted from the emulsion and freed from moisture by filtration through paper, and a phosphorus determination was made. This oil contained 0.032% of phosphorus (P), while the original oil contained about 0.045%, which showed that 29% of the total phosphorus had been removed by the water, as compared with the removal of 15% by the repeated extraction of the oil with alcohol. Another experiment was made in a similar manner except that after the addition of the water to the oil, the mixture was heated to about 55°C. The treated oil contained 0.024% of phosphorus (P) which indicated that this treatment had removed 44% of the phosphorus (P) present in the untreated oil. The caustic soda refining of cottonseed oil on the other hand removes practically all of the phosphorus from the crude oil. An analysis of refined oils showed that they contained from faint traces to 0.003% of phosphorus (P).

As yet no method has been found by which it would be possible to determine the distribution of the phosphorus in the oil between the glyceryl phosphatides, inosite phosphoric acids, etc., nor is there a method by which we can determine the total quantity of any one of these constituents present.

In order to obtain some information as to the quantity of a vegetable phosphatide which could be removed from cottonseed oil by heating with water as described, 500 cc. of refined cottonseed oil which contained 0.003% of phosphorus (P) was treated with a 50cc. ether solution which contained about 0.2 g. of soy bean lecithins. After mixing, the ether was removed by heating. The oil remained clear and brilliant. Experiments were previously made in which after addition of the lecithin directly to the oil the mixture was stirred for long periods and in some cases heated to 50°C., but in no instance was it possible to dissolve measurable quantities. The oil now contained 0.036% of phosphorus (P). After treatment with water and heat, the oil contained 0.024% of phosphorus (P). This experiment showed that 33% of the total phosphorus in the oil as lecithin had been removed by the water treatment. Some of the emulsion which formed when the oil was treated with water and stirred was kept for observation in a test-tube. After standing for 3 months about one-third of the emulsion remained unchanged. A similar experiment could not be made using the phosphatide which is present in the crude cottonseed oil because none of this phosphatide in a free condition had been isolated.

The treatment of the crude cottonseed oil with water and heat further removed the constituents which had been identified in the "settlings" that had previously separated from the oil, but no additional substances have been detected. As might be expected, however, the water-heat treatment partially decomposed some of the phosphatides with the formation of considerable glyceryl-phosphoric acid.

Two attempts were made to determine the percentage of the total substances removed when the crude oil from which the settlings had previously separated was treated with water and heated. Weighed quantities (202 and 650 g.) of this oil were treated with water and heated as described. After the mixture had stood for about 2 days as much as possible of the oil was separated by means of a siphon. In order to remove the rest of the oil floating on the aqueous layer, etc., several 75cc. portions of petroleum ether were added in turn so as not to disturb the lower layer. When the oil had dissolved, the petroleum ether was removed by the siphon. This treatment was repeated until practically all of this oil had been removed. Then the mixture remaining was evaporated to dryness and weighed. In the first experiment in which 202 g. of oil was taken, the residue was 1.64%, and in the second experiment (650 g. of oil) it was 2.7% of the oil treated. These experiments showed that it was impossible to get concordant results by this procedure, owing chiefly to the different quantities of oil being entrained and emulsified with the substances removed by the water treatment. The residue in each case was extracted and washed with petroleum ether until the washings were color-

less. The light yellow granular residues were dried at 110° and weighed. This residue in the first experiment was 0.028% of the original oil and in the second experiment 0.029%. The residues contained inorganic phosphates, glyceryl phosphates and organic phosphates of the inosite phosphate type, along with magnesium and calcium.

Experiments made in another laboratory, in which various samples of crude cottonseed oil were heated with 2% of water and the mixture passed through a super-centrifuge, showed that from 2.0 to 2.85% of the oil treated separated in the form of a thick dark sludge. It was reported that the sludge separated from the various oils contained from about 11 to 25% of oil.

In addition to the further study of the substances which have been discovered thus far, the investigation will be continued in the effort to detect and identify any other substances which may be present in the crude oil.

Summary

Crude cottonseed oil contains in addition to the constituents previously reported a lecithin type of phosphatide which gives an ether-soluble compound with cadmium chloride. This phosphatide can only be partially removed from the oil by extraction with alcohol. It has been found in the "settlings" from this oil. The treatment of the oil with water causes only a partial separation of this phosphatide.

The phosphatides, resins, and presumably other substances present in small quantities in the crude oil have emulsifying properties and are undoubtedly the cause in part for the retention of oil in the soap stock when the oil is refined by caustic soda.

EXTRACTION COMMITTEE REPORT

By G. K. Witmer

I do not occupy as prominent position in the analytical hall of fame as big brother ammonia, but I am extended quite a bit more consideration than little sister moisture, for which I am very deeply thankful. Recent years have witnessed a steady improvement in my various manipulations, and I am exceedingly hopeful that in due time I will earn a position alongside the honorable precision determinations.

If I arrive at the laboratory in the form of hulls, be very sure to mix me thoroughly before selecting a weighing portion. This is one respect in which I am often neglected, and very wrongfully so since I will vary throughout a hull sample much more than in a meal. And do not neglect to search for uncut seeds and meats. In this day of good oil-milling you are apt to ignore their presence, but they are very often to be found, and they are capable of affecting the accuracy of my figures to a very alarming degree.