Fluorescence as a Measure of Brown Substances in Soybean Lecithin[1,2]

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AN EARLIER publication in this Journal (3) has reported that the color of soybean lecithin is due to carotenoids, brown substances, and, sometimes, small amounts of chlorophyll or its degradation products. The amount of the brown substances, which are believed to be largely aldehyde-amine reaction products, was shown to be increased by heating either the lecithin itself or the oil before the gums are removed. Once this brown color is formed, it persists in the lecithin through the later steps of processing. For quality and competitive reasons most manufacturers of soybean lecithin prefer to produce a light-colored product. Therefore development of a rapid method for measuring the amount of brown substances is desirable. Since fluorescence was noted for the brown substances from soybean lecithin (3) and since fluorescence was found to correlate with browning in egg lipides (1), it seemed probable that the fluorescence of a solution of soybean lecithin might also provide a simple and useful measure of the brown color present. This paper describes the measurement of brown substances and of fluorescence in a number of samples of soybean lecithin and shows that there is a linear correlation between the two quantities.

Experimental

Commercial lecithins from a number of producers were studied. Samples weighing 2.5 g. were dissolved in redistilled technical-grade carbon tetrachloride. Those solutions which were not clear were filtered through Hyflo Super-Cel[4] in a small Hirsch funnel. They were then made to 50 ml. with carbon tetrachloride (5% weight-volume). Spectral absorbance was measured, using a Cary recording spectrophotometer with 1-cm. cells.

The absorbance by brown substances increases steadily with decreasing wave-length, and absorbance by carotenoids has a maximum and minimum at 455 mμ and 365 mμ, respectively, as shown in the previous paper (3). The spectrophotometric measurement of the amount of brown substances is based upon the absorbance at two wave-lengths, one corresponding to the minimum in the carotenoid absorption curve, *i.e.*, at 365 mμ, and another corresponding to a maximum (455 mμ). The absorbance at each wave-length can be expressed by an equation as follows:

$$A^{455} = a_x^{455}\, C_x b + a_B^{455}\, C_B b \qquad (1)$$

$$A^{365} = a_x^{365}\, C_x b + a_B^{365}\, C_B b \qquad (2)$$

where x and B refer to carotenoid and brown pigment, respectively; the numbers refer to the wave-lengths in mμ; and A, a, C, and b have the usual meanings of absorbance, absorptivity, concentration, and cell length (2). Equations 1 and 2 can be solved for C_B, the concentration of brown color. Then, since cell length, b, is 1 cm., the absorbance of brown color at 365 mμ can be written:

$$A_B^{365} = C_B a_B^{365} = \frac{A^{365}\left(\frac{a_x^{455}}{a_x^{365}}\right) - A^{455}}{\left(\frac{a_x^{455}}{a_x^{365}}\right) - \left(\frac{a_B^{455}}{a_B^{365}}\right)} \qquad (3)$$

A value of 0.144 is taken for a_B^{455}/a_B^{365}, the ratio of absorbances for the brown substances at 455 and 365 mμ. This is an average value obtained from measurements of double-bleached lecithins in which all carotenoids had been destroyed. A value of 6.50 is taken for a_x^{455}/a_x^{365}, the corresponding ratio of absorbances of carotenoids. The determination of this ratio is complicated by the instability and the complexity of the carotenoid mixture. Various samples of isolated carotenoid pigments gave ratios differing greatly from one another. The value above is based upon the absorbance of a solution of lyophilized wet gums in carbon tetrachloride. They had been prepared in the laboratory from commercial miscella and were relatively low in brown substances. An estimated amount of absorbance due to brown color was subtracted from the experimental absorbance curve for the lyophilized wet gums to give a calculated absorbance curve and the ratio a_x^{455}/a_x^{365}.

Using these values, equation (3) becomes:

$$A_B^{365} = \frac{6.50\, A^{365} - A^{455}}{6.36} \qquad (4)$$

For fluorescence measurements 1 ml. of the 5% solution described above was diluted to 50 ml. with carbon tetrachloride. For concentrations of this level and lower it was found that the fluorometer reading was directly proportional to the lecithin concentration. The fluorescence was measured in a Coleman 12A electronic photofluorometer, using the B-1 and Pc-1 filters supplied for use in thiamine assay. The instrument was adjusted to read 100 with a standard solution of 0.3 mg. quinine sulfate per liter of 0.1 *N* sulfuric acid. In order to balance the instrument with this standard quinine solution it was necessary to reduce the incident light by covering 4 of the 7 holes in the screen supplied with the B-1 filter. The fluorescence due to the carbon tetrachloride, which was about 12, was subtracted from the total fluorescence to give the fluorescence due to the lecithin.

Results and Discussion

The fluorescence value of the 28 lecithin samples is seen in Figure 1 to increase with increasing absorbance. In this figure the absorbance at 365 mμ due to

[1] The term "lecithin" is used throughout this paper to refer to the mixtures of phosphatides, oil, etc., known in the trade as soybean lecithin. It does not refer to phosphatidylcholine.

[2] Presented at fall meeting of American Oil Chemists' Society, October 11-14, 1954, in Minneapolis, Minn.

[3] One of the branches of the Agricultural Research Service, U. S. Department of Agriculture.

[4] The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

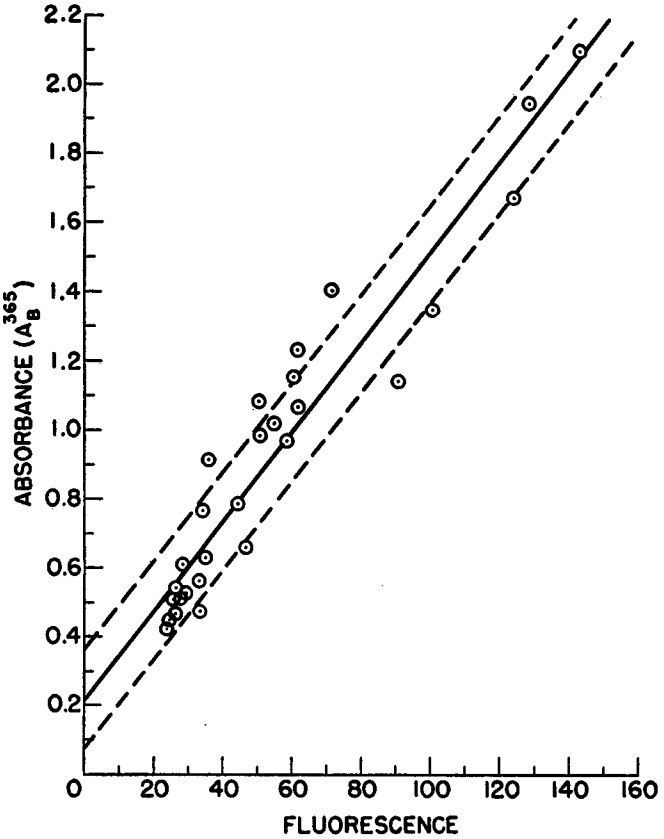

FIG. 1. Relation between fluorescence and absorbance due to brown substances in soybean lecithin.

brown color, A_B^{365}, calculated from measurements of the 5% solutions of lecithin in carbon tetrachloride in 1-cm. cells, is plotted against the fluorescence of 0.1% solutions measured as described above. A correlation coefficient is calculated at 0.95. The regression equation for the correlation line is:

$$A_B^{365} = 0.216 + 0.01313\,F \qquad (5)$$

where F (fluorescence) is the unit of scale reading. The standard error of estimate is 0.140. Lines indicating this standard error are shown in Figure 1 together with the line of the regression equation. This linear relation indicates that the fluorescence is mainly due to brown substances and that under the conditions used the fluorescence is little affected by such factors as self-absorption, absorption by carotenoids or chlorophyll and its degradation products, and fluorescence by constituents other than the brown substances.

Of the samples tested 9 were plastic lecithin and 19 were fluidized. Eleven were considered to be unbleached, 9 were single bleached, and 8 were double bleached. Inspection of the points on the curve indicated that neither source nor treatment had an effect on the behavior of the different types of samples. Two methods for measuring the extent of browning of soybean phosphatides are indicated by this work; one a spectrophotometric method requiring the measurement of absorbance at two wave-lengths and a calculation as prescribed by equation 4; the second a fluorophotometric method depending on the fluorescent property of browning reaction products. While the spectrophotometric method has been used successfully in the case of the data of Figure 1 in order to verify the expected linear relations between absorbance and fluorescence, it is believed that its use will be limited to research purposes. The instability, variability, and uncertainties of the absorption ratio for the carotenoid mixture introduces relatively large errors into corrections for carotenoid absorptions.

The determination of fluorescence is simple and rapid and appears to be uncomplicated by the presence of pigments and major components. In view of the linear relation between fluorescence and the calculated spectral absorbance, a measurement of fluorescence should prove a useful index of browning.

Summary

The fluorescence of soybean lecithin increases in a linear fashion with an increase in brown substances. Since the measurement of fluorescence is simple and rapid, this measurement should prove a useful index of browning.

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Direct Esterification of Phenols with Higher Fatty Acids[1,2]

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THE GENERALIZATION that phenols may not be directly esterified with fatty acids has long been incorporated in textbooks of organic chemistry. Thus Conant and Blatt (1) state that "the acyl derivatives (the esters) of phenols cannot be made by direct esterification. They are prepared by the use of acid anhydrides or acid chlorides." Similarly Hill and Kelly (2) have pointed out the following: "phenol reacts with an acid chloride or anhydride, but not with an acid, forming an ester." Ralston (3) in his exhaustive treatise on fatty acids does not mention a single instance where these compounds are used to esterify phenols directly.

The discovery of the direct esterification of phenols with fatty acids evolved from the attempt to devise

[1] Presented at the fall meeting of the American Oil Chemists' Society, Minneapolis, Minn., Oct. 11-13, 1954.
[2] Paper No. 155, Journal Series, Research Laboratories, General Mills Inc.