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# Color Index for Cottonseed Oils[1]

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The color index determined by the area under the absorption curve in the region of 400–500 millimicrons is preferred over the A.O.C.S. photometric method in research on cottonseed oil color because the color index gives the more accurate measure of the relative chromogen concentration in cottonseed oils. The evidence that the color index method is more reliable includes: (a) a demonstration that the area under the absorption curve may be used in place of absorbance in the Beer-Lambert equation; b) a panel score for cottonseed oil color intensity that agrees with the color index better than it does with the photometric color; and c) sources of error in the photometric method that do not occur in the color index method, including those contributed by the high emphasis on absorption at 550 and 670 millimicrons.

A QUANTITATIVE relationship between the red color intensity of darkly-colored, bleached cottonseed oils and the concentration of the chromogens in these oils is basic to research on the genesis, the identity, and the elimination of the red-colored bodies that create a color problem for about one-fourth of the cottonseed oil produced in the United States. The methods currently used for measurement of color do not satisfy the research requirements for determining the chromogen concentration in off-colored cottonseed oils. The A.O.C.S. Wesson method (1), using Lovibond glasses, is subjective, and it is an adaptation of the Lovibond method, which was developed originally for use in measuring the color of beer. The photometric A.O.C.S. method (1) was devised to give color values from spectrophotometric data that would match those obtained by use of Lovibond glasses.

It has long been established that the percentage of incident light that is transmitted through most transparent solutions is an exponential function of the concentration of the solution. This principle is known as the Beer-Lambert law, and it forms the basis of colorimetric procedures that find wide use in analytical chemistry. Normally, where the system is defined and where the absorption characteristics of the solute are known, the determination of the percentage of the incident light that is transmitted through the solution is made in a narrow wavelength band. It is obvious however that, when the system is poorly defined and where several unidentified solutes of unknown absorption characteristics are present, the use of a narrow wavelength band may not be relied upon for an accurate measure of the solute concentration.

The effort is made here to use the area under the absorption curve,[3] where the absorbance is plotted against the wavelength, as a measure of the relative chromogen concentration of the unidentified pigments in cottonseed oil. Thus, if the Beer-Lambert law describes the absorption behavior of the solutes in cottonseed oil, the relationship

$$C_t = \frac{R \sum_{\lambda=400}^{\lambda=550} \left(\log \frac{I}{Io}\right)}{b \sum_{\lambda=400}^{\lambda=550} \left( k_p \right)_{p=1}^{p=n}}$$

where $C_t$ is the total concentration of the absorbing solutes, $R$ is a proportionality constant, $b$ is the length of the light path, $\lambda$ is the wavelength in millimicrons, $I$ and $Io$ are light intensities, $p$ is the number of solute components, and $k_p$ is the absorptivity for the $_p$th component at wavelength $\lambda$, can be used to estimate the total concentration of solutes in the oils. The summation

$$\sum_{\lambda=400}^{\lambda=550} \left(\log \frac{I}{Io}\right)$$

is essentially the area under the absorption curve in the 400–500 millimicrons region in which the problem pigments absorb.

The denominator of the equation should remain constant in any dilution experiment with bleached

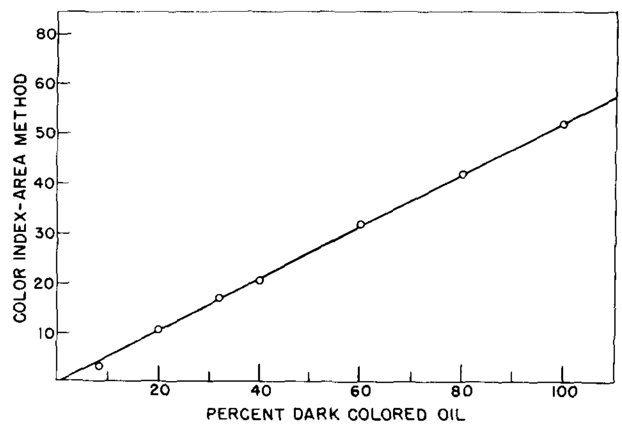


FIG. 1. Color index (area method) *vs.* percentage of dark-colored, bleached cottonseed oil mixed with a lightly-colored, bleached cottonseed oil.

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[3] This principle was proposed by Jacini and Carola (3) as a color index for olive oil and more recently was used by Frampton *et al.* (2) as a measure of egg yolk discoloration.

cottonseed oils, and the concentration at the various dilutions in such an experiment must be directly proportional to the contribution by the oil to the area under the absorption curve if the Beer-Lambert law is followed.

That the Beer-Lambert law is followed is demonstrated by the data plotted in Figure 1, where the percentage of concentration of darkly colored cottonseed oil in a very lightly colored oil is plotted as the abscissa against the contribution by the dark oil to the area under the absorption curve as the ordinate. Note that the direct proportionality demanded by the Beer-Lambert law is obtained.

## Materials and Methods

Nine refined and bleached cottonseed oils with A.O.C.S. photometric color values (Cc 13c-50), ranging from 0.14 to 1.49 (Table I), were used.

TABLE I

Rating of Bleached Cottonseed Oils by Three Color Methods

| Oil | Visual rating | | A.O.C.S. photometric color | | Color index | |
|---|---|---|---|---|---|---|
| | Score [a] | Rank | Value [b] | Rank | Value [b] | Rank |
| A | 21 | 1 | 0.14 | 1 | 8.7 | 1 |
| B | 42 | 2 | 1.00 | 6 | 10.0 | 2 |
| C | 65 | 3 | 0.67 | 4 | 10.9 | 3 |
| D | 86 | 4 | 0.53 | 2 | 11.3 | 4 |
| E | 101 | 5 | 0.71 | 5 | 11.7 | 5 |
| F | 126 | 6 | 0.64 | 3 | 13.3 | 6 |
| G | 150 | 7 | 1.31 | 8 | 14.3 | 7 |
| H | 166 | 8 | 1.18 | 7 | 15.6 | 8 |
| I | 182 | 9 | 1.49 | 9 | 16.4 | 9 |

[a] Sum of panel ratings for each oil from light (A) to dark (I).
[b] Average of duplicate determinations by two analysts.

The absorbances of the filtered oils were determined at 10-millimicrons intervals by use of a Beckman Model B Spectrophotometer, 1-cm. cell, and cyclohexane as the reference solvent. The sum of the 16 absorbances multiplied by 10 was taken as an approximation of the area under the absorption curve, and the product was designated as the color index.

For visual rating by a panel, flat-bottomed glass tubes [A.O.C.S. color tubes (1)] were filled to a depth of 67 mm. with the refined and bleached oils. The tubes were placed vertically in a rack and were transilluminated with a daylight lamp. Each member of a panel of 21 observers viewed the oils from the top, looking down, and arranged the oils in order of increasing color intensity. The panel color-score assigned to a given oil in the series of experimental samples was obtained by summing the relative positions assigned to it independently by each member of the panel. Thus, if an oil were assigned to position 2 by 7 members of the panel, to position 3 by 10, and to position 4 by 4, the score assigned was $60 = 14 + 30 + 16$.

## Results and Discussion

The color ratings of the bleached oils by panel scoring, A.O.C.S. photometric color method, and color indices (area method) are all recorded in Table I. The coefficient of correlation between the panel score and the A.O.C.S. photometric data was found to be 0.76, with 8 degrees of freedom. On the other hand, the coefficient of correlation between the color index and the panel score was found to be 0.99, also with 8 degrees of freedom. Furthermore the regression curves plotted in Figures 2 and 3 indicate a much higher divergence between the panel score and the A.O.C.S. data than between the panel score and the color index.

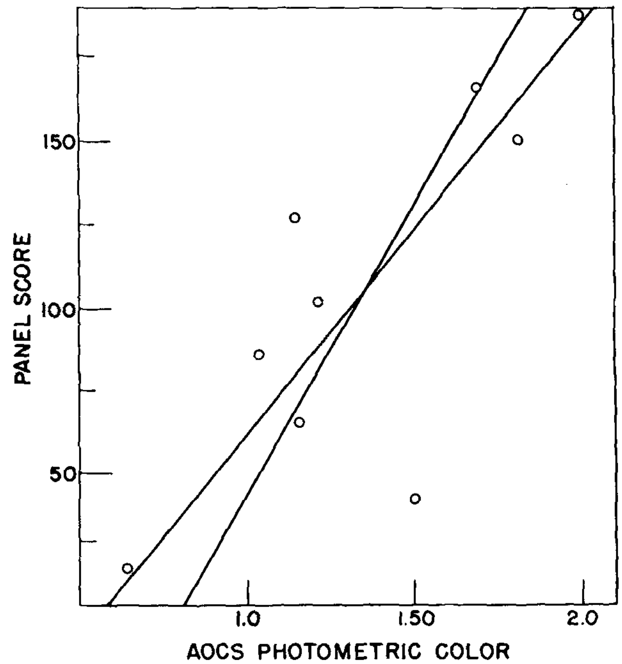


FIG. 2. Relation between panel scores and A.O.C.S. photometric color values.

A more quantitative comparison of the reliability of the area method and the A.O.C.S. method for obtaining color indices is found in the analyses of variance of the regression of the panel score on the A.O.C.S. photometric color and of the regression of the panel score on the color index. These analyses are shown in Table II. The order of magnitude of the variance about the regression for the A.O.C.S. photometric color is very much larger (24-fold) than

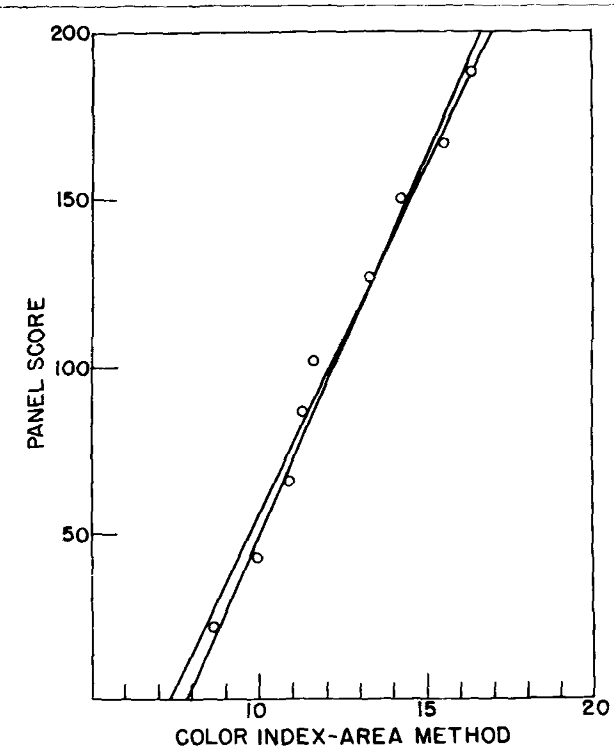


FIG. 3. Relation between panel scores and color indices-area method.

TABLE II
Analysis of Variance

| Source of variance | Character | | | |
|---|---|---|---|---|
| | Sums of squares | d | Variance | F |
| Panel score on photometric color | | | | |
| Due to regression | 14,900 | 1 | 14,900 | 9.32 |
| About regression | 11,178 | 7 | 1,599 | |
| Total | 26,078 | 8 | | |
| Panel score on color index | | | | |
| Due to regression | 25,593 | 1 | 25,593 | 39.4 |
| About regression | 485 | 7 | 654 | |
| Total | 26,078 | 8 | | |

the variance about the regression for the color index. Moreover the F value of 39 for 1 and 7 degrees of freedom indicates a very high order of significance for the regression of the panel score on the color index while the F value of 9.3 for 1 and 7 degrees of freedom for the regression of the panel score on the A.O.C.S. photometric color would imply that the odds are about 1 in 40 that the agreement between the A.O.C.S. photometric color and the panel score is owing to chance.

The sources of variance for the color indices were not all identified, but it may be noted from the data in Table II that the contribution to the variances by the experimental error is much greater for the A.O.C.S. photometric color than for the color index.

It may be seen from the absorption curves for refined and bleached cottonseed oils shown in Figure 4 that the absorption at 550 millimicrons is relatively insignificant in comparison with the absorption at lower wavelengths, yet the A.O.C.S. photometric method places high emphasis on the absorption at this wavelength. The absorption at 500 millimicrons is weighted by a 54-fold factor over the strong absorption at 460 millimicrons, as may be seen from the equation (1)

$$\text{Photometric color} = 1.29\ D_{460} + 69.7\ D_{550} + 41.2\ D_{620} - 56.4\ D_{670}$$

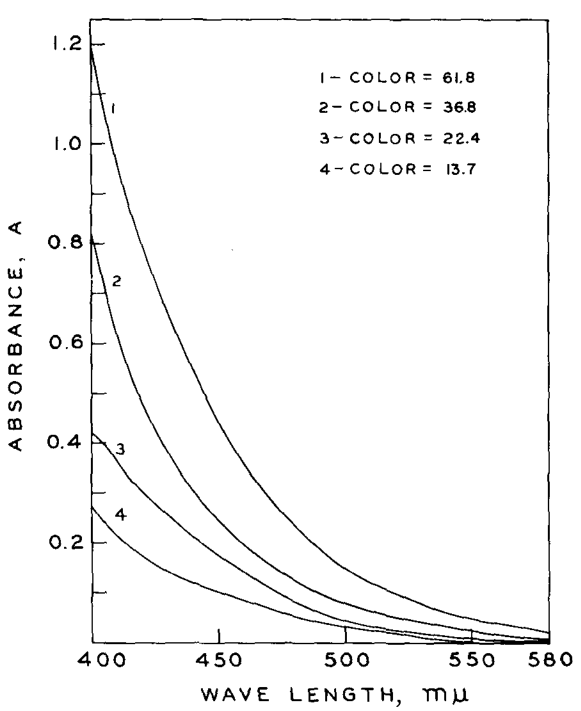


FIG. 4. Absorption curves for refined and bleached cottonseed oils.

which is specified in the A.O.C.S. procedure for calculating the color value.

Furthermore the coefficient for the absorbance at 670 millimicrons is negative in the equation specified by the A.O.C.S. photometric method. The extent to which the negative coefficient may distort the color value obtained by the A.O.C.S. photometric method is illustrated by the data recorded in Table III, where

TABLE III
Effect of Green Pigmentation on Apparent Oil Color

| Bleaching treatment | Adsorbent used | Oil color | |
|---|---|---|---|
| | | A.O.C.S. photometric | Color index |
| | % | | |
| Refined oil [a] | .. | 4.4 | 101 |
| Bleached with alumina [b] | 1 | 0.4 | 29 |
| Bleached with alumina [b] | 2 | 0.0 | 25 |
| Bleached with alumina [b] | 3 | 0.0 | 25 |
| Bleached with alumina [b] | 4 | 0.0 | 24 |
| Bleached with alumina [b] | 5 | 0.0 | 23 |
| Bleached with alumina + charcoal [c] | 1 | 1.3 | 19 |
| Bleached with alumina + charcoal [c] | 2 | 0.8 | 18 |
| Bleached with alumina + charcoal [c] | 3 | 0.9 | 16 |
| Bleached with alumina + charcoal [c] | 4 | 0.2 | 15 |
| Bleached with alumina + charcoal [c] | 5 | 0.6 | 15 |

[a] Contains green pigmentation, bleachable oil.
[b] No effect on green pigments, greenish visual color.
[c] Removes green pigments, yellow visual color.

the color values obtained for a cottonseed oil with a greenish color are tabulated. The color values obtained with the A.O.C.S. photometric method are zero for the oils bleached with 2,3,4, and 5 g. of alumina per 100 g. of oil, yet these oils were definitely colored, as may be seen from the color index values. Note that a positive value for the A.O.C.S. photometric color was obtained in each case when the green pigments were removed from the oils through the use of a small quantity of charcoal.

The precision of the color index measurement, as determined by 12 observations on each of a refined and a refined-and-bleached cottonseed oil, is compared in Table IV with the precision of the A.O.C.S. photometric method as determined by Pohle *et al.* (4). Included in the table are the precision data

TABLE IV
Precision of Three Methods for Estimating Color of Cottonseed Oil

| Method and statistic | Type of cottonseed oil | |
|---|---|---|
| | Refined | Bleached |
| Curve area color | | |
| Mean | 100.7 | 27.8 |
| Standard deviation | ± 0.68 | ± 0.67 |
| Coefficient of variation | 0.7% | 2.4% |
| A.O.C.S. photometric color (4) | | |
| Mean | 10.5 | 2.17 |
| Standard deviation | ± 1.30 | ± 0.23 |
| Coefficient of variation | 12.4% | 10.6% |
| Lovibond Red (4) | | |
| Mean | 10.9 | 2.34 |
| Standard deviation | ± 1.09 | ± 0.26 |
| Coefficient of variation | 10.0% | 11.1% |

for the Lovibond data, also determined by Pohle *et al.* The coefficient of variation for the color index method is very much less than these for the A.O.C.S. photometric and the Lovibond methods.

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