the water jacket on the motor to supply heat for the evaporator. A new type flaking machine which extrudes the flakes was supplemented for the rolls.

Although the extractor removed is not obsolete, this new extractor is more simple in design, the cost is very low and a steam plant and steam engine are unnecessary. The new extractor will handle 100 bushels a day. We are still experimenting with this type of extractor and hope to have it for distribution by the first of the year.

PHOSPHATIDES IN AMERICAN SOY BEANS AND OIL

By George S. Jamieson and Robert S. McKinney

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

During 1932, our attention was first called to the trouble experienced with soy bean oil expressed from seed grown in North Carolina. Shortly after expression the clarified crude oil became turbid and gradually a sizable precipitate separated. Obviously, this precipitate had to be removed before the oil would be acceptable to the purchaser. On account of its gelatinous character the removal of the precipitate by filtration was extremely difficult. Also, after filtration the precipitate retained a large quantity of oil. It was found preferable, therefore, to hold the crude oil in settling tanks at the mill until the precipitate had settled, and then carefully withdraw as much as possible of the clarified oil. In so far as could be ascertained, practically no trouble of this character was experienced by the soy bean oil mills in the Middle West States.

To determine the composition of this precipitate, we examined a gallon sample of the clarified soy bean oil[1], freshly expressed from a mixture of Mammoth Yellow, Tokio Green, Hollybrook, Mammoth Brown and Tar Heel beans. The Mammoth Yellow and Tokio Green varieties predominated. Several days after expression it was observed that the oil became turbid. After standing for several weeks a brownish-yellow gelatinous precipitate amounting to 2.1 per cent of the weight of the original oil had settled to the bottom of the bottle. At first, it was believed that the precipitate separating from the oil was a stearine because upon warming it dissolved in the oil. Upon cooling to room temperature the precipitate gradually reappeared. Consequently the oil was examined to see whether or not it contained an abnormal quantity of saturated acids. It was found to contain 11.12 per cent of saturated acids, which is within the usual range, indicating that the precipitate in question was not stearine. A test for phosphorus indicated that it consisted largely of phosphatides.

The precipitate was collected by centrifuging the oil and transferred to a Buchner funnel fitted with a filter paper. After three days no more oil could be separated by filtration. The precipitate was then removed from the funnel to a flask and dissolved in a small quantity of chloroform, and about 20 volumes of acetone was added to the solution. The precipitated phosphatides were easily filtered on the Buchner funnel and washed with acetone until all the oil was removed. After the phosphatides were dried for several weeks over calcium chloride in a desiccator they were readily reduced to a powder. Analysis showed that the substance contained 3.9 per cent of volatile matter, 1.15 per cent of nitrogen and 3.20 per cent of phosphorus (the average of four closely agreeing determinations). The phosphatides calculated in terms of lecithin, using the average figure for phosphorus, amounted to 81.6 per cent.

Tests made for sulphur and carbohydrates were both negative. The fact that the precipitated substances separated from the oil were completely soluble in chloroform precluded the presence of peptones and pentoses, as well as carbohydrates, such as were found in cottonseed oil settlings*. After the oil from the precipitate had stood about eighteen months, analysis showed that it still contained 0.016 per cent of phosphorus, which is equivalent to 2.70 per cent of lecithin.

In attempting to discover the cause for the different behavior of the oils from soy beans grown in the Eastern and Middle States, samples of the more important varieties were examined with regard to their phosphatide content. After experimenting with the various methods proposed for the extraction of phosphatides from seeds, it was found that a single treatment with boiling 95 per cent alcohol was satisfactory. The method used is as follows:

Accurately weigh one-gram portions of finely ground samples of beans. Spread each weighed portion in a thin layer on a 150-mm. filter paper (Reeve-Angel No. 211 or equivalent grade); fold paper at a point about one-quarter of the distance from each of two opposite sides over the sample to the center of the paper; wrap into a cylinder by coiling the paper from one of the unfolded sides and fold a second filter paper around this (to prevent the escape of fine meal), leaving one end open like an extraction thimble. Place a piece of absorbent cotton lint in the opening to facilitate the even distribution of the solvent, and transfer the cartridge or thimble to a Butt type extraction tube and connect it with a reflux condenser. Add 40 cc. of 95 per cent alcohol to a 150-cc. wide mouth extraction flask, connect it with the extraction tube and extract for four hours, preferably by heating the flask in a sand bath. The alcohol should drop from the condenser on the center of the cotton plug at the rate of about 150 drops per minute. After the extraction is completed, remove the flask, evaporate the alcohol as completely as possible on a steam bath and heat it in an oven at 100 to 105° for one hour. In addition to the phosphatides this residue contains some other alcohol extractable substances, including a portion of the oil.

Add 4 cc. of sulphuric acid and about 0.2 gram of potassium nitrate to the flask containing the residue. Hold the flask at about a 45° angle and rotate it over a small gas flame until the mixture thickens and chars. After it has stood for 5 or 10 minutes cautiously add 3 to 4 cc. of nitric acid, invert over the neck of the flask a porcelain crucible

[1]Sent by the Eastern Cotton Oil Company from their Elizabeth City, N. C., expeller mill.

*Jr. Oil and Fat Ind. *3*, p. 352, 1926.

cover and heat on an asbestos covered wire gauze so that the mixture boils gently. Continue the heating until copious fumes of sulphuric acid are evolved. If the solution at this stage is colored, cool somewhat, add 1 cc. more of nitric acid, and heat again. Sometimes one or more additional portions of nitric acid are necessary. Finally remove the crucible cover from the flask, concentrate the solution to a volume of about 2 cc., cool to room temperature, and dissolve the residue in 10 cc. of water. After the neutralization of the sulphuric acid with ammonium hydroxide, determine the phosphorus either by the gravimetric phospho-molybdate method, as described on pages 153-155 of the second English edition of Pregl's Quantitative Organic Microanalysis, or the magnesium (pyrophosphate) method. After the digestion is completed according to our directions, if the phosphorus is to be determined by the method described by Pregl, make the solution ammoniacal and heat it on the steam bath until the excess of ammonia is volatilized; then proceed with the precipitation of the phosphomolybdate. In using the other method it is important that after the filtration of the ammonium magnesium phosphate it be washed with 1:2 ammonium hydroxide and not with a more dilute solution as frequently recommended. Using the latter method, the weights of magnesium pyrophosphate obtained from the analysis of the alcoholic extracts of one-gram portion of ground samples of different varieties of soy beans ranged for the most part from 2.7 to 3.4 milligrams. The corresponding phosphomolybdate precipitates are notably heavier than those of magnesium pyrophosphate. The results obtained are given in Table I. The factor (25.50) used to calculate the percentages of phosphorus into terms of lecithin is based on Levine's formula for lecithin, $C_{44}H_{86}PO_9N$.

Comparing the phosphatide phosphorus figures given in the table for beans grown in the Eastern States with those from the Middle West States, it is evident that the trouble experienced at the eastern oil mills cannot be attributed to the quantity of phosphatides in the beans. With few exceptions, the beans from the Eastern States contain less than those from the western localities, the oils of which deposit upon standing little or no phosphatides. It should also be noted that no particular differences could be found

TABLE I—ANALYSIS OF SOY BEANS FOR PHOSPHATIDES

Variety	Phosphorus Percent	Calculated as Lecithin Percent
Soy Beans from North Carolina and Virginia		
Mammoth Yellow ..	0.150	3.82
Tokio Green	0.096	2.44
Mammoth Brown ..	0.080	2.04
Tar Heel	0.087	2.41
Biloxi	0.083	2.31
Herman	0.100	2.55
Lorida	0.078	2.00
Dixie	0.078	2.00
Haberlandt	0.080	2.04
Soy Beans from Illinois, Indiana and Ohio		
Illini	0.097	2.47
Mammoth Yellow ..	0.086	2.39
Tokio Green	0.097	2.47
Monsay	0.098	2.49
Blackeye	0.098	2.49
Peking	0.108	2.77
Ohio No. 13	0.114	2.90
Hollybrook	0.080	2.04
Mukden	0.098	2.49
Kingwa	0.111	2.83
Macoupin	0.097	2.47
A. K. No. 125	0.097	2.47
A. K. No. 146	0.105	2.67
Oil Mill Stock*.....	0.098	2.49

*A mixture consisting largely of yellow varieties of beans.

in the oil mill technic between the eastern and western plants. So far it has not been possible to discover what causes the separation of phosphatides in the oil from beans grown in the East. From the unusually high phosphatide content of the eastern grown Mammoth Yellow beans it was believed that their oil was probably the chief contributor causing the separation of phosphatides in the commercial product. The oil from one hundred bushel lots of Mammoth Yellow, Mammoth Brown, Tokio Green and Tar Heel beans was expressed by the Allied Mills Company. A sample of each oil freed from press foots was sent to us for observation and study. After standing a few days the oils expressed from the Mammoth Yellow and Tokio Green beans became turbid, and gradually sizable precipitates settled from the oil. The precipitate from the oil of the Mammoth Yellow beans, however, was notably larger than that from the Tokio Green beans.

After standing for some months the oil from the Mammoth Brown deposited a minute quantity of phosphatides, but even after standing for eight months not a trace of phosphatides had separated from the oil from the Tar Heel beans. These experiments showed that the Mammoth Yellow and Tokio Green beans caused the phosphatide trouble. From the table it will be observed that the Tar Heel beans contain nearly as much phosphatides as those of the Tokio Green beans grown in North Carolina.

It then became of interest to determine the phosphatide content of the oils from these varieties of beans. As soy bean oil does not contain measurable quantities of phosphorus compounds other than phosphatides, it is only necessary to determine their phosphorus content. The following procedure was used:

Weigh accurately one gram of oil into a 150-cc. wide mouth Pyrex flask. Add 0.5 gram of potassium nitrate and 10 cc. of sulphuric acid. Rotate the flask inclined at a 45° angle over a small gas flame until the mixture clears and thickens. After it has stood for about 10 minutes, add 5 cc. of nitric acid, cover and proceed with the digestion as directed for the alcoholic extract of soy beans. From time to time it will be necessary to add 1-2 cc. of nitric acid to facilitate the digestion. When the solution has a yellow color, concentrate to a volume of about 2 cc. If during this process the solution turns brown or a deep yellow allow it to cool for about 10 minutes; then add 2 or 3 drops of nitric acid and continue the heating. Finally determine the phosphorus by the Pregl microsphospho-molybdate method, as described.

In the acid digestion of the oils it was found that upon concentrating the sulphuric acid to a small volume, a persistent yellow color remained. This is due entirely to the presence of a small quantity of iron.

The results obtained with the four oils already described are given in Table II.

TABLE II

Oil (Variety of Bean)	Phosphorus Percent	Calculated as Lecithin Percent
Mammoth Yellow ..	.0463	1.18
Tokio Green	.0447	1.14
Mammoth Brown ...	.0633	1.62
Tar Heel	.0490	1.25

Although as previously mentioned, the first two oils given in table II deposited phosphatides, it will be observed that they still retained notable quantities in solution. Another oil from Virginia was recently found to contain in solution 0.100 per cent of phosphatide phosphorus, which is equivalent to 2.79 per cent of lecithin. This oil has stood for about three months without the separation of any phosphatides. The reason why phosphatides separate in the case of some eastern oils and not in others has not been discovered. Two commercial so-called "Non-break" soy bean oils, one of which was from eastern beans, were examined, and in terms

of lecithin, each contained 0.22 per cent. It would appear desirable to make all domestic soy bean oil intended for technical use as a drying oil a "non-break" product, particularly the oil from eastern beans, not waiting for the partial separation of phosphatides which, as has been found recently, does not always occur even after standing for several months.

SUMMARY

It has been found that the precipitate separating from clarified expressed crude oil from soy beans grown in North Carolina and Virginia is composed chiefly of phosphatides. Methods have been described for the determination of phosphatide phosphorus in soy beans which is applicable to seeds in general and in the oil. Soy beans of the more important varieties used by the oil mills both in the Eastern and Middle West States have been examined for their respective phosphatide content. With but few exceptions it was found that the beans grown in the East contained less phosphatides than those from the West, which indicated that the quantity of these substances present is not a factor in causing a partial separation of them in some oils but not in others. Regardless of whether or not a separation of phosphatides takes place, all the crude soy bean oils which have been examined so far have contained notable quantities of them.

Report of The Fat Analysis Committee

By W. H. IRWIN

Chairman

At a meeting of the Fat Analysis Committee held October 10, the committee considered certain points which have been brought up from time to time during the past year or two.

Unsaponifiable:

Attention was called to the fact that in the unsaponifiable matter method for oils and fats, there is no correction made for fatty acids which might be present in the unsaponifiable matter, whereas in the soap analysis methods in the last chapter of the American Oil Chemists' Society methods this correction is made. The correction ordinarily does not amount to more than one-tenth or two-tenths percent. After some discussion, it was decided to make the Fat Analysis Committee Unsaponifiable Method for oils and fats conform with the soap methods.

Fatty Acids Combined as Mineral Soap:

The point was brought up as to whether the fatty acids combined as mineral soap should be added to the M.I.U. or whether a credit should be given for the free fatty acids so combined. The point was made that fatty acids combined as mineral soap are of no value to the soap maker unless he acid-washes the material.

In view of the lack of complete information, it was agreed to make further study of this matter before reaching a decision.

Wiley Melting Point Method:

The cooperative results on the sample sent out in May were studied and it was agreed that they were not satisfactory enough to warrant adoption of the method without some further modification Dr. Vollertsen, who has had more experience with the method than any other member of the committee, agreed to draw up the details of the method and the committee will then do further cooperative work.

Free Fatty Acids:

Mr. Long pointed out the fact that the free fatty acids method, as now written, is not detailed enough. It was, therefore, agree to add a table indicating the amount of sample, the strength of the standard solution, etc., to be used in making the determination on products of various fatty acids content.

Titer Determination:

It was agreed to make clear the method of stirring in the titer determination so that there would be no possibility of misinterpretation. The method agreed upon was to stir with a circular motion in one plane at 100 r.p.m.

FAC Color Standards:

Little progress has been made in changing over to the more permanent standards, suggested by Mr. Doherty, for the reason that the committee has been unable to locate a sufficient supply of chemically pure uranyl chloride to take care of the requirements for the replacements of the sets now being used. It was also pointed out that it would be necessary to put up the standards in ampoules on account of the acid nature of the material, the action of which causes discoloring by action on the rubber stopper used in the present set. The substitution of ampoules will also probably necessitate a change in the kind of case used in protecting the set from the bleaching action of daylight. It was agreed to follow the matter further with a view of making a change as soon as the necessary materials are available.

Liquid and Solid Fatty Acids:

The cooperative results submitted on the liquid and solid fatty acid determinations in shortenings varied so widely that there seemed to be no hope of getting the results together without drawing the details and setting the conditions much closer than they are in the methods now in use. After some discussion, it was agreed that Mr. Long would draw up details of the Twitchell Method and that the Committee would make a study of this method during the coming year the iodine number and thiocyanogen number to be used in calculating fatty acids. Each laboratory will take one sample and follow the method through carefully at least three times at intervals of one to two weeks, reporting all figures in order to see how the individual laboratories can check their results by the method.

W. H. IRWIN, Chairman.
R. W. BAILEY.
T. C. LAW.
C. P. LONG.
M. L. SHEELY.
H. J. MORRISON.
L. M. TOLMAN.
H. P. TREVETHICK.
J. J. VOLLERTSEN.