Suggested Spectrophotometric Methods for Grading Green Soybean Oil[1]

EUGENE H. MELVIN, DUNCAN MACMILLAN, and FREDERIC R. SENTI, Northern Regional Research Laboratory,[2] Peoria, Illinois

ALMOST every year a small part of the soybean crop is damaged by frost and the oil obtained from frosted beans is more or less green. In 1939, and again in 1942, unusually early frosts damaged a large part of the crop. Approximately one-quarter of the 1942 beans were frost damaged. Green soybean oil does not respond to ordinary bleaching processes so it was necessary to set up a grading system to determine which oils were sufficiently green to require special bleaching. Late in 1942 the Trading Rules Committee of the National Soybean Processors Association devised such a system (1). The method is given in the Yearbook and Trading Rules of the National Soybean Processors Association (2). It consists of visual comparison of crude oil against each of two aqueous nickel sulphate solutions. One is called Standard A and contains 7 grams of nickel sulphate hexahydrate in 100 ml. of solution; the other, Standard B, contains 9 grams of nickel sulphate hexahydrate in 100 ml. of solution. During comparison the oil and nickel sulphate solutions are viewed through a specified red glass filter. This method was an emergency measure and, although it puts oils into three grades, only Grade 2 has limits.

Since most laboratories concerned with soybean oil technology are now equipped with spectrophotometers, it seemed desirable to employ such an instrument for grading green soybean oil. The green color is caused by the presence of porphyrins which have sharp absorption peaks in the vicinity of 640 to 680 mμ. If a spectrophotometer is used rather than the Wesson colorimeter prescribed in the existing method, an estimate of the amount of pigments is possible. Thus a program was begun to find a correlation between spectral absorption and the visually determined green grade of oils which could be the basis for simple, rapid, spectrophotometric methods. The program included a study of crude, refined, and refined and bleached oils to provide data on which to base possible methods of grading. The refined and bleached oils were included in the study because it has been suggested that all oils be bleached with activated earth.

Experimental

Because it is difficult to obtain naturally frosted soybeans in most seasons, eight samples of beans obtained from two farms in Peoria County were artificially frozen at various stages of maturity. Six of these samples were of the Adams variety and were picked from the same field over a period of 26 days. The earliest beans were very immature while those in the last sample were mature. Some lots of the pods were frozen at the Northern Laboratory in a room kept at 0°F. while others were frozen with solid carbon dioxide in the field. The pods were then dried and threshed, and the beans flaked. Oil was extracted from the flakes with hexane and the extracted oil stripped of solvent. A portion of each lot of oil was refined by the appropriate American Oil Chemists' Society method, and part of the refined oil was bleached by natural or activated earth as the green grade required.

Analytical data which characterize the beans and oils are presented in Table I. The methods used were the appropriate A.O.C.S. or N.S.P.A. methods.

In the work reported in this paper, a Duboscq colorimeter with the special red filter was used rather than the Wesson instrument prescribed in the N.S.P.A. method. The vernier scales on the Duboscq colorimeter permitted more accurate adjustment to the 1-in. depth required for comparison against the standards. In addition, since the depths can be varied and read from the scale, it was possible to set up a continuous scale of depth of nickel sulphate solution required to match a 1-in. column of oil. Had a Duboscq colorimeter been chosen for the official visual green grading method, a single solution would have been sufficient.

To provide a basis for a spectrophotometric system of grading, it was necessary to measure the absorption spectra of the oils and to match the oils visually against the nickel sulphate standards as viewed through the red filter. Absorption spectra of crude, refined, and refined and bleached oils were measured on a Coleman Model 6B instrument[3] and also on a Cary recording spectrophotometer, a double mono-

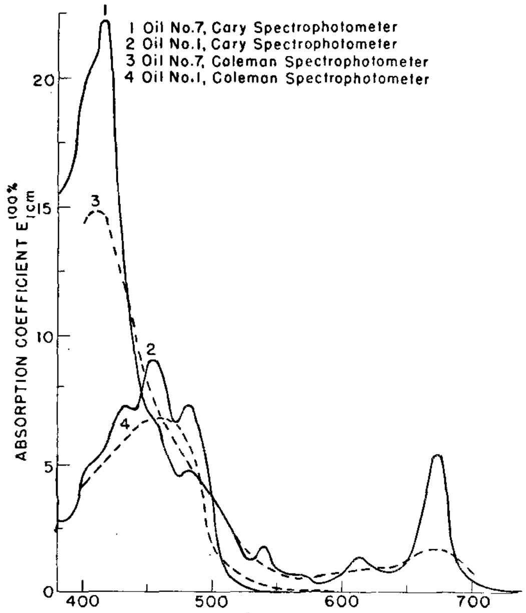

FIG. 1. Absorption curves of a crude light yellow oil (No. 1) and of a crude green oil (No. 7), measured with a Coleman Model 6B Spectrophotometer and with a Cary Recording Spectrophotometer.

---

[1] Presented at the American Oil Chemists' Society meeting, Cincinnati, O., Oct. 20-22, 1952.

[2] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

[3] The mention in this article of firm names or commercial products under a proprietary name or names of their manufacturer does not constitute an endorsement of such firms or products by the U. S. Department of Agriculture.

TABLE I

Analytical Data

Variety and oil number	Date of harvest	Frost damage in Beans	Oil content of beans	Iodine value of oil	Free fatty acid	Green crude grade	Refining loss	Refined bleached color	
								Wesson Lovibond glasses	A.O.C.S. photometric
		%	%		%		%		
Adams 9	9/19/51	100.0	16.2	126.4	2.2	3	16.0	70Y–5.0R 1"	26.4
Adams 6	9/24/51	100.0	17.6	127.4	1.9	2	12.2	70Y–4.2R 1"	17.8
Adams 7	9/28/51	100.0	17.4	129.8	1.4	2	8.6	20Y–2.2R 1"	15.2
Adams 4	10/ 2/51	61.1	19.5	133.8	0.9	1	6.7	70Y–4.5R 5¼"	5.5
Adams 3	10/ 8/51	14.7	19.2	131.6	0.7	1	6.2	70Y–5.9R 5¼"	7.0
Adams 1	10/15/51	2.5	20.4	134.0	0.5	1	5.3	20Y–1.9R 5¼"	2.8
Hawkeye 8	9/ 6/51	44.4	21.6	119.6	1.0	3	8.9	70Y–4.2R 1"	21.6
Hawkeye 5	9/12/51	23.9	21.4	125.2	0.7	2	6.7	70Y–10.4R 5¼"	11.3

chromator instrument of high resolving power and narrow band width. The absorption spectra of two oils (Nos. 1 and 7) are shown in Figure 1. The lack of detail in the curve obtained with the Coleman is due to its broad band width.

To determine the effect of the band width of the Coleman spectrophotometer, green oil No. 9 was chosen for study. The oil was diluted with carbon tetrachloride to give eight concentrations from 5 to 100% oil. Optical densities of these solutions at 670 mμ measured in 21.8-mm. I.D. tubes are shown in Figure 2. It will be seen that the plot of optical density against concentration does not give a straight line. This results from the breadth of the band width of the Coleman instrument and the narrowness of the porphyrin absorption band.

Absorption curves of crude and refined oils were measured in the Coleman instrument in standard 21.8-mm. I.D. tubes and in tubes of 16.1, 11.2, and 8.0 mm. I.D. Because the optical densities of the several crude green oils were greater than 2 over much of the spectrum when measured in 21.8-mm. I.D. tubes, the curves from 630 to 700 mμ, as measured in 8-mm. I.D. tubes only, are presented in Figure 3. Full curves for the eight refined oils measured in 21.8-mm. I.D. tubes are given in Figure 4. Optical densities at 670 mμ of the darkest oil, No. 9, as crude and after refining, are plotted against thickness of tube in Figure 5. It was found that over a period of several weeks crude oils had faded while refined oils had not. In order to relate optical densities as actually measured after the lapse of 45 days to what they were at the time the green crude grade was first established, the measured densities were multiplied by the ratio of nickel sulphate values at 0 days to the values at 45 days.

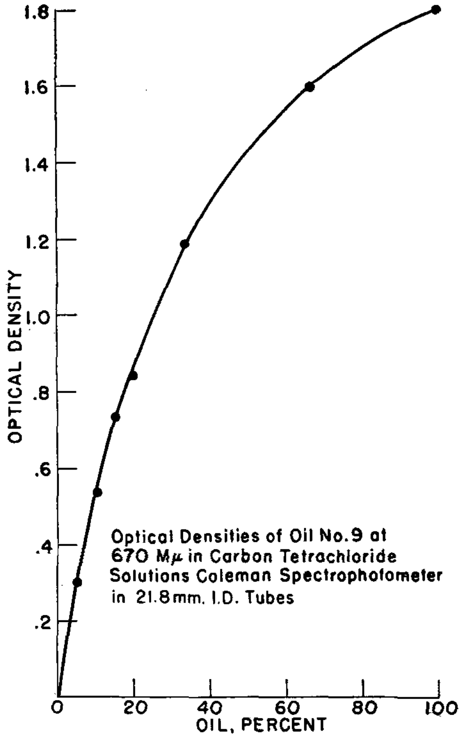

FIG. 2. Optical densities at 670 mμ as a function of concentration of oil No. 9, measured with Coleman Model 6B Spectrophotometer.

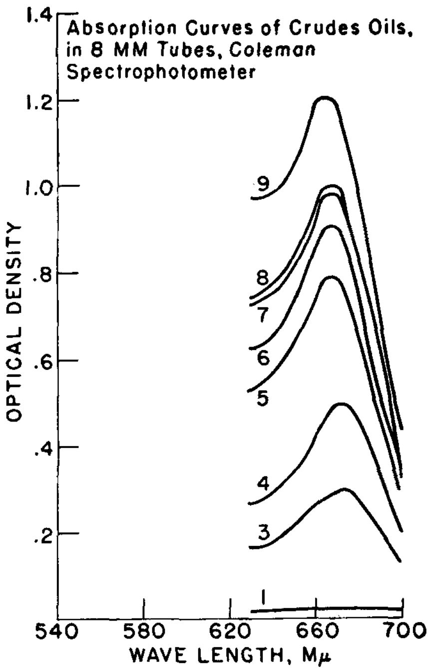

FIG. 3. Absorption spectra from 630 and 700 mμ of crude oils measured in 8-mm. I.D. tubes, using Coleman Model 6B Spectrophotometer.

Discussion

The analytical data presented in Table I show some changes which occur in the ripening of the soybeans. It will be noticed that the oil content and the iodine value of the oil increase with maturity whereas the free fatty acid and refining loss decrease.

The relation between the depth of a typical crude green oil, No. 9, and that of nickel sulphate required to match in the Duboscq colorimeter, as seen through the red filter, is shown in Figure 6. The line is curved rather than straight because the absorption band of

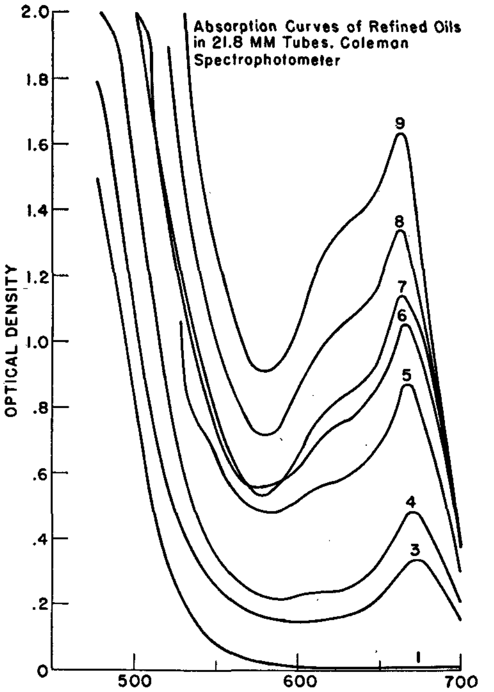

FIG. 4. Absorption spectra of refined oils in 21.8-mm. I.D. tubes as measured with Coleman Model 6B Spectrophotometer.

nickel sulphate is very broad whereas that of the green pigments in oil is fairly narrow.

The Coleman Model 6B spectrophotometer was chosen for a new grading system because it meets the requirements of the A.O.C.S. photometric method whereas narrow-band spectrophotometers do not. The most favorable part of the scale of the Coleman instrument is at optical densities less than 0.8. Above this point the scale readings become increasingly less accurate. Therefore 0.8 was chosen as the upper limit of Grade 2.

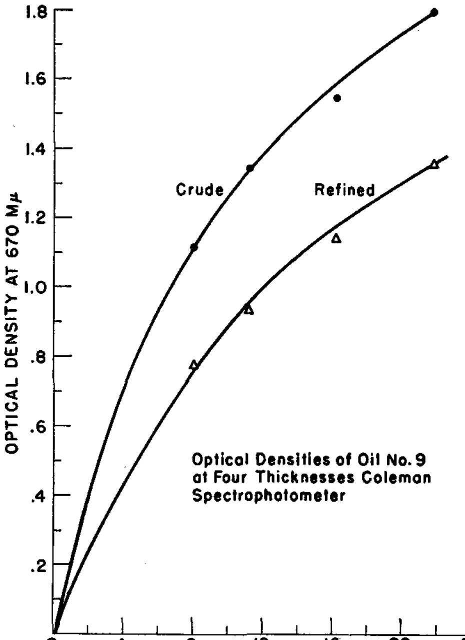

FIG. 5. Optical densities at 670 mμ of a green oil (No. 9), crude and refined, as a function of diameter of tube, using Coleman Model 6B Spectrophotometer.

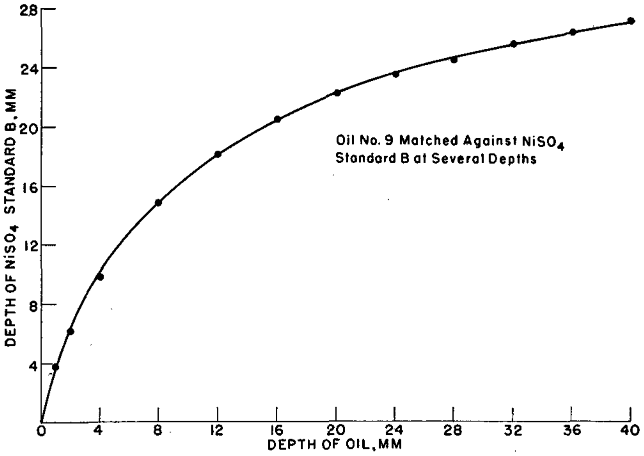

FIG. 6. Depths of nickel sulphate solution, Standard B, required to match several depths of a crude green oil (No. 9). Oil and solution viewed through specified red glass filter in Duboscq Colorimeter.

In Figure 5 the optical densities at 670 mμ of oil No. 9, which is near the break point between Grades 2 and 3, are plotted against the thicknesses of oil measured in the Coleman spectrophotometer. The refined oil exceeded an optical density of 0.8 in all thicknesses but 8 mm.; thus refined oils could be graded in tubes of approximately 8-mm. I.D. The limits of Grade 2 would be at optical densities of about 0.4 and 0.6. For the crude oil the optical density exceeded 0.8 at all thicknesses measured; therefore, if crude oil were to be used for green grading,

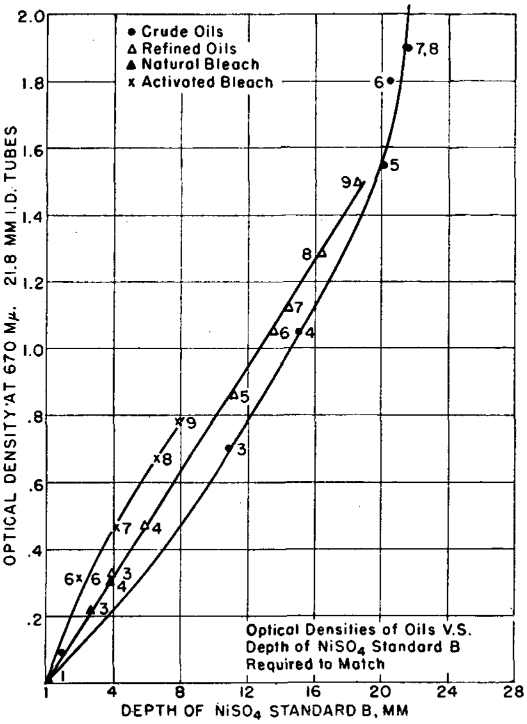

FIG. 7. Optical densities at 670 mμ of crude, refined, and refined and bleached oils measured with Coleman Model 6B Spectrophotometer in 21.8-mm. I.D. tubes.

it would be necessary to measure optical density in tubes of approximately 4-mm. I.D. The use of such a small tube however would be inconvenient. Break points would be at optical densities of 0.4 and 0.7. Optical densities at 670 mμ of the crude oil in carbon tetrachloride solution plotted against concentration, as shown in Figure 2, are less than 0.8 in solutions below 17%. If solutions of crude oil were to be measured, concentrations of about 15% would be required, but preparation of dilutions with reasonable accuracy is time-consuming. The limits of Grade 2 would be at optical densities of about 0.5 and 0.7.

In order to relate optical density measurements to the visual system of grading a graph was made, shown in Figure 7, of optical density at 670 mμ of crude, refined, and refined and bleached oils against the depth of nickel sulphate standard B, which was equivalent to a 1-in. depth of each oil. The three lines represent the oils at different stages of treatment. Values for crude oils of high optical density are somewhat scattered. Possible reasons for this scatter are that complex and varying mixtures of porphyrins are present which would produce varying widths and peak positions of the absorption bands and that it is difficult to use the Coleman instrument at high densities. The actual peak is usually not at 670 mμ; for example, the peak positions of the absorption bands shown in Figure 3 are, starting at the top curve, 665, 668, 668, 670, 670, 673, and 673 mμ.[4]

The middle line of Figure 7 represents refined oils and refined oils which have been bleached with natural earth. Although the peak position varies as in crude oils, the absorption is sharper and the optical densities are in a range more favorable for the Coleman spectrophotometer. The curve at the left in Figure 7 is for the green oils, refined and bleached with official activated earth.

Using the Duboscq colorimeter with standard nickel sulphate solution B, the break point between Grades 1 and 2 is equivalent to a thickness of 19.8 mm.; the break point between Grades 2 and 3 is 25.4 mm. (1 in.). The crude oil curve in Figure 7 intersects these break point values at optical densities of 1.5 for standard A and above the limit of the Coleman scale for standard B. Using the crude green grades of the parent oils, the break points of refined oils would be at optical densities of about 0.9 and 1.25. If oils refined and bleached with activated earth were used, the values would be about 0.1 and 0.7, which are somewhat low, but the spread between the upper and lower limits for Grade 2 oils is the largest of any of the suggested methods of grading green oils. For more satisfactory readings the amount of bleaching earth could be slightly reduced.

Since the values of 670 mμ for crude and refined oils in 21.8-mm. I.D. tubes are in a poor part of the scale on the Coleman instrument, the lower optical densities at 700 mμ and nickel sulphate values were plotted in Figure 8. In the upper curve, circles represent the values for crude oils calculated from the ratio of nickel sulphate values at 0 to 45 days. Crosses show densities and corresponding nickel sulphate values at time of actual measurements. These data show less scatter than those at 670 mμ. The optical density for the lower limit of Grade 2 is about 0.5 while that for the upper limit is about 0.7. A larger number of samples might alter these values slightly, but it is not likely that any major change would occur. The lower curve in Figure 8 is a plot of optical densities of refined oils at 700 mμ against the nickel sulphate solution values of the parent crude oils. The limits for Grade 2 would be at 0.2 and 0.4, which are undesir-

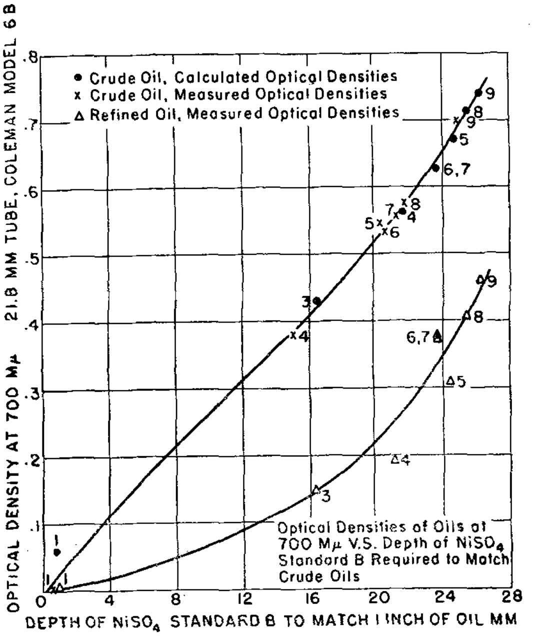

FIG. 8. Depths of nickel sulphate solution, Standard B, required to match 1 in. of the oils plotted against optical densities at 700 mμ of the oils, measured in 21.8-mm. I.D. tubes with Coleman Model 6B Spectrophotometer.

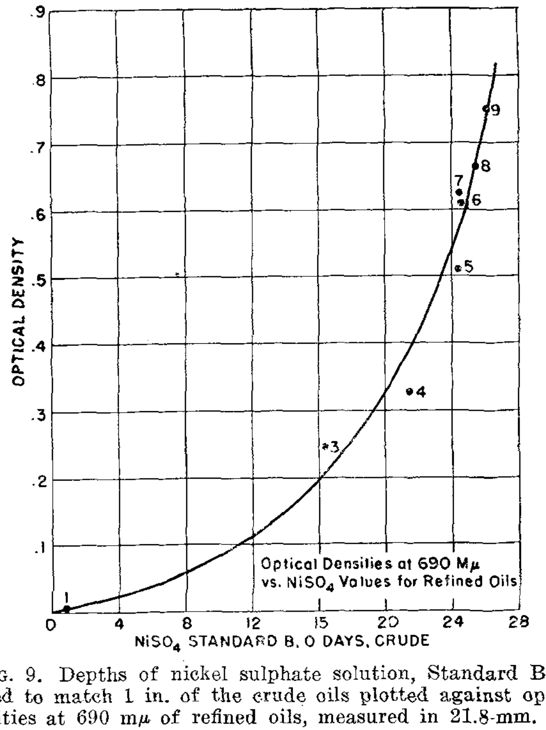

FIG. 9. Depths of nickel sulphate solution, Standard B, required to match 1 in. of the crude oils plotted against optical densities at 690 mμ of refined oils, measured in 21.8-mm. I.D. tubes with Coleman Model 6B Spectrophotometer.

---

[4] In agreement with the Coleman data the absorption curves obtained with the Cary spectrophotometer show variation in the wavelength of the peak absorption in the red. For some oils the peak in the region of 670 mμ is narrow, and in others it is quite broad, indicating that a mixture of porphyrins is present. Refining narrows the absorption band considerably, showing that some porphyrins are altered more readily than others.

ably low optical densities. One pair (Nos. 6 and 7) did not refine to give oils of as low optical density as would be expected from the curve, while another (No. 5) which, as a crude, had a higher value than the first pair, refined to give an oil of much lower optical density. There is then appreciable variation in response to the refining process. Bleaching however does not change the order of optical densities from those of refined oils. It is somewhat surprising that the upper curve in Figure 8 is so nearly a straight line, but reference to Figures 5 and 6 shows that the two curvatures representing the crude oil are similar.

To increase the optical densities, readings at 690 mμ could be used. These optical densities of refined oils are plotted in Figure 9 against nickel sulphate values of the parent crude oils at 0 days. Limits for Grade 2 oils would be approximately 0.4 and 0.7.

Summary

Possible spectrophotometric methods which could replace the present system of grading crude green soybean oils are given below in order of convenience. The limits are approximate values.

a) Measurement of optical density of crude oils at 700 mμ in 21.8-mm. tubes. Grade 2 limits would be at optical densities of 0.5 and 0.7.

b) Measurement of refined oils at 690 mμ in 21.8-mm. tubes. The limits for Grade 2 would be at optical densities of 0.4 and 0.6.

c) Measurement of refined oil at 670 mμ in 8.0-mm. tubes. Limits would be at optical densities of 0.4 and 0.6.

d) Measurement of refined and bleached oils at 670 mμ in 21.8-mm. tubes. Limits would be at optical densities of 0.1 and 0.7.

e) Measurement of optical density of crude oils at 670 mμ in approximately 4-mm. I.D. tubes. Limits would be at optical densities of 0.4 and 0.7.

f) Measurement of optical density at 670 mμ of crude oils in 15% solutions of oil in carbon tetrachloride in 21.8-mm. tubes. Limits would be at optical densities of 0.5 and 0.7.

Acknowledgments

The authors are indebted to J. J. Curtis and J. E. Hubbard for harvesting and processing the soybeans; to R. E. Beal and E. B. Lancaster for extracting the oil; and to F. R. Earle and T. A. McGuire for chemical analyses.

REFERENCES

1. Freyer, Egbert B., Oil and Soap, 22, 13 (1945).
2. Yearbook and Trading Rules, National Soybean Processors Association, page 60, 1952 edition.

[Received December 29, 1952]

Single Number Systems for the Color of Transparent Objects[1]

PROCTER THOMSON, Chemical Division, Procter and Gamble Company, Cincinnati, Ohio

THE American Oil Chemists' Society has sponsored three color systems: the Lovibond, the Fat Analysis Committee, and the photometric. There are a great many such systems as will be noted. The color comparison chart (Figure 1) shows the approximate relations between a number of such systems. The relations are not exact, but the chart has value in showing about the amount of color present.

Color is a quantity with three dimensions. Two of these are immediately perceived. The first and most important dimension of color is lightness. From water white to inky black, transparent objects can be arranged in a series with little disagreement between observers. The other obvious characteristic is hue. Violet, blue, green, yellow, red, and purple are perceived by all persons with normal vision.

COLOR COMPARISON CHART

GARDNER-HOLDT COLOR STANDARDS 1933	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
GARDNER-HOLDT COLOR STANDARDS 1921	1			2			3		4		5		6	7		8		9
LOVIBOND ANALYSIS 1¼" COLUMN RED YELLOW	0.23 2.3	0.32 3.2	0.45 4.5	0.65 6.5	0.80 8.0	1.20 12.0	1.70 17.0	2.15 21.5	2.90 29.0	4.00 40.0	5.50 55.0	7.30 73.0	10.0 100.0	14.9 149.0	20.0 200.0	25.0 250.0		
UNION COLORIMETER A.S.T.M.	0.9	1.1	1.3	1.5	1.7	1.8	2.1	2.3	2.5	2.8	3.3	3.8	4.3	4.6	5.0	6.0	7.3	9.0
U.S. OFFICIAL ROSIN GRADE STANDARDS										X	WW	WG	M	K-H	H	G	F-G	F
HELLIGE COLOR COMPARATOR 1930	-1	-1	-1	-1	1L	1-2L	2	3L	3	4	5L	5	6	7L	7	8L	8	9L
HELLIGE STOCK FONROBERT COLORIMETER	1	1	2	3	4	5	6	7	10	20	30	40	70	100	150	230	310	730
PFUND COLOR GRADER HONEY TYPE					0.5	2.0	3.0	4.0	5.5	8.0	9.0	12	15					
PRATT & LAMBERT COLOR STANDARDS					1	2	3	4	5	6	7	8	9	12	14	16	17	18
F.A.C. COLOR STANDARDS				1	3		5	7	11	11A 13	11B 15	11C 17	19		21 33	25 37	29 41	43
$K_2Cr_2O_7$ - H_2SO_4 GMS $K_2Cr_2O_7$ PER 100ML H_2SO_4	.0039	.0048	.0071	.0112	.0205	.0322	.0384	.0515	.0078	.0164	.0250	.0380	.0572	.0763	1.041	1.28	2.22	3.0

FIG. 1

The third dimension of color is a little more difficult to visualize. Imagine a rotating disc with a red sector and a grey sector of equal brightness. Revolving it with nearly all grey, a certain hue is perceived. As the red is increased and the grey is decreased, the basic hue does not change and the lightness does not change, but the color gets more pure. This characteristic is called saturation or purity. To define a color, then, three values are necessary.

The problem of defining color was undertaken by the International Commission on Illumination, properly known by capital letters of its name in French, C.I.E. Due partly to the fact that they were describing lights and light sources, the C.I.E. did not use the hue, saturation, and lightness, but three colors, blue, green, and red. These C.I.E. coordinates of transparent objects are calculated from the transmittancy. Having the X (red), Y (green), and Z (blue) values, they are ordinarily shown as the fraction of red + the fraction of green and the original green. Models in this scale are shown in Figures 3 and 4, which are described more fully as their purpose is explained.

Why then do we struggle to define color with a single number? In this group of oil chemists we see

---

[1] Presented at the 26th annual fall meeting, American Oil Chemists' Society, Oct. 20-22, 1952, Cincinnati, O.