This brief study, which was originally undertaken in order to confirm the Halphen and Bechi tests for cotton-seed oil in lards from hogs fed upon cotton-seed meal, has been found to present so many subjects of interest upon which as yet very little has been published that we are now making still further investigations upon the problems involved.

CONCLUSIONS.

These several samples of lard rendered from the fat of cotton-seed meal-fed hogs, were found to give the following tests for vegetable oils: (I) Welman's reaction; (II) Tollen's pentosan reaction; (III) Salkowski's cholesterin reaction; and (IV) crystals resembling phytosterin. The samples also gave the following tests for cotton-seed oil: (I) the Bechi; (II) the nitric acid; and (III) the Halphen reactions. From this it is evident that two statements can be made, first that the lards contain a vegetable oil, and second, if we agree with the most recent authorities, they contain three distinct constituents of cotton-seed oil. Hence, it seems safe to say that a part at least, of the oil existing in cotton-seed meal, is absorbed, in the case of hogs fed upon this ration, by the animal body and transmitted in its unaltered condition to the fat cells.

[CONTRIBUTION FROM THE LABORATORY OF THE BUREAU OF INTERNAL REVENUE, U. S. TREASURY DEPARTMENT.]

THE DETECTION OF PALM OIL WHEN USED AS A COLORING MATERIAL IN OILS AND FATS.

BY C. A. CRAMPTON AND F. D. SIMONS.

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THE high natural color of palm oil, together with the difficulty of detecting its presence when mixed with other oils and fats in small proportion has led to its use as a means of imparting a color to oleomargarine. In most of the cases that have occurred under the Federal law, the palm oil has been incorporated in the product by the use of cotton-seed oil, to which 2 to 5 per cent. of palm oil had been added. Two oils of this character, sold in the trade as "butter oils," gave the following figures:

	No. 6685.	No. 6732.
Specific gravity, at 15.5° C.	0.9119	0.9127
Refractive index, at 25° C.	1.4701	1.4706
Iodine value	107.8	110.3
Free acid (cc. normal alkali required for 100 grams oil)	3.1	1.2
Color (taken in 1 inch cell of Lovibond's tintometer):		
Brewer's scale. Too dark to read		116
Oil scale { Red	32	24
Oil scale { Yellow	280	72

The high color of the samples indicated the presence of some coloring-matter foreign to cotton-seed oil, but tests for the yellow coloring-matters ordinarily used in oleomargarine and butter (annatto, azo dyes, etc.) gave negative results. The high acid value of the samples (0.01 cc. normal alkali for 100 grams fat, or 0.25 per cent. calculated as oleo-palmitic acid, is the commercial limit in cotton-seed oils when used for edible purposes) indicated that an oil or fat containing a considerable quantity of free fatty acid had been added to the cotton-seed oil.

One hundred grams of each sample were dissolved in petroleum ether and shaken out with 50 cc. of alkali solution, containing 0.5 per cent. of potassium hydroxide. The watery layer was drawn off, made faintly acid with hydrochloric acid, and the precipitated fatty acid separated by filtration through filter-paper.

The melting-point, refractive index and iodine value of the fatty acids were determined, with results as follows:

	No. 6685.	No. 6732.
Melting-point	43° C	42°
Refractive index, at 25° C.	1.4642	1.4664
Iodine value	34.8	37.3

These figures showed the presence of a considerable portion of the higher fatty acids, and indicated that a fat of a much higher melting-point had been added to the cotton-seed oil. The only fat that could have carried the free acids of high melting-point, and also the color, was palm oil, and accordingly mixtures were made of "summer yellow" cotton-seed oil, the grade usually sold as "butter oil," and palm oil. These mixtures, when subjected to the above-described treatment, gave exactly the same results as the samples under examination, yielding solid, free acids which melted at from 43° to 45° C., with a refractive index of about 1.4626.

Very little free fatty acid could be obtained from any sample of cotton-seed oil alone, and the little that was obtained was liquid at ordinary temperatures; a sample of corn oil, which had undergone a treatment of oxidation to fit it for use in paint, gave a small quantity of free fatty acid, which, however, melted at 21° C., more than 20° lower than the temperature at which the free acids from the samples melted. The conclusion was positive, therefore, that the palm oil had been added to the cotton-seed oil for the purpose of imparting a color, and by matching the color of the samples in the tintometer with known mixtures an approximate determination of the amount of palm oil present was obtained. In this way, No. 6732 was found to contain about 2 per cent., and No. 6685 about 5 per cent. of palm oil.

For the identification of palm oil in the finished product, oleomargarine, the above methods would not suffice. The free acids of the fats present would be separated, and could not be distinguished from the free acids of palm oil. In the samples under examination, the percentage of palm oil present was probably about 0.5 per cent., certainly not over 1 per cent., and for the detection of this small quantity a resort to color reactions was imperative. Two tests of this kind were worked out, which, applied with certain precautions, and with care as to the use of pure reagents, were sufficiently pronounced and characteristic to establish positive proof of the presence of palm oil even in the small quantities mentioned.

FIRST METHOD.

The reagent used in this test was similar in character to Halphen's reagent, used to detect the presence of rosin oil in mineral oils.[1]

The method of applying it was as follows: One hundred cc. of the fat are dissolved in 300 cc. petroleum ether, and shaken out with 50 cc. of 0.5 per cent. potassium hydroxide. The watery layer is drawn off, made distinctly acid with hydrochloric acid, and shaken out with 10 cc. of carbon tetrachloride. The carbon tetrachloride solution is separated and part of it tested with the following reagent: Two cc. of a mixture of one part crystallized phenol in two parts carbon tetrachloride is added to it in a porcelain crucible, then 5 drops of hydrobromic acid (sp. gr. 1.19), and the contents mixed by gently agitating the dish.

[1] *J. Soc. Chem. Ind.*, **21**, 1474 (1902).

The almost immediate development of a bluish green color is indicative of palm oil.

SECOND METHOD.

For this test an adaptation was made of the reagent used in the Liebermann-Storch test for rosin oil.[1]

The following is the detailed procedure: Ten cc. of the melted and filtered fat are shaken with an equal volume of acetic anhydride (chemically pure and colorless), then one drop of sulphuric acid (sp. gr. 1.53) is added, and the mixture shaken a few seconds. If palm oil be present, the lower layers on settling out will be found to be colored blue with a tint of green.

The test was applied to all the oils and fats ordinarily used for edible purposes, and none were found to give the characteristic color, except that sesame oil and mustard oil gave colors which might be confused with the color obtained from palm oil. Fortunately, these are not oils having a high natural color, consequently they would be present, if at all, in some considerable quantity, and their presence may easily be demonstrated by characteristic tests, the sesame oil by the furfural reaction, and the mustard oil by the high refractive index of the fatty acids extracted by the alkali solution.

The coloring-matter in sesame oil, which is the cause of this color reaction, may also be separated by repeated extractions with alcohol, when the oil left will not give the blue color. A similar number of extractions of cotton oil containing 1 per cent. of palm oil had no effect on the formation of the color. Mustard oil has not yet been tested after shaking out with alcohol.

The following precautions should be observed in the application of both of the color tests described above:

(1) The reagents used must be chemically pure and colorless. A tint of yellow in the carbon tetrachloride, acetic anhydride, or carbolic acid has a marked influence on the development of the color.

(2) The sample to be tested must have been *freshly* filtered and the filtering done at a temperature not exceeding 70° C. The duration of the heating must be as brief as possible.

(3) The sample should be kept in a cool, dark place until filtered and tested. Undue exposure to air and light, or the presence of water, alcohol, ether, or similar reagents interfere with the color reaction.

[1] Lewkowitsch : Vol. 1, p. 384.

(4) It will be noted that the bluish-green color developed in each test is transient. Changes occurring after the lapse of several minutes are to be disregarded.

(5) As a further corroboration of the presence of palm oil, the refractive index of the fatty acids extracted by the alkali should be determined. The refractive index of the fatty acids extracted in the above manner from an oleomargarine made up of oleo oil, neutral, cotton-seed oil, and a small amount of palm oil, will not exceed 1.4615 at 25° C. On the other hand, if corn, mustard, or, in fact, almost any of the other vegetable oil be used in the manufacture of the oleomargarine, the refractive index of the fatty acids extracted will be much higher, depending on the quantity of the oils used.

Bleached palm oil does not give the color reactions described. They are, therefore, dependent, either upon the coloring-matter of the palm oil itself, or upon some constituent which is destroyed by the process of bleaching.

ON A GLOBULIN OCCURRING IN THE CHESTNUT.

BY WILLIAM EDWARD BARLOW.

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THIS paper presents the first results of an investigation which the writer hopes shortly to continue. It is offered as a slight addition to the sum of our knowledge of the vegetable proteins —a knowledge for which we are indebted chiefly to the well-known researches of Osborne, Harris, Campbell and others.

The globulin studied by the writer was obtained as follows: Spanish chestnuts, of the edible variety (*Castanea vesca*), were peeled, bruised in a mortar, and treated with ether in an extraction-apparatus until most of the oil was removed. The resulting mass was then finely powdered and again extracted with ether until a fat-free meal was obtained. This meal was treated with a 10 per cent. solution of sodium chloride for several hours at 50° C. The resulting solution, strained and filtered until almost clear, gave a flocculent precipitate on saturation with ammonium sulphate. This precipitate was filtered out, drained free from the liquor, washed, and dissolved in 10 per cent. sodium chloride solution. The solution was filtered repeatedly until perfectly clear and bright and then dialyzed for four days against running