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A Spectrophotometric Method for the Evaluation of Vegetable Oil Colors

W. D. POHLE and S. E. TIERNEY, Swift and Company, Chicago, Illinois

THE TWO METHODS most commonly used at the present time for the evaluation of vegetable oil colors are a visual technique (1) employing color glasses and a spectrophotometric procedure designed to correlate with the visual method (2). There are certain disadvantages in these methods. The visual method has the inherent weaknesses of all subjective methods. Duplication of sets of color glasses is not adequate, and it is difficult (and at times impossible) to match the color of the glasses with the oil samples.

Since the photometric method was designed to give color values comparable to the visual method, many of the faults of the latter have been perpetuated. Furthermore the system of using nickel sulphate solution for the standardization of the spectrophotometer permits sufficient variation in the adjustment of the wavelength scale to cause appreciable differences in optical density measurements, particularly at 550 millimicrons.

In measuring the color of light-colored oils, experimental errors in reading the optical density at 550 millimicrons are of the same magnitude as significant differences between oil because of the excessive weight given to the optical density values at this wavelength in the equation. High chlorophyll content oils have an unrealistic color by this method because of the negative factor used for optical density measurements at 670 millimicrons.

Because of the serious drawbacks of the visual method a satisfactory instrumental procedure for oil-color evaluation would be very desirable. The disadvantages of the present photometric method can be overcome and the usefulness increased by selecting a color measurement that expresses more exactly the amount of color in oil and is more reproducible among laboratories.

In this study the color measurements were made with a Bausch and Lomb Spectronic No. 20, using 25 x 105-millimeter cylindrical cuvettes, except where otherwise stated. The percentage of transmittancy and optical density were read directly, and data below 10% transmittance (above 1.0 optical density) are not presented in the graphs because they would not be used in evaluating the color of the oils. The percentage of transmittance is plotted on a semilogarithmic scale with the optical density shown on the opposite side of the graph. Since optical density values are proportional to the amount of color, graphical presentation of the curves show the relative differences in the amount of color.

Transmittancy-wavelength curves for a refined cottonseed oil and a refined soybean oil as well as curves obtained from these oils bleached with various percentages of earth are shown in Figure 1. In evaluating these oils, an index of the total color can be obtained from a measurement or measurements between 450 and 550 millimicrons, and the amount of chlorophyll can be determined accurately from optical density measurements at 630, 670, and 710 millimicrons when the equation has been developed as described by Stillman (3).

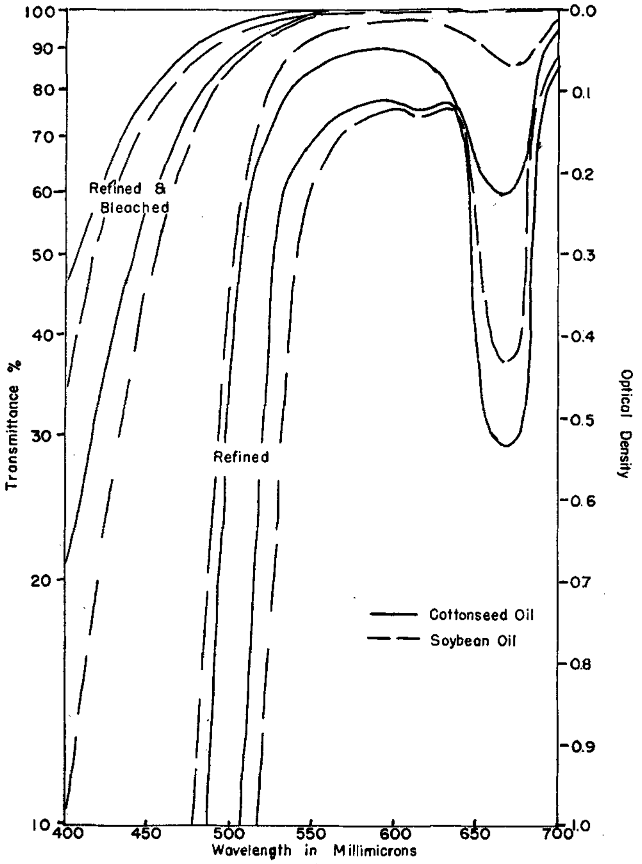

FIG. 1. Transmittancy-wavelength curves for cottonseed and soybean oil, both refined and refined and bleached.

If the total color of the refined oils and the bleached oils are to be measured on the same scale and good distinction is to be maintained for bleached oils, it is evident that the optical density must be measured between 450 and 500 millimicrons. In this region the optical density of refined oils cannot be measured without using a cell of shorter light path or by diluting the sample. Dilution was employed because it provided greater flexibility with available equipment. If cells of shorter light path were used, impracticably thin cells would be required in some cases.

Transmittancy-wavelength curves for refined oil,

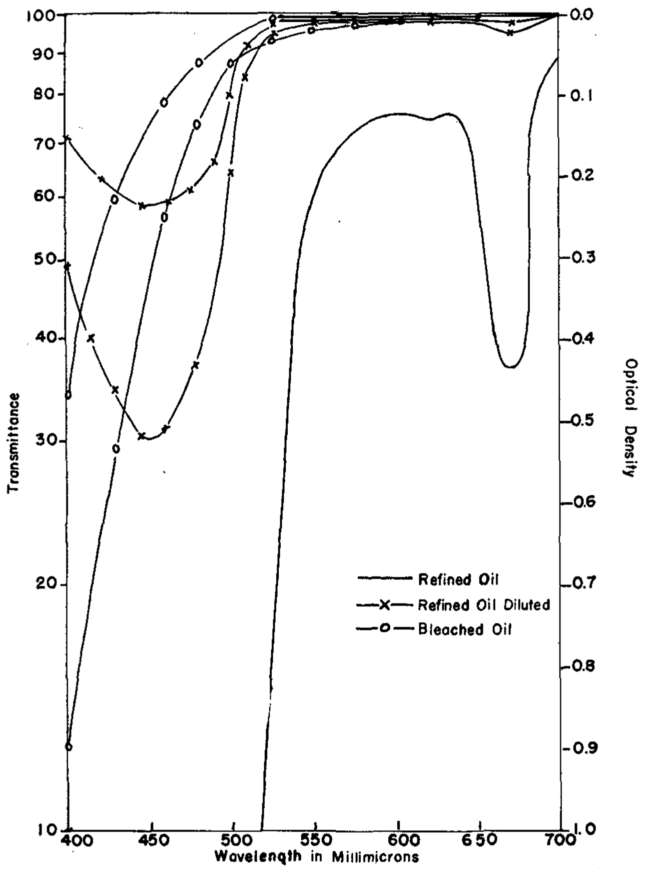

FIG. 2. Transmittancy-wavelength curves for soybean oil.

refined oil diluted with carbon tetrachloride, and bleached cottonseed and soybean oils are shown in Figures 2 and 3. The characteristic absorption between 400 and 460 millimicrons in the diluted, refined oils is not evident in the bleached oil. Evidently the color constituents responsible for this absorption are readily removed and are responsible for the difference in the characteristic of the transmittance-wavelength curve for refined cottonseed and soybean oils in the region mentioned above. The marked difference between the characteristics of the curves for the refined oils and the bleached oils in the region of 400 to 460 millimicrons makes comparison more difficult so 500 millimicrons were selected as the wavelength to be used for measuring optical density of the bleached oils and the diluted refined oils. While optical density measurements on the slope of a curve are not desirable, better results have been obtained and had the advantage that the same wavelength could be used for both cottonseed and soybean oils.

To reduce the optical density of refined cottonseed oil to 0.2 to 0.3, one volume of the sample was diluted to five volumes with carbon tetrachloride whereas one volume of refined soybean oil was diluted to 20 volumes. The optical density of the refined cottonseed oil solution is multiplied by 5 to get the optical density of the oil, and the optical density of the refined soybean oil solution is multiplied by 20. To eliminate fractional values for expressing color the optical density value is multiplied by 100, and this figure is called the color value of the oil. The color values are rounded off as follows:

Color values	Rounded off to nearest
0 to 20	Unit
20 to 100	2 Units
100 to 250	5 Units
250 to 1,500	10 Units

This gives a much broader scale for expressing color, and the values represent more nearly the relative amount of color in refined oils and bleached oils. The following table shows the approximate relationship between the color values and the Lovibond Red values.

Product	Color value	Lovibond Red
Bleached Oil	0 to 20	0 to 3
	20 to 70	3 to 5
Refined Cottonseed Oil	70 to 150	5 to 10
Refined Soybean Oil	270 to 820	6 to 12

Estimate of Precision Between Laboratories. Color measurements have been made in seven laboratories, using the Lovibond Method (1), the Photometric Color Method (2), and the Photometric Color Method (2) after adjustment of the instruments, using a potassium dichromate solution and the Color Value Method. These spectrophotometric measurements were made with Coleman Model 6 B spectrophotometers. The color measurements were made on the refined oil, directly and after dilution, and directly on the bleached oil.

The mean, standard deviation and coefficient of variation for each sample tested by the seven laboratories are given in Tables I and II. The data for the

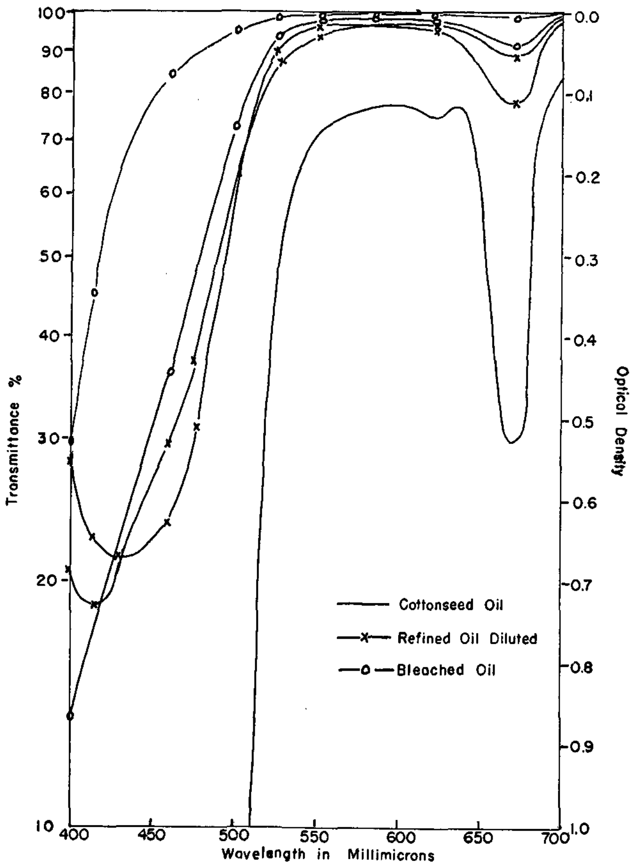

FIG. 3. Transmittancy-wavelength curves for cottonseed oil.

TABLE I
Reproducibility of Lovibond, Photometric Color Using Nickel Sulfate and Potassium Dichromate Solution for Standardization

	Lovibond			Photometric color (Std. with $NiSO_4$ soln.)			Photometric color (Std. with $K_2Cr_2O_7$ soln.)		
	Mean	Std. dev.	Coeff. Var. %	Mean	Std. dev.	Coeff. Var. %	Mean	Std. dev.	Coeff. var. %
Refined oils									
1 S/B	12.77	0.81	1.3	14.26	1.08	7.6	14.43	0.49	3.4
3 S/B	11.91	0.77	6.5	12.73	1.00	7.9	13.24	0.84	7.3
10 C/S	10.51	1.30	12.4	10.93	1.09	10.0	11.14	0.92	8.3
7 C/S	8.43	0.87	10.3	9.00	0.74	8.2	9.47	0.67	7.1
8 C/S	7.94	0.73	9.2	8.60	0.68	7.9	8.60	0.61	7.1
Avg.	......	0.90	......	......	0.91	......	......	0.71	......
Bleached oils									
4 S/B	6.87	0.72	10.5	7.44	0.53	7.1	7.43	0.31	4.2
2 S/B	4.61	0.54	11.7	4.91	0.38	7.7	5.00	0.39	7.8
5 C/S	4.56	0.44	9.6	4.51	0.29	6.4	4.68	0.39	8.4
6 C/S	4.49	0.54	12.0	4.47	0.42	9.4	4.54	0.36	7.9
9 C/S	2.17	0.23	10.6	2.34	0.26	11.1	2.27	0.25	11.0
Avg.	......	0.49	......	......	0.38	......	......	0.34	......

Lovibond and photometric color are given in Table I and that for the color value in Table II.

TABLE II
Reproducibility of Color Value

	Optical density at 500 mμ × factor		
	Mean	Std. dev.	Coeff. var. %
Refined oils			
1 S/B	741.9	10.8	1.5
3 S/B	742.1	20.8	2.8
10 C/S	134.9	5.2	3.9
7 C/S	126.6	7.2	5.7
8 C/S	113.6	3.3	2.9
Bleached oils			
4 S/B	79.5	3.4	4.3
2 S/B	53.9	1.4	2.6
5 C/S	19.3	1.1	5.7
6 C/S	18.9	0.70	3.7
9 C/S	9.9	0.44	4.5

The data in Table I show that, when a potassium dichromate solution was used for standardization of the instruments in different laboratories, the variation between laboratories was less.

Comparison of the coefficient of variation for the photometric color determined under the best conditions with those for the color values in Table II show that better agreement was obtained between laboratories for color value than for photometic color.

Removal of Oil Color by Bleaching. The technique which has been described is more suitable for following the removal of color from refined oil and evaluating the bleaching properties of earths than existing methods.

The color removed from three oils by varying amounts of bleaching earths was followed. The percentage of color removed was calculated from the color value (A) of the refined oil and the color value (B) of the bleached oil according to the following equation:

$$\text{Percentage of color removed} = \frac{A - B}{A} \times 100$$

The three oils used were a refined, solvent-extracted, soybean oil, a refined, hydraulic, cottonseed oil, and a refined, Expeller cottonseed oil. The three earths were the Official 1954 A.O.C.S. earth, the Official 1955 A.O.C.S. earth, and Special Filtrol.

Typical data for the color value and the percentage of color removed when the refined cottonseed oils were bleached with varying amounts of earth are shown graphically in Figure 4, and similar data for the refined soybean oil are shown in Figure 5. The color values and the Lovibond values are given in Table III.

These data show a) a close similarity between the cottonseed oils, b) the marked difference in the bleaching earths, and c) the lack of correlation between the Lovibond values and color removal. The color value indicates the relative amount of color in the bleached oils more accurately than the Lovibond values.

The laboratory bleaching of refined cottonseed and soybean oil, under routine plant conditions with 1954 A.O.C.S. Bleaching Earth during the 1954 season, 1954 A.O.C.S. Bleaching Earth on two samples during the 1955 season, and 1955 A.O.C.S. Bleaching Earth during the 1955 season, are given for some samples in Table IV. These data indicate that the oils were similar in color both seasons and that lighter colored bleached oil and better color removal were obtained in 1955. This indicates that the level selected was not matched as well with the 1954 A.O.C.S.

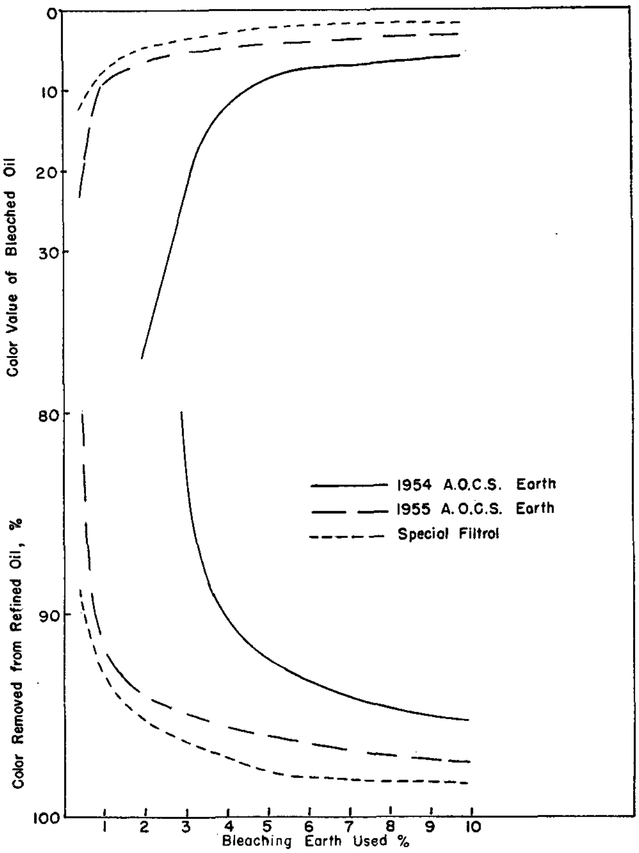

FIG. 4. Color value of bleached oil and percentage of color removed from refined cottonseed oil when different amounts of bleaching earth are used.

TABLE III

Color Values and Lovibond Values for Refined Oils and Bleached Oils

Bleaching earth used	% of earth used	Ref. hydraulic cottonseed oil		Ref. expeller cottonseed oil		Extracted soybean oil	
		C.V.	L.V.	C.V.	L.V.	C.V.	L.V.
Orig. ref. oil	......	125	5.5	116	5.5	840	11.0
A.O.C.S. 1954	0.5	92	5.8	110	5.8	650	12.0
	1.0	69	5.0	90	5.0	450	12.0
	2.0	27	4.0	44	4.5	240	11.0
	3.0	15	2.5	23	2.8	140	7.0
	4.0	10	2.0	11	2.5	69	5.0
	5.0	9	1.4	9	2.0	38	3.5
	7.0	7	1.0	7	1.0	11	1.5
	10.0	6	1.0	6	1.0	6	1.1
A.O.C.S. 1955	0.5	17	4.0	25	4.0	110	7.3
	1.0	9	2.2	10	2.5	26	4.0
	2.0	8	2.0	7	2.0	11	2.2
	3.0	6	1.0	6	1.0	9	1.6
	4.0	6	1.0	5	1.0	7	1.4
	5.0	5	1.0	5	1.0	6	1.1
	7.0	4	1.0	4	1.0	5	1.0
	10.0	3	1.0	3	1.0	4	1.0
Special Filtrol	0.5	9	3.0	13	3.0	60	5.0
	1.0	8	1.0	8	2.0	12	2.2
	2.0	6	1.0	5	1.0	8	2.0
	3.0	5	1.0	4	1.0	5	1.0
	4.0	4	1.0	3	1.0	5	0.9
	5.0	4	1.0	2	0.9	3	0.8
	7.0	3	0.8	2	1.0	3	0.9
	10.0	3	0.7	2	0.9	2	0.8

C.V.—Color value.
L.V.—Lovibond red value.

Bleaching Earth as might be done by measuring the color value and determining the amount of earth required to bleach refined oils to a specified color value.

For example, it might be determined what amount of bleaching earth is required to bleach refined cottonseed oil with a color value of 110 to 125 to a color value of 8 to 9 and refined soybean oil with 400 to 500 to 9 to 10. Optimum bleaching conditions might be selected on the basis of color removed, for example, the mid-point of a range for a 2% increase in bleaching earth which does not increase the percentage of color removed 1%. The color value of the refined oil bleached at the optimum level for a given bleaching earth can also be estimated. Table V shows an evaluation of the three bleaching earths as indicated above, using the data from Figures 4 and 5 and Table III. This is presented as an example of the type of data that can be obtained for comparative purposes when selecting a suitable earth and the optimum bleaching level.

TABLE V

Type of refined oil	Bleaching earth	Percentage of earth required to give a color value of 8 or 9	*Optimum* concentration for using earth	Approximate color value of oils bleached at the optimum level
C/S	A.O.C.S. 1954	5.5	5.0	9
C/S	A.O.C.S. 1955	1.5	2.0	7
C/S	Special Filtrol	0.8	2.0	5
S/B	A.O.C.S. 1954	6.0	8.0	9
S/B	A.O.C.S. 1955	2.5	3.0	7
S/B	Special Filtrol	1.5	3.0	5

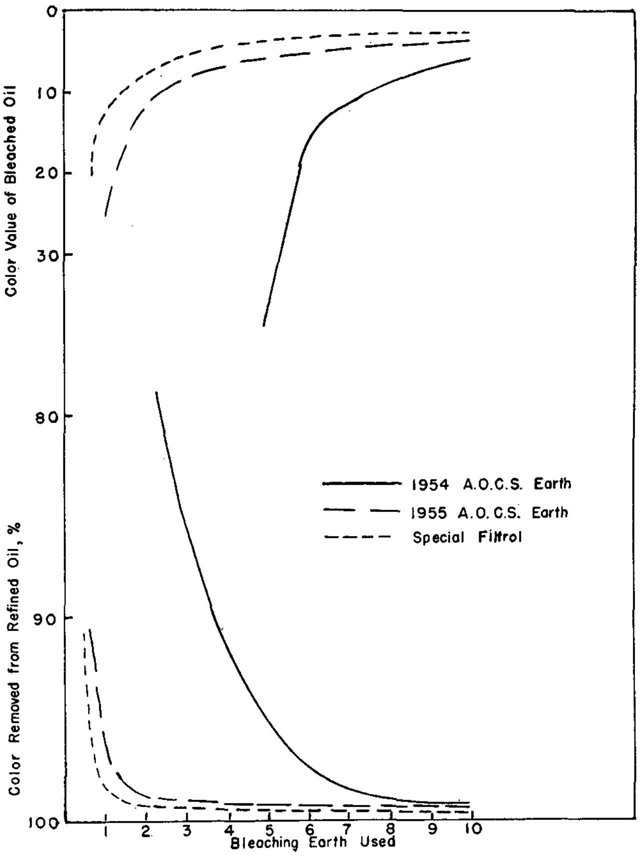

FIG. 5. Color value of bleached oil and percentage of color removed from refined soybean oil when different amounts of bleaching earth are used.

Estimation of Bleached Color from Refined Color. Color values for cottonseed and soybean oils refined and bleached by the Official Methods in 1955 are plotted in Figure 6. Similar data was obtained during past seasons using a Coleman Model 6B Spectrophotometer and the Official Earth used at that time. These and past data indicate that the color of the refined oil can be used as an index of the color of the bleached oil in most cases.

Some oils are difficult to bleach and the graph in Figure 6 fails to predict the bleached color in these cases. Only two oils of this type have been encountered during this study. The transmittance curves of these oils were different from the normal oils but data is too limited at present to predict whether it will be possible to identify abnormal oils that cannot be satisfactorily bleached, other than by an actual bleaching test.

TABLE IV

Some Data on Color Value of Laboratory Refined and Bleached Oil for 1954 and 1955 Seasons

Type of Oil	A.O.C.S. earth	Used, %	During season	No. of samples	Range for color value		Color % removed
					Refined oil	Bleached oil	
C/S..	1954	5.67	1954	10	73–150	6–16	89–93
C/S..	1954	5.67	1955	2[a]	116–125	8–9	93–94
C/S..	1955	4.67	1955	10	78–118	4–6	93–96
C/S..	1955	4.67	1955	2[a]	116–125	5	96
S/B..	1954	5.67	1954	8	340–460	8–10	97–98
S/B..	1954	5.67	1955	1[a]	840	18	97
S/B..	1955	3.00	1955	16	270–430	5–8	98–99
S/B..	1955	3.00	1955	1[a]	840	9	99

[a] Same refined oils used with both 1954 and 1955 bleaching earths.

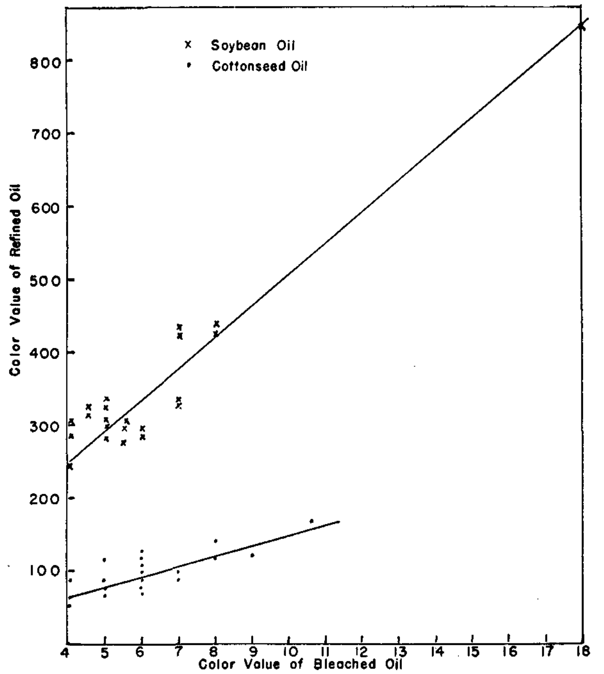

FIG. 6. Color value of laboratory refined and bleached oil.

Summary

A means for evaluating oil color on the basis of an optical density measurement at 500 millimicrons has been proposed. The amount of chlorophyll would be taken into account by present methods or an adaptation of these methods.

The advantages of the proposed means of evaluating oil color are that it expresses more exactly the amount of color in the oil, provides a more accurate estimate of color removal during bleaching, and makes possible more exact evaluation of bleaching earth.

Data have been presented on the color value (Optical Density × 100) of refined and bleached oils, possible variation in value obtained from different laboratories, estimation of color removed during bleaching, evaluation of bleaching earth, and relationship between color of refined and bleached oils prepared in the laboratory by the Official Method.

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The Fatty Acid Composition of the Seed Fat from Swietenia Macrophylla

M. M. CHAKRABARTY and D. K. CHOWDHURI, Department of Applied Chemistry, Calcutta University, Calcutta, India

THE SEED FATS of the *Meliaceae* family have not been studied in any great detail. One member of this family, *Swietenia macrophylla*, is an important timber tree in various parts of India, particularly in the Himalayan regions. Its seeds are rich in fat and contain swietenolide, a bitter principle, which has possibilities in pharmacy. The composition of the seed fat from *S. macrophylla kina*, grown in Mexico, was reported by Munguia and coworkers (1), who found possibilities for the utilization of this oil. Further investigation of this oil by the methods of low temperature crystallization and ultraviolet spectrophotometry seemed desirable.

The seeds are oblong in shape and have a thin pericarp. Extraction with petroleum ether (b.p. 40–60°C.) yielded 50% of a clear yellow oil, having a bitter taste. Properties of the oil were as follows:

Refractive index at 40°	1.4692
Saponification equivalent	292.4
Iodine value (Wijs)	109.7
Free fatty acids, percentage as oleic	0.6
Unsaponifiable matter, percentage [a]	1.1

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[a] A.O.C.S. method (2).

Mixed fatty acids isolated from the saponified oil had a saponification equivalent of 280.3 and an iodine value of 114.9. Linoleic and linolenic acid contents were estimated by the alkali isomerization-spectrophotometric method of Hilditch and coworkers (3). The oleic acid content was calculated from the iodine value, after allowing for linoleic and linolenic acids. It was assumed that no palmitoleic acid was present and that the only monounsaturated, diunsaturated, and triunsaturated acids were oleic, linoleic, and linolenic acids, respectively.

The mixed acids were first fractionated by crystallization from a 10% solution in acetone at −60°C. The solid fraction was further fractionated by crystallization from a 10% solution in ether at −20°C. The crystallization scheme, yields, and iodine values of the fractions are shown in Figure 1.

Each fraction was analyzed spectrophotometrically, and the results are summarized in Table 1.

Fraction A (acetone-soluble) was converted into methyl esters, which were then distilled under vac-

TABLE I

Spectrophotometric Analysis [a] of Acid Fractions

Fatty acid fraction	Total	A	B	C
Iodine value	114.9	38.9	102.2	156.9
$E^{1\%}_{1\text{cm.}}$ at 268 mμ (170°/15 min.)	64.0	18.0	56.0	87.0
$E^{1\%}_{1\text{cm.}}$ at 234 mμ (180°/60 min.)	350.0	112.0	302.0	473.0
Acid composition, %				
Linolenic	11.5	3.2	10.1	15.7
Linoleic	31.3	10.3	38.0	42.3
Oleic	29.4	12.6	6.3	41.4
Saturated	27.8	73.9	45.6	0.6

[a] According to method of Hilditch and coworkers (3).