The residual toxicity of treated cottonseed meals cannot be explained on the basis of their free gossypol content as analyzed for meals with high values gave better growth performance than some with lower levels of free gossypol. There were also very marked differences in final body weight after 8 weeks of feeding six different treated cottonseed meal samples having practically the same free gossypol content.

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Determination of the Phosphorus Content of Lipids[1]

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NUMEROUS procedures, including gravimetric, titrimetric, and colorimetric, have been suggested for the determination of phosphorus in organic materials. Of these, colorimetric procedures are usually preferred for their rapidity and adaptability to microanalysis. A considerable amount of work has been done on colorimetric methods and in particular, on the "molybdenum blue" reaction suggested by Bell and Doisey (1) and developed by Fiske and Subbarrow (2), which appears to be the one most generally accepted. This method is extremely sensitive, and very small samples may be used without loss of accuracy.

However an examination of the literature on this subject reveals the existence of many variations and modifications of the molybdenum blue method. Experience with the most authoritative procedures (3, 4) has shown that color development is very critical and varies with the nature and the concentration of the reducing agent, acidity, time, and temperature. Because of this, the analyst has had to do considerable experimentation in order to develop a technique which would give reliable results.

In this work a number of molybdemum blue procedures were investigated, and a method was developed for the determination of phosphorus in cottonseed lipids which considerably reduces the effect of the variables. The method utilizes perchloric acid for sample digestion and p-methyl-amino-phenol sulfate (elon, metol) as a reducing agent for color development. Samples analyzed included crude and semi-refined cottonseed oil as well as phospholipid fractions separated from the oil.

Digestion of Lipid Samples

Perchloric acid has found considerable favor for the digestion of organic materials due to its high oxygen content and its speed of reaction. It has been recommended by King (5) and Frampton (6) for the digestion of lipids for phosphorus determination. A disadvantage however is its incompatibility with stannous chloride, which is an excellent reducing agent for the molybdenum blue reaction. Moreover perchloric acid is a hazardous chemical, and explosive conditions may occur unless proper care is taken.

The hazard may be reduced by a) the use of a minimum size sample which reduces the heat liberated, b) the use of sufficient acid to avoid an explosive mixture, and c) the use of a small amount of nitric acid to supplement the perchloric acid.

Since the sensitivity of the molybdenum blue method permits the use of small samples, a maximum of 0.1 gm. of oil and a minimum of 1.0 ml. of 72% perchloric acid have been found desirable. Larger samples are much more difficult to digest, and less perchloric acid will form an explosive mixture.

It was found that one drop of concentrated nitric acid would initiate the reaction at a lower temperature and, after the major action had subsided, two or three more drops helped to complete the oxidation. The digestion procedure involved four stages which were as follows: first, a foaming reaction involving the nitric acid; second, the major oxidation which liberates considerable heat; third; a clean-up with additional nitric acid; and fourth, a final clarification with strong heating.

It is most important that the final clarification be complete, otherwise erratic results will be obtained. Yet it is well to avoid excessive heating since some phosphoric acid may be vaporized.

The use of hydrogen peroxide has been suggested for clarification of the digest (7), but it was not found as satisfactory as the nitric acid. A test has shown that residual nitric acid will not interfere with the color reaction. However Greenberg (8) has found that the nitrite ion interferes when the phosphoric-molybdic complex is reduced with $SnCl_2$ and recom-

[1] The findings reported in this article were determined in research dealing with the separation and properties of the constituents of crude cottonseed oil by the use of liquid-liquid solvent extraction, which is being conducted by the Texas Engineering Experiment Station in cooperation with the Cotton Research Committee of Texas.

mends the use of sulfamic acid in the molybdate reagent as a remedy. It is possible that reduction products of nitric acid were the cause of erratic results when the digest was inadequately heated, but no test was made on the effectiveness of sulfamic acid with elon.

The digestion is best conducted in 30-ml. Kjeldahl flasks although 20-ml. Pyrex test tubes were used satisfactorily. It has also been found feasible to insert one or two glass beads in the flasks to aid in mixing and to prevent spattering. In addition to the foregoing recommendations, it is necessary to use considerable caution when heating the reaction mixture, and it is recommended that the mixture be shaken continuously while heating! With some experience the digestion can be completed within five minutes.

The Molybdenum Blue Method

The molybdenum blue method as applied to the determination of phosphorus in lipid materials consists of digesting and oxidizing the sample with a suitable oxidizing agent, formation of a phosphoric-molybdic acid complex, and reduction of this complex with a suitable reductant to give the molybdenum blue color. The phosphorus content of the sample is then obtained by comparing the degree of absorption of red or infrared light by the blue solution with that of a solution of known phosphorus content. The maximum absorption occurs at a wavelength of approximately 820 mμ although the peak is quite broad and the measurement of optical density may be made as low as 650 mμ with reasonable accuracy. Although the spectrophotometer was used in this work, the simpler photometers should give satisfactory results.

Though apparently quite simple, this method is complicated by a number of factors which affect the intensity of color development. These factors include the time of development, the temperature of development, the molybdate-acid ratio, the pH, the presence of interfering ions, and the nature of the developing agent. Woods and Mellon (9) discuss allowable concentrations of interfering ions. However, in the work with vegetable oils, concentrations of interfering ions were too low to have any effect.

It is desirable to maintain conditions which will produce the maximum stable color and yet avoid the reduction of any excess molybdate reagent to molybdenum blue. With low acidity the molybdate is reduced in the absence of phosphorus, and at high acidity the color development is reduced or inhibited.

The optimum acidity and tolerance to acid variation appear to depend on the molybdate-acid ratio and the strength of the reducing agent. In most procedures a buffering agent, such as sodium sulfite, sodium succinate, or sodium acetate, is recommended. Sodium sulfite and sodium bisulfite appear to supplement the reducing agent as well as increase its stability.

The literature contains many articles describing the use of various agents for the reduction of the molybdenum-phosphorus complex. The most commonly used agents include stannous chloride (8, 10, 11, 12, 13), hydroquinone (1), 1,2,4-amino-naphthol-sulfonic acid (2, 3), hydrazine and hydrazine sulfate (4, 12, 14, 15). It was found that all of these had certain serious disadvantages.

Stannous chloride develops a stable color when sulfuric acid is used for sample digestion but produces a precipitate when perchloric acid is present. The actions of hydroquinone and 1,2,4 amino-naphthol-sulfonic acid were found to vary with acidity. Also the rate of color development was slow and varied considerably with time and temperature. Unreliable results were obtained with each of these reducing agents when the solution was heated to bring about or speed up color development. Hydrazine sulfate is quite unstable, and fresh reagent must be prepared immediately before using. Heating, which is essential for color development in this case, is also undesirable for previously mentioned reasons. When perchloric acid was used instead of sulfuric acid for oxidizing the sample, hydrazine sulfate gave erratic results.

In the search for a reducing agent that could be satisfactorily used for color development in the presence of perchloric acid, several photographic developing agents were tested. Mees (16) discusses the "bromide potentials" of various reducing agents; p-methyl-amino-phenol sulfate (elon), 2,4 diaminophenol hydrochloride (amidol), and thiourea are given high values. These substances were tested for color producing qualities and compared with the more standard 1,2,4 amino-naphthol-sulfonic acid.

Thiourea was found to require heating in order to develop color, and no conditions could be found in which the blank did not also develop color. Elon and amidol were very similar in action. Both produced an immediate color which remained stable for a considerable time.

A review of the literature on these two substances revealed that Fiske and Subbarow (2) had tried both of these reducing agents but, for unstated reasons, had not found them satisfactory. However Gtomori (17) used elon very satisfactorily for color development in the presence of sulfuric acid. Allen (18) describes the use of amidol as a reducing agent in the presence of perchloric acid.

The present investigation confirmed the findings of Gtomori as well as of Allen. Although both elon and amidol were almost equally satisfactory, elon was selected because of its ready availability and greater stability.

Effect of Variables with Elon as Reducing Agent

The reducing agent used was Eastman photographic grade elon. The solution was prepared by dissolving 0.5 gm. of elon, 12.5 gm. of sodium bisulfite, and 2.4 gm. of sodium sulfite in distilled water and diluting to 100 ml. This solution has very good keeping qualities whereas elon by itself in aqueous solution does not keep well. The presence of sodium bisulfite in the solution was found to give considerably better color stability.

Measurements of optical density were made with a Model DU Beckman Spectrophotometer at a wavelength of 820 mμ, using 1.000-cm. cells. Readings were reproducible within $\pm$ 0.002 in the range used with this instrument. The reference blank was prepared identically to the test solution except for the phosphorus content, and the tests were conducted at room temperature, which was approximately 25°C. A test showed that properly digested oil samples containing molybdate but without reducing agent had the same optical density as the above blank.

A comparison of color stability between elon and 1,2,4-amino-naphthol-sulfonic acid is shown in Figure 1. These readings were obtained from samples

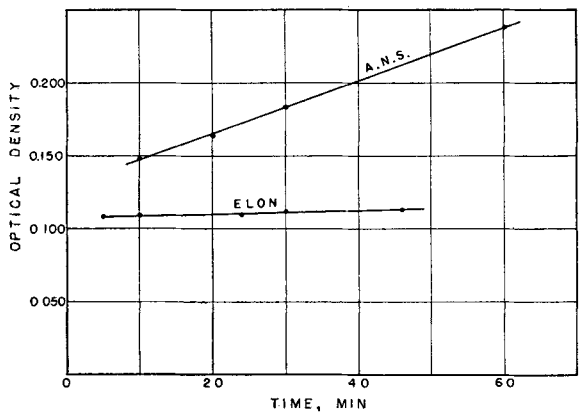

FIG. 1. Change of optical density with time.

containing 0.20 mg. of phosphorus, 1.0 ml. of 72% perchloric acid, 1.0 ml. of 5% ammonium molybdate, 2.0 ml. of the reducing agent, which were diluted to 25 ml. The 1,2,4 amino-naphthol-sulfonic acid solution was prepared according to the A.O.C.S. method (3). It contained 0.25 gm. of reductant, 12.5 gm. of sodium bisulfite, and 2.4 gm. of sodium sulfite per 100 ml. of solution.

It may be seen that the optical density produced by elon was considerably more stable than that produced by the amino-naphthol-sulfonic acid.

Since some perchloric acid is lost during sample digestion, a test was made to determine the effect of varying acid content on the elon color development. The color readings were found to be constant for the range of 0.8 to 1.5 ml. of 72% perchloric acid in 25 ml. of solution.

The effect of the presence of nitric acid was also checked. The addition of 2 to 3 drops of concentrated nitric acid was found to have no effect on the optical density readings.

A test of the effect of reducing agent concentration indicated that 2 ml. of the 0.5% elon solution was adequate.

The adherence to Beer's law is good although there is a slight downward curvature in the plot of optical density vs. phosphorus content as shown in Figure 2.

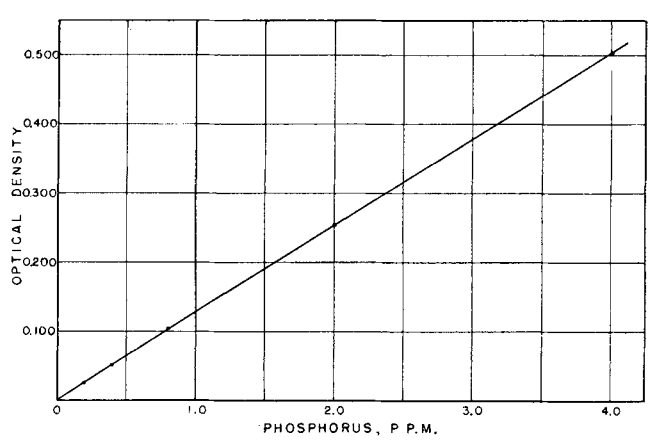

FIG. 2. Optical density *vs.* phosphorus content.

The readings used in this figure were taken 10 min. utes after addition of the reducing agent.

Consistency of Results and Range of Phosphorus Content

The following table illustrates the consistency of optical density readings and shows that the perchloric acid digestion of the sample does not affect accuracy:

TABLE I

Consistency of Results

Run	Phosphorus Added	Optical Density
	mg.	
1	0.05	.257
2	0.05	.257
3	0.05	.265
4	0.10	.507
5	0.10	.505
6	0.10	.505
7	0.05	.260
8	0.05	.251
9	0.05	.252
10	0.10	.500
11	0.10	.504
12	0.10	.508

In each of runs 1-6 inclusive the known amount of phosphorus was digested with 3 drops of phosphorus-free refined cottonseed oil. In runs 7-12 no oil was added.

From experimental results the expected accuracy will be within 1% of the reading for phosphorus concentrations in the range of 1.0-5.0 ppm. This corresponds to a range of 0.025-0.125 mg. in 25 ml. of solution and to a range of 0.025-0.125% with an 0.100-gm. sample.

The practical lower limit of optical sensitivity is estimated at 0.1 ppm. of phosphorus which corresponds to 0.0025% P with an 0.100-gm. sample. The average optical density reading at this concentration was 0.012 with deviations of $\pm$ 20% of the reading.

When the material has a high phosphorus content, it is preferable to use a smaller sample. However it was found that good results could be obtained by diluting an aliquot portion of the blue solution to a readable optical density provided the blank was diluted to the same degree.

Suggested Procedure

REAGENTS:

1. Perchloric acid, phosphorus free, 70-72%
2. Ammonium molybdate solution 5.0%
3. Reducing solution:
   P-methylaminophenol sulfate (elon) 0.5 gm.
   Sodium bisulfite 12.5 gm.
   Sodium sulfite 2.4 gm.
   Dilute to 100 ml. It is preferable to keep solution in dark bottle and in refrigerator although the shelf life at room temperature is at least one week.
4. Standard phosphorus solution:
   Dissolve 0.4393 gm. of dry c.p. potassium dihydrogen phosphate in water and dilute to one liter. This solution contains 0.1 mg. of phosphorus per ml. Further dilution of this solution to 0.01 mg. per ml. should be used for standardization at low concentrations of phosphorus.

PROCEDURE:

Weigh 0.07-0.10 gm. of oil sample into digestion flask. Add 1.0 ml. of 70-72% $HClO_4$, one drop of concentrated HNO_3 and one or two glass beads and heat gently until the oxidation reaction subsides. Add 2

more drops of concentrated HNO_3 and heat until the digestion is complete and white fumes of $HClO_4$ appear. Cool the digest and transfer it to a 25-ml. volumetric flask. Dilute somewhat with water, add 1.0 ml. of the molybdate solution, and mix. Then add, without delay, 2 ml. of elon solution and dilute the mixture to 25 ml.

Prepare the blank by diluting a mixture of 1.0 ml. of perchloric acid, 1.0 ml. of molybdate solution, and 2.0 ml. of elon solution to 25 ml.

Reading:

Set the spectrophotometer at 820 mμ and adjust the instrument to read zero with the blank as a reference. Read the optical density of the sample and compare with a reference curve prepared by use of the standard phosphorus solution. Readings should be taken at the same time interval after addition of the reducing agent.

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Interim Report of the Bleaching Methods Committee. The Proposed Official Bleaching Earth of United States Origin

Historical

THE purpose of this part of the report is to review the background of the circumstances leading to action taken by the Society to abandon the use of the XLOOO brand English natural bleaching earth, employed for a number of years as the A.O.C.S. official natural bleaching earth, for one of domestic origin and more similar to the material now used by the vegetable oil industry.

At the Atlanta meeting of the Society in May 1950 a recommendation was made by the Uniform Methods Committee to the effect that future stocks of official natural bleaching earth should be of United States origin. J. T. R. Andrews, chairman of the U.M.C., in his report (J.A.O.C.S., Vol. 29, p. 47) states in part "the wisdom of this decision is becoming quite apparent since a survey of our supply of official natural bleaching earth, bearing the 5% dosage level, shows some indication of non-uniformity." Further mention of this survey will be made elsewhere in this report.

The Uniform Methods Committee reported in their 1947-48 report to the Society the acceptance of the responsibility from the Chemists' Committee of the N.C.P.A. and the Technical Committee of the N.S.P.A. for the preparation and standardization of official bleaching earth (natural and activated) and filter aid. Elaborating further, the report states that they procured and standardized through the cooperation of The Procter and Gamble Company a new lot of official natural bleaching earth, the first new lot of earth to be prepared in nine years. With increased usage of the natural bleaching earth, especially by the soybean oil industry, this supply was rapidly depleted, consequently it was necessary to procure a new lot of earth, which was certified for use as A.O.C.S. official natural bleaching earth on August 1, 1949. It will be recalled by some, due to the unprecedented quantity of earth required to meet the needs of the industry, that considerable difficulty was experienced in an attempt to match the bleaching potency of the previous lot of natural earth, which had carried a dosage figure of 6%. The undertaking was one of much greater magnitude than heretofore experienced. The problem to find somebody equipped and willing to mix to uniformity, screen, and pack some 12 tons of earth was no simple problem.

It should be pointed out that the above-mentioned earth was divided into two lots. It was found that 5% of the first lot of the new earth was the equivalent of 6% of the old standard in bleaching efficiency. This lot was depleted by July 31, 1951. However, prior to the expiration date (July 31, 1951), there existed indications of a lack of uniformity in bleaching response. The Bleaching Methods Committee, through the cooperation of Swift and Company, sampled all of the existing stock of earth in the Chicago warehouse of the Central Scientific Company, namely, the remainder of lot 1 and all of lot 2. Bleaching tests were conducted on both lots. The first lot received, designated to be used at the 5% level, was found to be non-uniform in bleaching efficiency. The second lot designated as 5.67%, was found to be of satisfactory uniformity in bleaching response, using of course, as a basis for the conclusion, the results obtained on the cans of earth sampled. This earth was subsequently certified by the Chemists' Committee of the N.C.P.A. and the Technical Committee of the N.S.P.A. for the year beginning August 1, 1951 and expiring July 31, 1952.

Expiration dates of July 31, 1953 and July 31, 1954 were subsequently certified. At the present time there is an indication that there is a sufficient supply of natural earth remaining to meet the requirements until current expiration date, namely July 31, 1954. The Society has had a few complaints during the past year concerning non-uniformity of the 5.67% dosage earth. In each case, upon investigation, the