Color Tests for Kapok Oil

From the Laboratories of Swift & Company, Los Angeles, California

By VIRGIL C. MEHLENBACHER

THE advantages of the use of qualitative color reactions for purposes of detection and identification of oils and fats have long been recognized. In years past many such tests have been proposed by various workers. Although it is our specific purpose in this paper to consider and compare the various colorimetric tests which have been proposed for the detection of kapok oil, a short discussion of the general nature of such tests might not be amiss.

It is safe to say that by far the majority of the color or turbidity reactions for fats and oils are produced by impurities of one form or another which normally occur within the oils. Unfortunately, these chromogenetic substances are apt to be removed, or altered in their characteristics during processing. Naturally, we may expect that any such alteration might be reflected in any subsequent chromatic phenomena which these natural impurities would manifest, and such is actually the case. Consideration of these weaknesses, inherent in color methods, prevents us from placing too great faith in their unfailing reliability; however, they can be made to furnish good confirmatory evidence.

Color tests which have been proposed at various times include the Halphen test for cottonseed oil, kapok oil, and baobab oil, the Baudouin test for sesame oil, the modified Villavecchia test for olive oil, the Becchi test for differentiating between cottonseed and kapok oils, the Bellier test for hydrogenated cottonseed oil (1), the Ghose Pal test for hydrogenated fish oil (2), the Besson test for kapok oil, and others.

Few, if any, of these reactions will define one oil, and one oil only, under any or all circumstances The Baudouin test, while probably the most reliable color test we have, has very definite limitations with which any analyst using it should be thoroughly acquainted.

It is generally known that kapok oil responds to the characteristic Halphen test with an even greater intensity than does cottonseed oil. Therefore, a positive Halphen test will confirm the presence of either kapok or cottonseed oil. Baobab oil, while it, too, is said to respond to this test, need not be considered in industrial practice unless it attains commercial significance. Different lots of oils react with varying intensities with almost any color test, so, of course, it is impossible to make a definite statement as to the exact minimum amount of any oil which can be detected. A few tests which have been conducted in this laboratory will give a general indication of the relative sensitivity of the Halphen test to kapok and cottonseed oils.

TABLE I.

Comparison of Sensitivity of Cottonseed and Kapok Oils with Halphen Test.

Sample	Reaction
0.5% kapok oil	Very positive
0.1% kapok oil	Positive
0.025% kapok oil	Slightly positive
0.01% kapok oil	Very slightly positive
0.5% cottonseed oil	Very slightly positive
0.1% cottonseed oil	Doubtful
0.05% cottonseed oil	Negative

In order to differentiate between kapok and cottonseed oils several methods have been used by various workers most of which are modifications of the Becchi test. Fryer and Weston (3) describe the Becchi-Milliau test as follows:

> "The mixed fatty acids from the oil are obtained, and 5 c.c. dissolved in a test-tube in 15 c.c. of 90 per cent alcohol. 2 c.c. of 3 per cent silver nitrate solution are added.
>
> "The mixture is heated on the water-bath till about one-third of the alcohol is evaporated. In the presence of cottonseed oil, darkening occurs, and the fatty acids, as they separate, are colored brownish or black."

According to the same authors, heating of the oil before testing diminishes the intensity of the reaction.

Various observers have reported on this test, or some modification of the same. Wiley (4) reported favorably on the Milliau modification of the Becchi reaction, but Lewkowitsch (5) considers it too capricious to be recommended.

Tortelli and Rugger (6) have also proposed a modification and claim that 10 per cent cottonseed oil can be detected in the presence of olive oil.

Durand and Baud (7) again modified the Becchi test to distinguish between cottonseed and kapok oil. Their method is as follows:

> "Fifteen ml. of the oil are saponified with caustic soda and alcohol in the usual manner, 200 ml. of boiling water are added and the whole boiled till the alcohol has evaporated. The fatty acids are then thrown out by the addition of N/ 10 H_2SO_4 in slight excess. The fatty acids are skimmed off, and shaken twice with 15 ml. of cold distilled water, the water being then drained off and the fatty acids dried rapidly in an oven at 150° C. Five ml. of these fatty acids are shaken with a 5 ml. of a 1 per cent solution of silver nitrate in absolute alcohol.
>
> "Under these circumstances cottonseed oil produces only a barely perceptible brown color, while kapok oil rapidly develops a deep coffee coloration. By means of this test it is possible to recognize 1 per cent of kapok oil in other liquid oils."

Besson (8) describes a short method for identifying kapok oil whereby the sample is shaken with a 2 per cent solution of silver nitrate in absolute alcohol, when kapok oil gives an almost immediate coffee brown coloration, while cottonseed oil yields a yellow color only after some hours. Besson further claims that 1 per cent kapok oil may be detected in cottonseed oil, and as little as 0.25 per cent kapok oil may be detected in oils such as olive, sesame, etc.

When investigation began in this laboratory in an effort to find some reliable means of identifying kapok oil consideration was given to several of the tests referred to in the foregoing. At the outset the Besson test appeared to have the distinct advantage of simplicity.

In order to examine the Becchi-Milliau reaction, as described by Fryer & Weston, a large number of samples of different kinds of oils were tested. The results of these tests are tabulated in Table II.

TABLE II

Sample	Becchi-Milliau Test Color of Mixture	Acid Layer	Decision
Sardine	Very slight darkening	Light	Negative
Whale	Very slight darkening	Light	Negative
Lard	No change	Light	Negative
Beef tallow	Very slight darkening	Light	Negative
Olive	Very slight darkening	Light	Negative
Linseed	Very slight darkening	Light	Negative
Walnut	Very slight darkening	Light	Negative
Kome	Very slight darkening	Light	Negative
China wood	Reddish	Light	Negative
Perilla	Very slight darkening	Light	Negative
Coconut	Very slight darkening	Light	Negative
Palm	Reddish	Light	Negative
Kapok	Very slight darkening	Light	Negative
Domestic cottonseed	Very slight darkening	Light	Negative
Oriental cottonseed	Very slight darkening	Light	Negative
English cottonseed	Very slight darkening	Light	Negative
Peanut	No Change	Light	Negative
Babassu	No Change	Light	Negative
Mustard	No Change	Light	Negative
Sesame	Very slight darkening	Light	Negative
Hemp seed	No Change	Light	Negative
Soya bean	Very slight darkening	Light	Negative
Rape	No Change	Light	Negative
Coconut	Very slight darkening	Light	Negative
Teaseed	No Change	Light	Negative
Corn	No Change	Light	Negative

It will be noted that there is no evidence or defining characteristic which might be used as a guide to differentiation. In fact, we have tested quite a number of samples of cottonseed and kapok oil, and whereas at times we have been able to obtain positive reactions, we have been forced to conclude that the test as described is unreliable.

Many samples of cottonseed and kapok oils were tested by Durand and Baud's modification. Results of a few of these tests are shown in Table III.

TABLE III

Durand and Baud's Modification (Becchi-Milliau Test)

Sample	Reaction
No. 1—Kapok oil	Positive
No. 2—Kapok oil	Negative
No. 3—Kapok oil	Positive
No. 4—Kapok oil	Negative
No. 1—Cottonseed oil	Negative
No. 2—Cottonseed oil	Negative
No. 3—Cottonseed oil	Negative
No. 4—Cottonseed oil	Positive

The results which we obtained with this test were not only inconsistent as indicated in Table III, but the color reactions obtained were not nearly as pronounced or intense as those obtained with the Besson test.

In our investigation of the Besson test, the method which we finally adopted is as follows:

REAGENTS:

1. Chloroform.
2. Alcoholic silver nitrate, prepared by making a 2 per cent solution of silver nitrate in absolute alcohol. (Absolute alcohol made from specially denatured formula No. 30 was used in all of our work.) The silver is slow to dissolve, consequently should be prepared at least 24 to 48 hours previous to use. The solution must be maintained in a dark-colored or amber bottle.

METHOD:

Dissolve from 5 to 10 cc.'s of the liquid sample of oil, or melted fat, in a volume of chloroform slightly greater than that of the sample used. This test should be performed in a 1x10 test tube. Shake for a few seconds, or until the fat is completely dissolved, then add an amount of the silver nitrate reagent equal in volume to that of the sample used. Shake the mixture for 30 seconds, and allow to set without disturbing for 30 minutes. At the end of 30 minutes, examine for the presence of kapok oil. If kapok oil is present, the solution at this time will have assumed a brownish-black appearance, with a general muddiness and turbidity. This turbidity is characteristic of kapok oil, and is caused by a separation within the test solution and is subsequently followed by precipitation. In the case of very small amounts of kapok oil only a deep reddish-brown coloration will be noticed. Usually pure cottonseed oil will only produce a deep yellow color after standing for 30 minutes. This is also true of most vegetable oils.

We recommend making the test on refined samples only and then after filtering with some diatomaceous earth.

Sometimes pure cottonseed oil produces a deep red color, and for this reason it is very necessary that extreme care be taken in dealing with small amounts of kapok oil.

A large number of samples have been investigated with this test the results of which are tabulated in Table IV.

Study of this data will show that none of the samples examined gave a positive reaction excepting the one oriental cottonseed oil, and of course kapok oil. The history of the sample of oriental cottonseed oil could not be definitely traced.

To the date of this writing about two thousand samples of domestic cottonseed oil have been tested with this reaction, as well as many samples of oriental cottonseed oil, including oil originating in Brazil, Egypt and China. In only two cases out of the number of domestic oil samples tested have doubtful results been obtained, and in both of these cases we were unable to definitely ascertain the preceding history of the oil. Had this oil been pumped through pipe lines or into tank cars which had previously carried kapok oil a positive test could be expected to result. As a matter of fact, both of these samples came from vegetable oil refin-

TABLE IV

Sample	Besson Test. Color after 30 Min.	Turbidity	Decision
Teaseed	Pale yellow	None	Negative
Corn	Yellow	None	Negative
Rape	Pale yellow	None	Negative
Soya bean	Pale to deep yellow	None	Negative
Hempseed	Dark yellowish-green	None	Negative
Raisin	Pale yellow	None	Negative
Sesame	Pale yellow	None	Negative
Mustard	Pale yellow	None	Negative
Babassu	Pale yellow (Slight white precipitate)	None	Negative
Peanut	Pale yellow	None	Negative
Palm	Green	None	Negative
Coconut	Water white (Slight white precipitate)	None	Negative
Lard	Water white	None	Negative
Olive	Pale yellow	None	Negative
Beef tallow	Pale yellow	None	Negative
Linseed	Deep yellow	None	Negative
Walnut	Deep yellow	None	Negative
Kome	Yellow	None	Negative
China wood	Pale yellow	None	Negative
Perilla	Deep yellow	None	Negative
English cottonseed oil	Pale yellow	None	Negative
No. 1—Oriental cottonseed oil	Reddish	None	Negative
No. 2—Oriental cottonseed oil	Yellow	None	Negative
No. 3—Oriental cottonseed oil	Brownish-black	Very	Positive
Domestic cottonseed oil	Deep yellow	None	Negative
Kapok oil	Brownish-black	Very	Positive
Sardine	Yellow	None	Negative
Whale	Yellow	None	Negative

eries which were or had been processing kapok oil.

At least a hundred samples of kapok oil have been tested without failure to obtain a positive reaction when using the test as described.

We wish to point out and emphasize the fact that we have found it necessary to use turbidity as the distinguishing characteristic rather than color differentiation. The coffee-brown coloration, as originally proposed by Besson, is inadequate inasmuch as many samples of cottonseed oil produce this same color. We have found no other oils which have a tendency to discolor the test solution other than palm oil. However, the color produced by palm oil is green, and so entirely different from that obtained with either kapok or cottonseed oil that there is no danger of mistake.

Besides these many samples of kapok and cottonseed oils which we have tested, we have also examined quite a number of samples of most of the other oils included in Table IV, again without failure of the test to respond. In each case when a positive test has been obtained, investigation of the previous history of the oil has revealed admixture with kapok oil.

It is to be noted that in all of the tests proposed for kapok oil, silver nitrate is the active reagent. The fundamental difference between the Besson test and the other tests is that the former is made on the glycerides, whereas the other tests are made on the fatty acids. It is our opinion that the failure of these tests to react under all circumstances when using the fatty acids is due to removal of the active color and turbidity forming substances during the process of saponification and subsequent washing of the fatty acids. This tendency makes the use of the fatty acids, or any test based on the fatty acids, hazardous, to say the least.

In order to obtain some idea of the sensitivity of the Besson test, dilutions of kapok oil in cottonseed oil were made. The results of these tests are shown in Table V.

Sample No. 8 was considerably darker after standing 30 minutes than No. 9, but our experience has indicated that some samples of cottonseed oil darken as much. The turbidity characteristic was absent and for this reason we believe that at this low dilution the Besson test should be interpreted with discretion. Five per cent kapok oil was easily distinguished.

TABLE V

Sensitivity of the Besson Test.

Sample	Color	Turbidity	Decision
1. 100% Kapok	Brownish-black	Very	Positive
2. 90% Kapok-10% Cottonseed	Brownish-black	Very	Positive
3. 75% Kapok-25% Cottonseed	Brownish-black	Very	Positive
4. 50% Kapok-50% Cottonseed	Brownish-black	Very	Positive
5. 25% Kapok-75% Cottonseed	Brownish-black	Very	Positive
6. 10% Kapok-90% Cottonseed	Brownish-black	Very	Positive
7. 5% Kapok-95% Cottonseed	Brownish-black	Very	Positive
8. 1% Kapok-99% Cottonseed	Reddish-brown	None	Doubtful
9. 100% Cottonseed	Yellow	None	Negative

To determine the effect of preheating the oil on this reaction several samples of kapok oil were heated, cooled, and tested, the results of which are shown in Table VI.

Samples deodorized in a laboratory glass deodorizer for from six to eight hours, and then tested with the Besson test, showed no lessening in sensitivity. We can only conclude from this that ordinary heating conditions such as would be encountered in normal plant practice do not destroy the Besson reaction.

TABLE VI

The Effect of Preheating of Oil on Besson Test.

Sample	Color	Turbidity	Decision
Heated @ 110° C. for ½ hour	Brownish-black	Very	Positive
Heated @ 200° C. for ½ hour	Brownish-black	Very	Positive
Heated @ 250° C. for ½ hour	Brownish-black	Very	Positive
Heated @ 196° C. for 6 hours	Brownish-black	Very	Positive

In order to determine the effect of hydrogenation upon this reaction a sample of kapok oil was hardened in a laboratory converter. The results of this are indicated in Table VII.

Consideration of the foregoing data would seem to indicate that the Besson test as described can be used for the identification and detection of kapok oil with about the same degree of reliability that can be placed in other color tests for oils and fats. Further than this the Besson test appears to be somewhat more reliable than other tests of a similar nature, but made on the separated fatty acids. Cottonseed oil seems to be the only other oil (that we have examined) which might give a test to be confused with that produced by kapok oil. However, our experience has been that if the Besson test is used as we have recommended, interpreted as we have suggested, and not expected to be used for the lowest dilutions, it is reliable as a means of identifying kapok oil as well as differentiating between kapok and cottonseed oils.

In spite of the large amount of evidence which we have been able to gather, a large portion of which could not be published here, it is recommended that others who are interested examine the test critically. The usefulness and reliability of any test such as the one described can only be definitely established after a great number of samples have been examined, and these of sufficient variation in character and origin so as to cover all circumstances which might arise in commercial practice.

TABLE VII

Effect of Hydrogenation on Besson Test.

	Iodine No.	Refractive Index (ZB @ 40C.)	Besson Test
Kapok oil-hardened	88.8	56.35	Sl. Positive
Kapok oil-hardened	86.3	55.75	Negative

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