HALPHEN'S TEST IMPROVED.

BY R. A. KUEVER.

Halphen's colorimetric test for cottonseed oil, according to Raikow, depends upon an unsaturated fatty acid which combines with sulphur, forming a wine-colored compound. It is the test of the United States Pharmacopoeia for the detection of cottonseed in olive oil. The procedure of the official test is as follows:

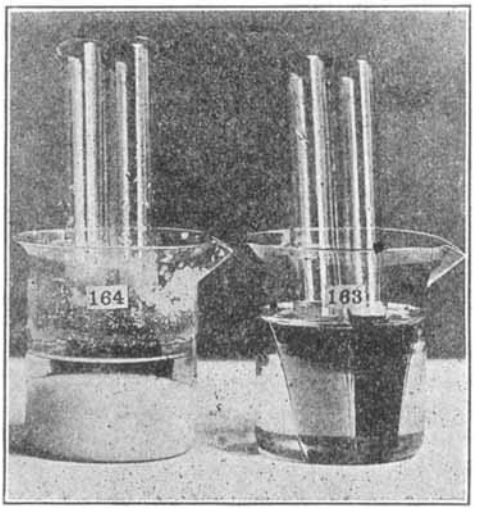
Mix 5 mils of the oil in a test tube with 5 mils of a mixture of equal volumes of amyl alcohol and carbon disulphide, which contains 1 percent of sulphur in solution, and immerse the test tube to one-third its depth in boiling, aqueous salt solution; no reddish color develops in fifteen minutes (cottonseed oil).

This test is not entirely satisfactory for several reasons. The Halphen reagent is not stable because the sulphur soon crystallizes after which the activity of the reagent is seriously impaired. The test is not sufficiently sensitive nor is it entirely dependable. The brine bath is unsatisfactory insofar that it sputters and crystallizes out salt as is shown in the photograph below.

Moreover, the temperature of a brine bath is not sufficiently high (106° C.) to bring out the color in the shortest possible time. It has been determined that a temperature as high as 175° C. does not interfere with the color formation.

Neither the reagent nor the procedure of the official test is entirely satisfactory and the following changes are proposed to overcome some of the difficulties. As a reagent the following has been found much more sensitive and permanent:

Carbon disulphide	100 mils
Pyridine	100 mils
Sulphur	2 Gm.



The above photograph shows the color and absence of color as produced by the improved Halphen's reagent. In each beaker the left-hand tube contains equal parts of pure olive oil and reagent, while the right-hand tube contains the same mixture excepting that the olive oil has previously been adulterated with ten percent of cottonseed oil. Beaker No. 164 shows the use of a brine bath at 106° C. in which the color did not fully develop for an hour. Beaker No. 163 shows the proposed hydrocarbon oil bath at 115° C. in which the color was completely developed almost instantly.

The pyridine and carbon disulphide are mixed and the sulphur, under a reflux, is dissolved by the aid of gentle heat.

The procedure may advantageously be changed by substituting for the brine bath a hydrocarbon oil bath and heating it to 115° C. before the tubes containing the suspected oil and reagent are immersed. If cottonseed oil is present the wine coloration will develop almost instantly.

This test is sensitive to one percent. If olive oil has been adulterated with as little as one percent of cottonseed oil, it may be detected by this method.

Pharmacists have successfully utilized this colorimetric test in their window displays of olive oil—one piece of apparatus showing a negative test on the brand of oil on display while another exhibited a positive test on some spurious brand. Since the heat may be removed a few minutes after the tubes are placed in the hydrocarbon oil, this apparatus lends itself well for window display purposes. The color is quite permanent. No change is perceptible in months.

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PEPSIN—METHODS USED FOR ITS QUANTITATIVE ESTIMATION—ITS PERMANENCE AND EFFECTIVENESS IN SOME OF ITS PREPARATIONS.*

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The oldest accurate and carefully recorded and carried out methods[1] for the quantitative estimation of pepsin, are those described by Bidde and Schmidt[3] in 1852. In the three-fourths of a century since, progress has been made in the quantitative estimation of the activity of this ferment, but it has been a progress in the art of manipulation and in the improvement of mechanical devices used rather than an advance in the understanding of the exact mechanism of the reactions involved in the process. Since the official or U. S. P. IX method for the quantitative estimation of pepsin has brought forth much criticism and many suggestions for its improvement, it was thought that time might well be spent in a study of the processes in an endeavor to improve the present method of assay. The older methods will be discussed more briefly and then a report of our success with them and also with the more recent ones suggested in the literature will be taken up.

At least two[1 & 2] excellent and comprehensive critical reviews of pepsin assay methods are to be found in the literature. Neither of these includes discussions of the more recent edestin methods (Fuld's and Brewster's), the recent modification of the electrolytic method[9] suggested by Northrup[20] or of the U. S. P. IX method and its development. Any method which requires more time for preparation and carrying out than the U. S. P. IX method does, with no advantage over it as to accuracy or reliability, need not be considered. This eliminates the Mett method which requires from twelve to twenty-four hours, Volhard's casein precipitation method which Geselschap[23] characterizes as "cumbersome and not particularly reliable," and the former U. S. P. method which required six hours for complete digestion. The Grützner and other colorimetric methods are not nearly as accurate as the U. S. P. IX method. With the Grützner carmine-fibrin method we have been able to detect only a 6 : 5 relationship between two solutions actually having a 2 : 1 relationship by the official test.

All the methods of the Jacoby, Solms, Fuld, Brewster "soluble protein" type

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