color to some extent, so that irregular results may be obtained with such substances as hydrogenated cottonseed oil, which melts at a high temperature.

The natural Kreis-reacting substances in cottonseed oil are not glycerides, and bear no relation to the decomposition of the glycerides, from rancidity or any other cause.

The work from which the above observations have been selected covers a period of eleven years' experience with the Kreis test, during which thousands of samples of edible fats have been examined. The portion which was done recently was carried out with the assistance of Mr. Isaac N. Jordan.

CONCLUSIONS

Refined cottonseed oils and their products sometimes contain chromogenetic substances carried over from the crude oil. These substances sometimes cause a positive Kreis reaction in a non-rancid oil, and hence interfere greatly with the detection of rancidity by this means. For this reason the Kreis test is not entirely dependable for the detection of rancidity in cottonseed oil.

MODIFICATION OF THE HOWARD METHOD FOR COUNTING YEASTS, SPORES, AND BACTERIA IN TOMATO PRODUCTS

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The modification here described is based on the fact that by boiling tomato pulp with Löffler's methylene blue and Ziehl-Neelsen's carbofuchsin the microorganisms are stained a slightly deeper color than the tomato tissues.

Aside from producing this slight differential effect, the combination of the two stains facilitates the observation of the yeasts, spores, and bacteria. Methylene blue, used alone, fails to properly stain some of the organisms, while carbofuchsin, without the addition of methylene blue, produces a reddish color which makes it hard to see clearly the outline of the bacteria when artificial light is used.

Take 20 cc. of tomato juice, pulp, or ketchup, or 5 cc. of paste or other highly concentrated products. Transfer the sample to a 100 cc. beaker using sufficient water to make the total volume about 30 cc. Heat to boiling, and add 2 to 4 cc. of Löffler's alkaline methylene blue. Stir well and continue the boiling for 3 min. The amount of methylene blue used depends on the concentration of the product under examination, enough being added to color the sample blue. A green color shows that insufficient stain has been used.

Next add 2 cc. of Ziehl-Neelsen's carbofuchsin, stir well, and again boil for 3 min. Let cool a minute, and add 5 to 10 drops of formaldehyde to reduce any excess of carbofuchsin; stir well and dilute with water to 60 or 120 cc., depending upon the concentration of the product. Shake well, transfer some of the sample to a beaker, prepare the microscopic slide in the usual manner and examine by the Howard method.¹

¹ Association of Official Agricultural Chemists, Report of Committee on Editing Tentative and Official Methods of Analysis, 1916, 324.

It is necessary to use artificial light since the blue color of the slide makes examination by daylight very difficult.

THE BEHAVIOR OF PHENOLPHTHALEIN WITH IODINE AND A METHOD FOR THE DETERMINATION OF PHENOLPHTHALEIN

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The question has frequently arisen as to the actual therapeutic strength of medicinal preparations containing phenolphthalein. Though phenolphthalein has had widespread use in medicine for many years, no method for its quantitative determination has been found satisfactory.

Among those investigated is a titration method proposed by Kollo,¹ based on the well-known reactions of phenolphthalein with iodine in which tetraiodophenolphthalein² is formed. Numerous experiments with Kollo's method failed to give concordant results and made it apparent that the reaction was subject to a number of errors, among which oxidation and incompleteness of conversion to the tetraiodo compound were later found to be most important.

In the present investigation a study was made of the effect upon this reaction of varying successively the concentration of iodine, temperature, and concentration of alkali, while that of the phenolphthalein was kept constant.

EXPERIMENTAL

The general method of carrying out the experiments enumerated in Tables I, II, and III consisted quite uniformly in the addition of a solution of iodine in potassium iodide to an aqueous alkaline solution of phenolphthalein. In nearly every case an approximately quantitative estimation of the principal reaction product, tetraiodophenolphthalein, was made by extraction of the reaction mixture with chloroform in a separatory funnel, after the excess of iodine had been removed with sodium sulfite and the whole acidified with hydrochloric acid.

The use of chloroform as an extraction medium was determined upon from a few preliminary experiments with a number of organic solvents immiscible with water. When freshly precipitated with acid from the cold alkaline solution, tetraiodophenolphthalein was found to be readily soluble in chloroform. The color of the weakly alkaline solution of the pure compound is wine-red by transmitted light and blue by reflected light. Phenolphthalein is only sparingly soluble in chloroform and the color of its alkaline solution needs no description.

In each experiment observations were made as to:

- (1) The character of precipitate formed.
- (2) The character, amount, and color of the alkaline solution of the portion of the precipitate remaining insoluble in chloroform.
- (3) Appearance, weight, and color of the alkaline

¹ *Pharm. Praxis, Bucharest*, 7 (1908), 341.

² *Claisen and Lob, Ber.*, 28 (1895), 1605.