than material dried at 80° C. in a vacuum oven. Also the antioxidant suffered no deleterious effects when heated in lard at 150° C. for a considerable time when lard extracts of the fruit were being made. This point is of considerable practical importance since it makes possible the treatment of lard with the Osage orange materials during the latter part of the rendering process. The undissolved plant material then may be filtered off with other materials following the rendering operation.

Studies are being conducted to determine the stability of the Osage orange antioxidant under conditions of deep fat frying and carry-over value into baked goods. Also studies are being followed to determine what chemical modifications of the antioxidant may be effected to improve its properties, uses, and potency.

Most of the antioxidant materials are located within the fibrous portion of the Osage orange fruit. Results reported in Table IV show that the fruit sap, seeds, and core contain only a small percentage of the antioxidant. The seed kernels, which constitute 20% of the dry fruit mass, contain 42% by weight of a pale yellow semi-drying oil. Removal of the oil leaves a meal containing 67% protein.

The Osage orange antioxidant responds well to the synergistic action of certain acids according to the results shown in Table V. Of these acids, tartaric, oxalic, malonic, citric, and ascorbic acids gave the best responses. It is of interest that these acids are either dicarboxylic or tricarboxylic. Ascorbic acid gave a slightly better result than citric acid, but the slight advantage would not justify its use because it is much more costly than citric acid.

Examination of the data in Table VII reveals that 0.1% of the Osage orange antioxidant approximately doubles the AOM stability values for cottonseed and soybean salad oils. It increases the stability of hydrogenated soybean oil 2.5 times and that of hydrogenated lard about four times. For some reason it failed to affect the stability of the samples of hydrogenated cottonseed oil tested.

It is reasonable to suppose that the Osage orange antioxidant will prove useful in such materials as oil-soluble vitamin concentrates, cosmetics, pharmaceuticals, lubricating greases, and many other substances that are subject to oxidative deterioration.

In concentrations required for antioxidant function the Osage orange antioxidant imparts no noticeable flavor or odor and is nontoxic. Large doses of the antioxidant have been fed to rats over a period of several weeks without any apparent ill effects on growth or metabolism of body tissues. Pomiferin is an isoflavone and represents a group of well known plant constituents, many of which are known to occur in plants used for foods.

Acknowledgments

The author wishes to express his appreciation to C. L. Lundell, director of the Texas Research Foundation, Renner, Texas, for provision of facilities for conducting much of the basic work on the Osage orange fruit constituents and to John Dendy for taking and developing photographs shown herein.

REFERENCES

1. Clopton, J. R., and Roberts, Ammaretto, J. Am. Oil Chem. Soc., *26*, 470-472 (1949).
2. Betts, H. S., "Osage Orange," Forest Service, U. S. Dept. Agric. Bull., "American Woods," 4 pp., Aug. 1945.
3. Wolfrom, M. L., Harris, W. D., Johnson, C. F., Mahan, J. E., Moffet, S. M., and Wildi, J. Am. Chem. Soc., *68*, 406-418 (1946).

[Received October 29, 1952]

The Effect of Chlorophyll on the Color and Value of Vegetable Oils

R. C. STILLMAN, Procter and Gamble Company, Cincinnati, Ohio

THE Color Committee of the American Oil Chemists' Society has worked for a number of years on the development of a spectrophotometric method for measuring oil colors. A.O.C.S. Tentative Method Cc 13C-50 revised October, 1951, is a direct outgrowth of this work. The A.O.C.S. Photometric Method has not proven acceptable to many persons because oil colors obtained by its use do not agree in all cases with oil colors obtained by the Wesson Method, A.O.C.S. Official Method Cc 13b-45 using Lovibond glasses. Disagreement between the two methods can be attributed mainly to variations in the chlorophyll content of oils. The Photometric Method just does not compensate for chlorophyll in the same amount and degree as does the Wesson Method. Figure 1 shows transmittance curves for two oils containing different amounts of chlorophyll. The chlorophyll contents of the oils, as well as A.O.C.S. colors, and Lovibond red values are shown. The disagreement between A.O.C.S. color and Lovibond red as chlorophyll changes is apparent. At low chlorophyll levels A.O.C.S. colors are slightly higher than Lovibond red values, and at high chlorophyll levels A.O.C.S. colors may be very much lower than Lovibond red values.

Effect of Chlorophyll on Apparent Bleachability of Oil. The problem of checking bleaching earth activ-

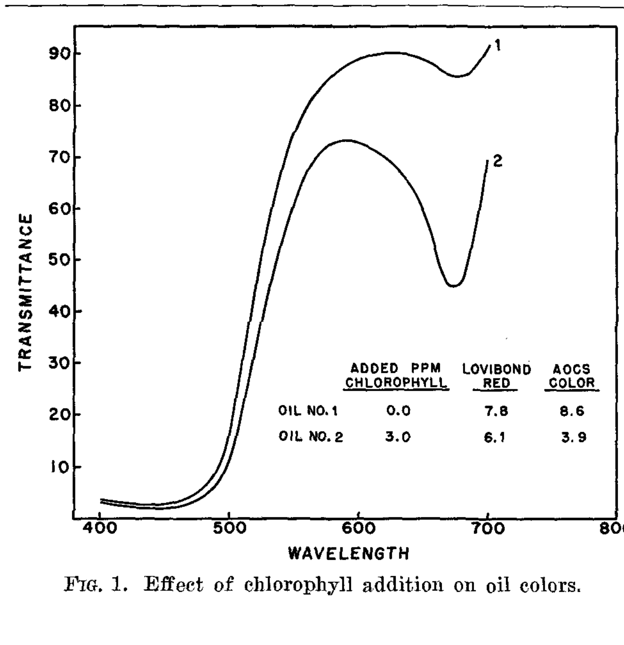

FIG. 1. Effect of chlorophyll addition on oil colors.

ity and the bleachability of oils is a continuing one faced by oil processors and makers of bleaching earths alike. Unquestionably the method of measuring oil colors is a factor in such evaluations. To study bleachability A.O.C.S. colors were determined on 24 regular production refined oil samples. The oils were then bleached with A.O.C.S. natural bleaching earth, and the bleached oil colors were obtained, using the A.O.C.S. photometric method. Original oil colors, bleached oil colors, the color drop, and p.p.m. chlorophyll are shown in Table I. It is clear that as the chlorophyll

TABLE I

Refined and Bleached Oils

Oil No.	A. O. C. S. Colors			P.p.m. Chloro.	$70.6\ D_{550}-10.7\ D_{670}$ Color		
	Refined	Bleach.	Drop		Refined	Bleach.	Drop
1	9.7	1.7	8.0	.09	8.2	1.4	6.8
2	10.6	1.8	8.8	.15	9.3	1.6	7.6
3	10.3	1.3	10.0	.22	9.3	1.0	8.3
4	9.1	1.3	7.7	.36	8.5	1.1	7.4
5	10.5	1.2	9.3	.36	9.9	1.0	8.9
6	7.5	1.9	5.6	.57	7.6	1.6	6.0
7	7.9	1.4	6.5	.69	8.4	1.1	7.3
8	9.7	2.0	7.8	.75	10.4	1.6	8.8
9	8.9	1.9	7.0	.75	9.6	1.6	8.0
10	7.5	1.7	5.8	.90	8.6	1.4	7.2
11	7.1	1.9	5.2	.90	8.2	1.7	6.6
12	7.7	2.3	5.4	.93	9.0	1.9	7.1
13	7.9	2.9	5.0	.96	9.3	2.4	6.8
14	8.7	1.9	6.8	.99	10.2	1.4	8.8
15	7.2	1.5	5.7	.99	8.6	1.3	7.3
16	9.0	2.9	6.0	.99	10.4	2.5	7.9
17	7.7	1.9	5.8	1.02	9.1	1.6	7.5
18	7.7	2.7	5.0	1.29	10.1	2.5	7.6
19	7.3	2.3	5.0	1.32	9.0	2.4	6.7
20	8.0	1.9	6.1	1.35	10.5	1.5	9.0
21	7.0	2.6	4.4	1.35	9.5	2.3	7.2
22	7.5	3.0	4.5	1.38	9.9	2.7	7.2
23	8.2	2.7	5.5	1.53	11.4	2.5	8.9
24	6.2	3.9	2.3	2.27	11.8	4.1	7.7

increases in the refined oil, the A.O.C.S. color decreases, the color of the bleached oil increases, and the color drop for a given amount of earth decreases markedly. The decrease in apparent color removal can be due to either faulty color measurements or to inefficiency of the earth in removing color when chlorophyll is present.

An experiment was designed to study the effect of chlorophyll on oil bleachability. Three oils were selected for the test, all three having an optical density at 550 millimicrons of close to 0.160. The three oils were characterized as follows:

	Lovibond Red	A.O.C.S. Color	P.p.m. Chlorophyll
Red Oil	12.1	11.5	0.0
Mix	3.8	1.8	2.6
Green Oil	0.0	—4.9	4.7

The mix sample was prepared by mixing equal amounts of the red and green oils. Chlorophyll was determined by the equation

$$\text{P.p.m. chlorophyll} = \frac{D_{670} - \frac{D_{550}}{3}}{.085}$$

All three samples were bleached with A.O.C.S. activated earth at several dosage levels. The effect of the bleach on the oil colors is shown in Figure 2. It must follow from the results obtained that the removal of chlorophyll is accompanied by an increase of A.O.C.S. color values, or conversely that A.O.C.S. color values are depressed extensively by the presence of chlorophyll. It is also suggested by the following table that neither Lovibond colors nor A.O.C.S. colors are of real value in judging either the bleachability of an oil or the bleaching power of an earth except on oils containing little or no chlorophyll.

COLOR DROP PRODUCED BY 1% BLEACH

	A.O.C.S. Color	Lovibond Red
Red Oil	4.7	5.7
Mix	—2.2	—2.0
Green Oil	—2.2	0.4

Derivation of a Rational Color Equation. A rational photometric color method based upon the value of the oil from a bleachability standpoint is desirable. To derive such a method the effect of chlorophyll on the measurement of oil color and the relative ease of removing chlorophyll by bleaching must be known. These two factors were determined in a series of experiments. It is unquestionably true that any equations derived from such experimental data will be peculiar to the oils studied and may require modification when extended for use on all oils. Such modification should however be small and of little real importance.

To study the effect of chlorophyll on oil color a series of 31 oils were prepared containing known amounts of chlorophyll and an ordered amount of red color. The first series of oils, 11 in number (0 to 10), contained no chlorophyll and were prepared by diluting a dark oil, No. 1 (13.4 Lovibond red), with varying amounts of a light oil, No. 0 (1.1 Lovibond red). The second series of oils (1-1 to 1-10) were prepared by adding 1.0 p.p.m. chlorophyll to oil No. 1 to give oil 1-1 and diluting with oil 0 in exactly the same way as in the original series to give oils 1-2 to 1-10. The third series of oils were prepared by adding 5.0 p.p.m. chlorophyll to oil 1 and diluting with oil 0 in precisely the same manner to give oils 5-1 to 5-10.

Photometric measurements were made on the 31 oils. Lovibond red colors and A.O.C.S. colors were determined. The whole series was arranged in an ordered series from dark to light by a number of observers and an average eye order grade assigned to each oil. All of these data are shown in Table II. Eye order grades vs. optical density at 550 millimicrons is plotted in Figure 3. All of the oils except 5-1, 5-2, 5-3, and 5-4 fall along a straight line. Since there are no non-green oils or light green oils in the same range

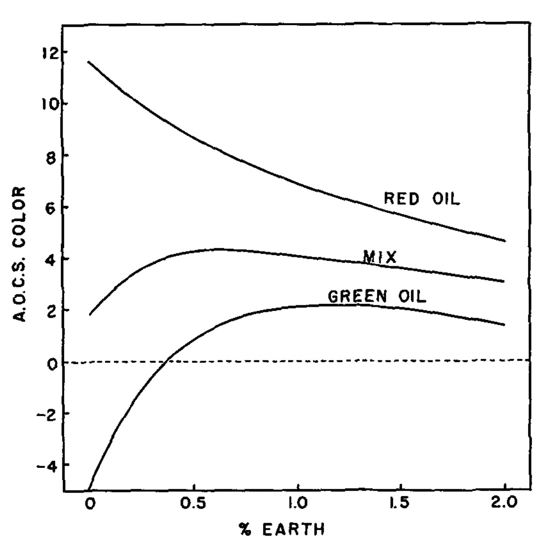

FIG. 2. A.O.C.S. color vs. % earth.

TABLE II
Prepared Non-Green and Green Oils

Oil. No.	P.p.m. Chloro.	D_{460}	D_{550}	D_{620}	D_{670}	Eye[a] Order (E.O.)	Lov. Red	A.O.C.S. Color	67 D_{550}	(E.O.+2) ×0.52	77.4 D_{550}— 31.2 D_{670}	70.6 D_{550}— 10.7 D_{670}
1	0	1.38	.195	.073	.063	25	13.4	14.8	13.1	14.0	13.1	13.1
2	0	1.32	.183	.074	.061	22	12.2	14.1	12.3	12.5	12.3	12.3
3	0	1.24	.168	.072	.061	21	11.0	12.7	11.3	12.0	11.1	11.2
4	0	1.15	.150	,062	.052	18	10.4	12.4	10.1	10.4	10.0	10.0
5	0	1.05	.137	.058	.045	17	9.1	10.8	9.2	9.9	9.2	9.2
6	0	.920	.111	.049	.038	12	7.4	8.3	7.4	7.3	7.4	7.4
7	0	.775	.087	.037	.030	11	6.4	6.9	5.8	6.8	5.8	5.8
8	0	.640	.072	.032	.026	7	4.8	5.7	4.8	4.7	4.8	4.8
9	0	.497	.056	.027	.020	5	3.7	4.5	3.8	3.6	3.7	3.7
10	0	.329	.037	.019	.015	2	2.5	2.9	2.5	2.1	2.4	2.5
0	0	.159	.017	.012	.010	1	1.1	1.3	1.1	1.6	1.0	1.1
1-1	1.0	1.49	.231	.121	.173	26	11.3	13.3	15.5	14.6	12.5	14.5
1-2	.9	1.45	.209	.109	.166	23	10.5	11.6	14.0	13.0	11.0	13.0
1-3	.8	1.32	.188	.100	.142	20	10.3	10.9	12.6	11.4	10.1	11.8
1-4	.7	1.24	.174	.092	.126	19	9.8	10.4	11.7	10.9	9.5	10.9
1-5	.6	1.12	.147	.078	.110	16	7.8	8.7	9.9	9.4	7.9	9.2
1-6	.5	.980	.124	.067	.092	14	6.3	7.5	8.3	8.3	6.7	7.8
1-7	.4	.830	.100	.054	.071	10	5.3	6.3	6.7	6.2	5.5	6.3
1-8	.3	.690	.081	.046	.058	9	4.5	5.2	5.4	5.7	4.5	5.1
1-9	.2	.520	.061	.035	.041	6	3.4	4.1	4.1	4.2	3.4	3.9
1-10	.1	.358	.041	.024	.023	3	2.5	3.0	2.8	2.6	2.5	2.7
5-1	5.0	1.80	.357	.289	.542	31(43)[b]	10.1	8.5	23.9	23.4	10.6	19.4
5-2	4.5	1.70	.320	.258	.495	30(38)	9.3	7.2	21.4	20.8	9.3	17.3
5-3	4.0	1.60	.285	.230	.445	29(34)	7.5	6.4	19.1	18.7	8.2	15.4
5-4	3.5	1.53	.255	.205	.398	28(30)	6.8	5.7	17.1	16.6	7.3	13.7
5-5	3.0	1.42	.225	.180	.358	27	6.2	4.7	15.1	15.1	6.2	12.1
5-6	2.5	1.27	.190	.152	.303	24	4.5	4.0	12.7	13.5	5.3	10.2
5-7	2.0	1.10	.156	.123	.248	15	3.6	3.4	10.5	8.8	4.3	8.4
5-8	1.5	.890	.115	.090	.186	13	3.1	2.4	7.7	7.8	3.1	6.1
5-9	1.0	.680	.087	.068	.132	8	2.5	2.3	5.8	5.2	2.6	4.7
5-10	0.5	.439	.053	.040	.070	4	2.0	2.0	3.6	3.1	1.9	3.0

[a]Average visual grade assigned by a number of individuals. [b]Estimated.

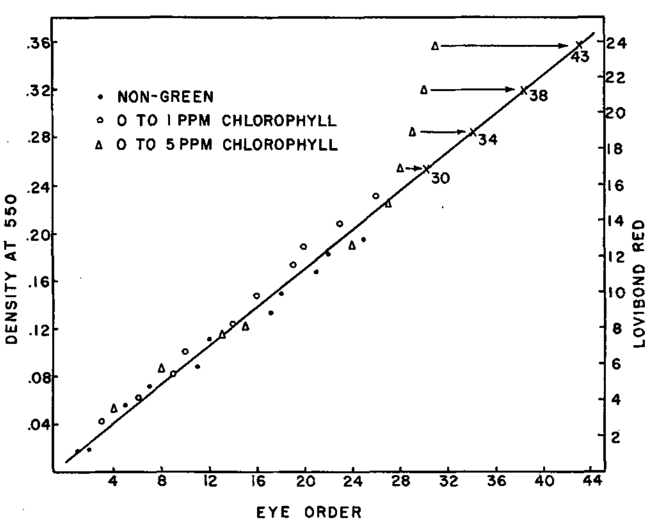

FIG. 3. Density vs. eye order.

of darkness, the four greenest oils cannot be expected to fall on the line nor are the values assigned to oils 0 and 1 necessarily exact for a similar reason. The Lovibond red scale plotted at the right ordinate corresponds to the colors of the non-green oils as given in Table II. Estimated eye order values for oils 5-1, 5-2, 5-3, and 5-4 are also given.

From the data given in the table it can be shown that, for the non-green oils,

a) A.O.C.S. colors are slightly higher than Lovibond red colors.
b) A.O.C.S. and Lovibond red = 67 D_{550} approximately.
c) A.O.C.S. and Lovibond red = 0.52 × (eye order + 2) approximately.

From oil 5-1, it can be shown that,

$$\text{p.p.m. chlorophyll} = \frac{D_{670} - \frac{D_{550}}{3}}{.085}$$

the constant .085 approaches 0.1 as the chlorophyll content decreases.

It can also be shown that the optical density at 550 millimicrons is increased by approximately 0.03 unit for each p.p.m. chlorophyll in the oil sample.

Further consideration will show the relationship, Lovibond red color or A.O.C.S. color, equals 67 D_{550}, which holds for the non-green oils, should be applied to the green oils as well. This is indicated by the eye order grades.

For the oils in this series,

$$\text{Color} = 67\ D_{550} = .52 \times (\text{eye order} + 2)$$
as judged by eye.

From the data at hand excellent agreement between photometric values and Lovibond red colors can be obtained at all chlorophyll and red color levels by the equation

$$\text{Photometric color} = 67\ D_{550} - 2.6 \times \text{p.p.m. chlorophyll}$$
$$= 77.4\ D_{550} - 31.2\ D_{670}$$

This equation would unquestionably show far less discrepancies with the Lovibond colors than does the equation specified in A.O.C.S. Method Cc-13C-50. For example, the oils shown in Figure 2:

	Lovibond Red	A.O.C.S.	77.4 D_{550}— 31.2 D_{670}
Red Oil	12.1	11.5	10.2
Mix	3.8	1.8	3.6
Green Oil	0.0	—4.9	—1.6

Values for the 31 oils prepared with known amounts of chlorophyll have been calculated and are shown in Table II.

The equation, color = 77.4 D_{550} — 31.2 D_{670}, takes into account only the effect of chlorophyll on the red color of an oil. No consideration is given to the relative ease of removing red and green color by bleaching. It is possible to develop in a similar manner a color equation based upon the bleaching data given. It has been shown that chlorophyll will add approximately 0.03 density unit to the 550 millimicron reading for each p.p.m. of chlorophyll present in the oil. From bleaching data (see Fig. 2) 1.0% earth will reduce the density at 550 millimicrons if due to red color by .074 unit and if due to green color by .124 unit. The actual measurements are:

	0% Bleach	½% Bleach	1% Bleach
Red Oil	.157	.107	.083
Green Oil	.160	.048	.036

The ratio of green to red removal is about 2 to 1 up to the point where all green color is removed.

From these considerations we can calculate an equation based upon the fact that green color is more easily removed than red color.

In a non-green oil

$$\text{Color} = 67\ D_{550}$$

In a green oil

$$\text{P.p.m. chlorophyll} = \frac{D_{670} - \frac{D_{550}}{3}}{.085}$$

The contribution of chlorophyll to density at 550

$$\text{millimicrons} = \frac{.027}{.085}\left(D_{670} - \frac{D_{550}}{3}\right)$$

If green color is removed twice as easily as red color, then

$$\text{Color} = 67\left[D_{550} - \frac{.027}{2 \times .085}\left(D_{670} - \frac{D_{550}}{3}\right)\right]$$
$$= 70.6\ D_{550} - 10.7\ D_{670}$$

In the ratio $\frac{.027}{.085}$ used in the chlorophyll contribution to optical density at 550 millimicrons, values from $\frac{.027 \text{ to } .032}{.085 \text{ to } .10}$ could be used, but the actual resultant equation would change very little.

Application of the Developed Equations. Two equations have been derived:

a) To give equivalent Lovibond red values
   Color = $77.4\ D_{550} - 31.2\ D_{670}$

b) To give a color value based upon bleachability of oils
   Color = $70.6\ D_{550} - 10.7\ D_{670}$

The color of a number of green and non-green oils using these equations have been calculated and shown in Tables I and II. In Figure 4 color values using equation 2 have been replotted against the amount of earth used in bleaching the red, green, and mix oils studied. In Table I it should be noted that, for 24 oils representing actual plant samples, a given amount of earth results in nearly a constant color and that the bleached colors are reasonably close to A.O.C.S. colors obtained on the same oils. In Table II, equation 1 gives colors very close to Lovibond red values while equation 2 gives values materially different from Lovibond values when chlorophyll is present in the oil, and exceptionally close when chlorophyll is low or absent entirely. In Figure 4 increasing amounts of earth produce a regular removal of color independent of whether the coloring material is red or green.

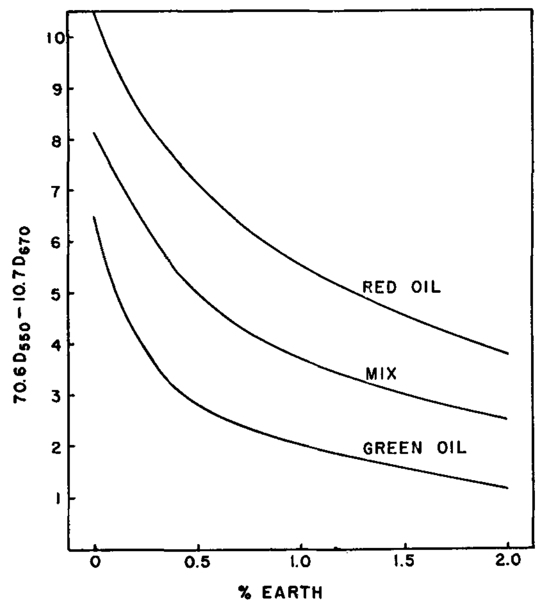

FIG. 4. Color vs. % earth.

Summary

To summarize the present status of the color situation and the implications of the present work, several conclusions can be drawn.

There are many ways of expressing the color of vegetable oils. The two most currently used are Lovibond red colors and A.O.C.S. colors. Both Lovibond and A.O.C.S. colors are unrealistic when oils containing chlorophyll are evaluated. Bleaching results indicate that chlorophyll is removed more readily than red color, but the removal of green is sometimes accompanied by an apparent increase in color as indicated by Lovibond or A.O.C.S. values. Hence neither Lovibond nor A.O.C.S. colors can be depended upon to evaluate correctly the bleaching power of an earth.

The A.O.C.S. Color Committee has been given, by the Uniform Methods Committee, the problem of developing a realistic color equation. The work herein reported indicates that the equation

$$\text{Color} = 70.6\ D_{550} - 10.7\ D_{670}$$

is such a realistic equation for evaluating the color of both green and non-green oils. The use of this equation also enables a reasonable evaluation of bleaching earths. Since measurements need be made only at 550 and 670 millimicrons, there is a considerable time saving over the use of the present A.O.C.S. equation and it should be possible to obtain better precision between laboratories. The equation is recommended to the Color Committee for further study.

The use of a second equation based upon measurements at 460 and 550 millimicrons for evaluating the color of bleached oils containing no chlorophyll will probably be found advisable. Similar work to that reported above in developing the equation for oils containing chlorophyll leads to a bleached oil color equation of

$$\text{Color} = 4.6\ D_{460} + 34\ D_{550}$$

The Color Committee may also desire to investigate this equation along with that given for green oils.

[Received October 30, 1952]