production of the oil would be on a relatively small scale because of the low oil content of the seed, nevertheless it should prove to be a valuable minor source of drying oil and worthy of some attention during the current shortage of drying oils.

2. Coumarin, comparable in quality to the synthetic product, representing 2% of the dry seed weight. It is useful in the manufacture of artificial vanilla flavoring, candy, pastries, baked goods, soap, tobacco, cosmetics, and perfumes.

3. A protein-rich meal which may be useful in the manufacture of adhesives, fibers, plactics, protective coatings, etc., and as a source of special amino acids. After being subjected to hot alcohol extraction (to remove the oils, glucosides, and other bitter principles, the sterols, phosphatides, resins, pigments, and coumarin) Hubam clover meal is assumed to be free of possible toxic compounds. Coumarin, which under certain circumstances gives rise to dicoumarol, the causative agent for the hemorrhagic sweet clover disease, is thus removed. The resulting product should be useful as a high protein supplement in foods and feeds. This meal averages about 47% protein. The alcoholic extract may be further processed for its useful ingredients including coumarin pigments (useful as indicators, antioxidants, medicinals, etc.), sterols (useful in the preparation of pharmaceuticals), phosphatides (useful in emulsifying agents, cosmetics, pharmaceuticals, etc.), and other constituents as yet unidentified.

Although Hubam clover has not been found to be especially rich in any one material, it is felt that the combined value of the various constituents present in the seed should ultimately make it an attractive source of basic raw materials.

REFERENCES

1. American Oil Chemists' Society, "Official and Tentative Methods" (1946).

2. Anon., "Chemical Composition of Hubam Clover," Iowa Agr. Expt. Sta. Ann. Rept. 1922, pp. 35-6.

3. Association of Official Agricultural Chemists, "Official and Tentative Methods of Analysis," 6th Edition (1945).

4. Hughes, H. D., and Henson, E. R., "Crop Production," The MacMillan Co., New York (1930).

5. Osborne, T. B., "The Vegetable Proteins," Longmans, Green and Co., London (1924).

6. Piper, C. V., "Forage Plants and Their Culture," The MacMillan Co., New York (1939).

7. Roberts, W. L., and Link, K. P., A Precise Method for the Determination of Coumarin, Melilotic Acid and Coumaric Acid in Plant Tissues, J. Biol. Chem. *119*, 269 (1937).

8. Treakle, L. J., and Sprague, H. B., Comparison of Methods of Harvesting Hubam Clover for Seed, Paper No. 2, Information Series, Texas Research Foundation (Jan. 2, 1948).

9. United States Dept. of Agr., "Agricultural Yearbook" (1937), p. 1204.

10. Willard, C. J., and Thatcher, L. E., "Experiments with Hubam Clover," Ohio Agr. Expt. Sta. Mo. Bull., Vol. 7, No. 1-2, p. 15 (1922).

A Spectrophotometric Method for the Determination of Color of Glyceride Oils

ALEXANDER K. PRESNELL, The Andrew Jergens Co., Cincinnati, Ohio

Introduction

NUMEROUS proposals have been made for methods to avoid the difficulties attendant upon visual methods of color measurement in the examination of animal and vegetable fats and oils. The most recent developments have been spectrophotometric methods, three of which are mentioned by Thomson (1). In two of these instances the spectrophotometric readings were correlated with Lovibond values and in the third with FAC standards. Such systems represent a distinct advance, but it is felt that expression of the photometric values in terms of a less arbitrary standard would be a more desirable procedure. Not only are Lovibond glasses difficult to obtain and frequently somewhat inaccurate, but they consist of an arbitrary series of filters whose transmissions do not correspond to the primary color sensitivities of the eye. Results obtained with the Lovibond system are appreciably dependent upon the color vision and judgment of the observer. If the procedure recommended by the A.O.C.S. (2) be followed, the Lovibond glasses used are often not those which give the best color match.

Due to varying quantities of colored substances in oils it is not possible to select any single wave length at which the transmittance will be an exact function of the color, total transmission, or the visual transmission of the oil. Inasmuch as color, rather than absorption at any specific wave length, is the value it is desired to measure, expression of the results in terms of tri-stimulus values would be the only entirely satisfactory method. If, however, it is necessary or desirable to express the color in terms of a single value, the Y of the tri-stimulus values may be used as it is a direct measure of the transparency in terms of visibility function (3).

Due to the general shape of the spectral transmission curves of glyceride oils it seemed probable that the tri-stimulus values could be closely approximated by functions of the transmission values at three properly selected wave-lengths. This is essentially the determination of the tri-stimulus values by the method of weighted ordinates but involves a great reduction in the number of ordinates.

Apparatus

The data presented here are derived from transmission curves drawn by a General Electric recording spectrophotometer having an effective slit width of 10 millimicrons and using absorption cells of 10.0 and 50.0 mm. sample lengths. Any instrument of corresponding slit width and accommodating cells up to 50 millimeters may be used. The Beckman quartz spectrophotometer, which is widely used in the oil industry, is satisfactory if equipped with the interchangeable cell compartments. If instruments with cell lengths of less than 50 millimeters be used, the transmissions may, when necessary, readily be calculated to a 50-millimeter basis with satisfactory results by the use of Bouguer's (or Lambert's) Law. This law may be stated as $T = t^x$, where t is the transmission factor of a material of unit thickness, and T the transmission factor of the material of x thickness.

The use of instruments with effective slit widths of much more than 10 millimicrons would introduce appreciable, but not necessarily serious, error. It is possible that a photometer equipped with the required interference filters might be used.

Procedure

Oils were clarified, if cloudy, by filtration through filter paper. In a few cases dehydration with anhydrous sodium sulfate was also necessary. At the temperatures required by the Methods of the A.O.C.S. (2) the transmission curves were run on the spectrophotometer. Fifty millimeter cells were used except in the case of very dark oils as noted below. The comparison cell may be filled with either water or carbon tetrachloride with practically identical results, but a pure, colorless sample of carbon tetrachloride is preferable because its refractive index is so close to those of most oils.

From the curves so obtained the tri-stimulus values for the ICI Illuminant C were calculated by the method of selected ordinates, using 30 ordinates in each instance. Illuminant C was chosen because it is the international standard for colorimetry and is the best representative of average daylight (4).

Observations

Oils from various sources and exhibiting the greatest variations in spectral transmission were examined. Three ordinates were then chosen in such a manner that they fulfilled two conditions. First, they were near the peak tri-stimulus values of Illuminant C. Second, readings at these ordinates were affected by specific absorption bands only to approximately the same extent as were the tri-stimulus values.

By trial and error, factors were found which could be applied to the transmission values at the ordinates selected to give close approximations to the tri-stimulus values previously computed. The following expressions were found to be nearly exact:

$$X=0.2\cdot T_{445}+0.15\cdot T_{555}+0.65\cdot T_{600}$$
$$Y=0.1\cdot T_{445}+0.7\cdot T_{555}+0.2\cdot T_{600}$$
$$Z=1.2\cdot T_{445}+0.06\cdot T_{555}$$

where X, Y, and Z are tri-stimulus values, and T is the per cent transmission at the wave length in millimicrons designated by the subscript. Table I shows the comparison of tri-stimulus values with those calculated from the formulas listed above. The transmission values are for 50 millimeter cells. Extremely dark oils are not represented in this table. Lovibond values are included for those samples on which they were determined. It is not intended to establish a correlation between these and the tri-stimulus values.

The table shows the rank order correlation of the Y value calculated from three ordinates with the true value to be .999, and if complete transmission be expressed as unity, the standard error of estimate for Y is 0.0147. The X is as good, but the Z value exhibits a lower, although still useful correlation. So far as is known, in both the case of X and that of Y,

TABLE I

Sample	Material	Undiluted Samples. Cell Length 50.0 mm.									Lovibond
		% Transmittance at			Value of X		Value of Y		Value of Z		
		445 mμ	555 mμ	600 mμ	From 30 Selected Ordinates	Cal. from 3 Ordinates	From 30 Selected Ordinates	Cal. from 3 Ordinates	From 30 Selected Ordinates	Cal. from 3 Ordinates	
1	A	72.7	93.5	94.6	88.25	90.06	89.30	91.64	89.90	92.85	1.3-13-.5
2	B	24.2	90.5	94.2	76.61	79.65	86.52	84.61	39.49	34.47	1.0-10-0
3	C	53.7	89.3	95.2	83.67	86.02	85.39	86.92	69.16	69.80	
4	D	41.5	85.3	91.0	79.92	80.25	83.14	82.06	55.45	54.92	1.5- 9-.5
5	E	5.5	86.7	89.7	69.77	72.41	77.12	79.81	10.43	11.80	2.1-21-.5
6	F	5.5	83.5	92.0	76.87	73.43	76.25	77.40	11.12	11.61	3.3-35-0
7	G	14.7	78.2	87.1	69.76	71.29	74.77	73.63	26.72	22.33	2.5-25-0
8	F	13.0	77.2	86.0	68.48	70.08	73.20	72.54	24.41	20.23	3.1-31-0
9	G	9.4	76.8	88.7	69.22	71.06	72.97	72.44	19.80	15.89	3.7-35-0
10	F	15.7	76.0	84.1	67.17	69.21	71.19	71.59	27.74	23.40	3.1-31-0
11	H	23.5	68.8	79.5	65.17	66.70	67.89	66.41	34.37	32.33	
12	F	6.0	68.7	82.2	64.91	63.94	65.44	65.13	14.69	11.32	4.6-35-0
13	F	5.5	69.8	78.3	61.04	62.47	64.14	65.07	9.63	10.79	3.6-35-1
14	F	1.5	71.5	70.0	56.17	56.53	60.75	64.20	5.53	6.09	10.2-70-0
15	D	5.5	55.3	73.5	56.69	57.17	54.62	53.96	11.95	9.92	7.9-40-0
16	F	4.4	56.0	70.0	55.03	54.78	54.62	53.64	11.56	8.64	5.6-70-0
17	F	3.5	57.2	72.0	54.67	56.08	54.58	54.79	8.80	7.63	6.4-70-.5
18	F	2.5	55.7	61.3	48.24	48.70	50.05	51.50	2.99	6.34	4.5-70-2
19	I	0.4	49.5	74.1	53.82	55.67	49.17	49.51	3.48	3.45	9.6-70-0
20	D	4.2	46.4	69.2	50.98	52.78	47.77	46.74	9.00	7.82	10.4-70-0
21	F	2.3	47.9	63.0	48.84	48.60	47.38	46.36	8.56	5.63	
22	F	2.0	47.4	62.0	46.53	47.81	44.72	45.78	3.68	5.24	8.1-70-1
23	F	0.0	47.8	52.8	41.50	41.49	41.58	44.02	1.99	2.87	
24	J	0.0	40.7	66.1	45.16	45.00	37.32	41.71	0.12	2.44	10.5-70-0
25	F	1.7	40.3	51.6	38.69	39.93	36.38	38.70	2.30	4.46	9.7-70-2
26	F	2.0	37.0	53.2	40.56	40.53	36.28	36.74	4.06	4.62	14.3-70-2
27	F	2.0	35.0	53.4	39.54	40.36	35.52	35.38	3.70	4.50	15.2-70-1
28	F	1.5	34.5	46.7	36.70	35.83	33.85	33.64	3.17	3.87	11.3-70-3
29	F	1.7	31.3	42.7	32.00	32.79	29.80	30.62	2.56	3.92	
30	D	2.2	26.0	42.8	31.72	32.16	27.63	26.98	4.15	4.20	18.0-70-2
31	F	1.8	27.7	40.6	30.78	30.91	27.22	27.69	2.84	3.82	13.3-70-4
32	D	1.7	21.5	38.2	29.03	28.40	23.58	22.86	3.25	3.33	21.0-70-2
33	G	1.7	22.3	36.6	26.87	27.48	22.92	23.10	2.28	3.38	
34	K	0.0	15.0	46.4	30.02	32.41	21.25	19.78	0.00	0.90	22.7-70-2
35	G	1.7	18.7	35.0	25.81	25.90	20.72	20.26	2.28	3.16	22.9-70-3
36	F	1.7	14.5	31.5	23.77	22.99	17.83	16.62	2.42	2.91	36.0-70-3
37	G	1.6	14.9	25.6	19.10	19.20	15.73	15.71	2.05	2.81	20.9-70-6
38	I	1.7	9.0	27.0	21.29	19.24	13.75	11.87	2.26	2.58	
39	I	1.7	8.5	23.0	16.59	16.57	10.38	10.72	1.87	2.55	
40	F	1.5	5.0	10.7	8.77	8.01	6.73	5.79	2.03	2.10	too dark
41	I	2.5	2.5	11.0	10.00	8.03	5.87	4.20	2.78	3.15	too dark
42	F	1.7	3.5	8.4	7.25	6.33	5.28	4.30	2.30	2.25	too dark
Standard error of estimate						1.43		1.47		2.08	
Rank order correlation						.999		.999		.964	

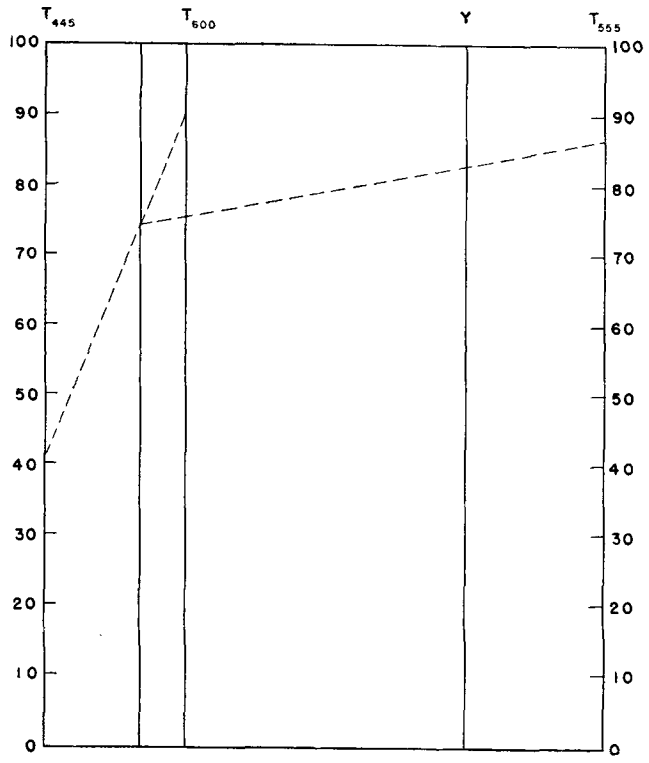
A=bleached tallow; B=linseed oil; C=distilled fatty acids; D=coconut oil; E=olive oil; F=tallow; G=grease; H=castor oil; I=fatty acids; J=soya oil; K=mackerel oil.

TABLE II

Sample	Material	Samples Diluted 1:25 With CCl_4. Cell Length 10.0 mm.								
		% Transmittance at			Value of X		Value of Y		Value of Z	
		445 mμ	555 mμ	600 mμ	From 30 Selected Ordinates	Cal. from 3 Ordinates	From 30 Selected Ordinates	Cal. from 3 Ordinates	From 30 Selected Ordinates	Cal. from 3 Ordinates
43	L	0.0	53.5	69.1	52.09	52.94	48.94	51.27	0.39	3.21
44	M	3.4	39.7	55.4	41.74	42.65	39.60	39.21	6.79	6.46
45	N	2.2	26.0	33.6	25.71	26.18	24.97	25.14	4.39	4.20
46	O	0.0	9.4	18.4	13.66	13.37	11.13	10.26	0.20	0.56
47	P	0.0	0.4	1.7	2.01	1.17	0.90	0.62	0.00	0.02
Standard error of estimate					0.71		1.14		1.28	

L=acidulated soya foots; M=corn oil foots; N=garbage grease; O=refuse palm oil; P=acidulated cottonseed foots.

NOMOGRAM FOR THE DETERMINATION OF "Y"



the correlation is much better than any that has been worked out for Lovibond values over a comparable range.

Dark Oils

To cover the entire range of color of oils it is necessary to use more than one cell thickness, or to dilute the sample, or both. The only disadvantage of this procedure is that it renders difficult the comparison of samples measured in different dilution or different cell thickness. It is possible, but not practical, to express the results obtained on a thin layer of a diluted oil in terms of the transmission through a thicker, undiluted layer of the same oil. In this work it was found that all those oils too dark in a 50-millimeter layer could be examined satisfactorily by diluting them to 25 volumes with carbon tetrachloride and measuring the transmission of the solution in a 10-millimeter cell. Intermediate procedures, such as the examination of the undiluted oil in the 10-millimeter cell, and the 1:25 dilution in the 50-millimeter cell may be used if considered advisable.

Under the conditions mentioned, dark oils yield transmittance curves similar to the lighter oils. Tristimulus values, calculated from three ordinates as were those for the light oils, show good correlation with those derived from thirty selected ordinates. This is shown in Table II.

Calculations of Results

Although the time required to calculate the value of Y is not excessive, the process may be made still faster by the use of an easily constructed nomogram. At the same time the possibility of introducing a relatively large error by mistakes in calculation is reduced. Such a nomogram is illustrated in the accompanying figure, and others for the X and Z values may readily be made (5).

Summary

A spectrophotometric method is described which permits an accurate estimation of the color of oils from transmittance values at three wave lengths. The color may be expressed in terms of tri-stimulus values or simply as visual transparency and is independent of the arbitrary color systems in wide use at present.

Acknowledgment

The author is indebted to Robert Van Tuyle of Emery Industries and Procter Thomson of Proctor and Gamble for some of the oil samples.

REFERENCES

1. Thomson, P. J. Am. Oil Chem. Soc. *24*, pp. 387-8 (1947). See also Report of Oil Color Committee, J. Am. Oil Chem. Soc. *25*, 271-6 (1948).

2. Official and Tentative Methods of the American Oil Chemists' Society. Second edition (1946). Cc 136-45, B.

3. Hardy, A. C. Handbook of Colorimetry, pp. 9, 33. The Technology Press (1936).

4. *Ibid.*, p. 4.

5. Levens, A. S. Nomography, pp. 68-75, Wiley and Sons (1948).