Sources of Color in Soybean "Lecithin"[1, 2]

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THE dark color of soybean "lecithin" is a characteristic which frequently affects the utilization of this product. In commercial practice this defect is partially counteracted by bleaching with hydrogen peroxide (17). The chemical nature of the lecithin pigments and their contribution to color seems never to have been adequately described, nor have the specific effects of bleaching and processing conditions. The study of these problems is the subject of the present paper.

In Figure 1 is plotted the absorbance of 5% solutions of lecithin in carbon tetrachloride. Curve 1 is from a commercially dried gum not treated with peroxide. Curves 2 and 3 are from commercial single bleached and double-bleached lecithins, respectively. Although these samples were not produced from identical wet gums, certain qualitative comparisons are valid. It is seen in Curve 1 that there is a large carotenoid-type absorption with peaks at 429, 454, and 483 mμ. In Curves 2 and 3 this absorption is seen to be largely destroyed by the action of peroxide. It is apparent that the carotenoid absorption is superimposed upon a general background absorption of a type which increases at shorter wavelengths. This analysis of absorption is similar to that found in ether extracts of dried eggs in which a carotenoid absorption is superimposed upon the general absorption due to brown aldehyde-amine reaction products (1). In addition to the color bodies just described there is a slight absorption maximum in Figure 1 at 670 mμ due to porphyrins. In certain other samples of commercial lecithins examined, this absorption maximum was absent.

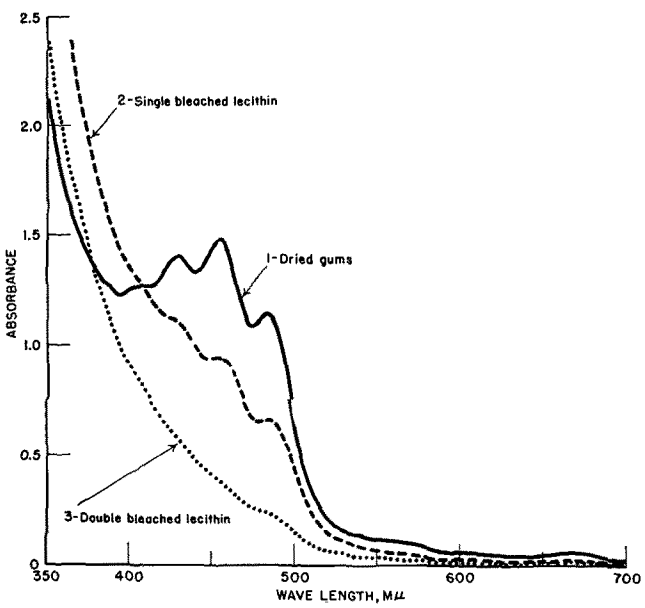

FIG. 1. Absorption spectra of lecithin solutions in CCl_4 (5 g./100 ml.) measured in 1-cm. cell in Cary recording spectrophotometer.

Carotenoid Pigments

Very little information is available concerning the identity of the carotenoids in soybean lecithin. Several workers reported that β-carotene was the principal carotenoid in soybeans (5, 9, 10). Nakamura and Tomita (7) have found lutein, taraxanthin, a fucoxanthin-like pigment, and an eloxanthin-like pigment, as well as several other carotenoids in the unsaponifiable fraction of soybean oil. Obata, Nukata, and Zama (8) stated that the yellow pigment from soy lecithin was mainly β-carotene.

To obtain a solution of carotenoid pigments, 20.1 g. of the dried gums, whose absorption is shown in Figure 1, Curve 1, were saponified and processed in a manner similar to that used by Dutton and Edwards for egg lipides (2). The ether solution of the pigments was evaporated at room temperature under a stream of nitrogen to a volume of 514 ml. and was left overnight in a glass-stoppered cylinder at 0°C. to allow some white insoluble material to settle out. The clear supernatant liquid was then decanted, the insoluble material was washed by decantation, and the residual ether removed by filtration. After concentrating the ether solution of the pigments under vacuum, it was made to 100 ml. in ether.

The absorption of these pigments in carbon tetrachloride is compared with that of the original gums in Figure 2. In this figure is also shown the absorption of the fatty acid fraction obtained from the saponification mixture and of material from the aqueous

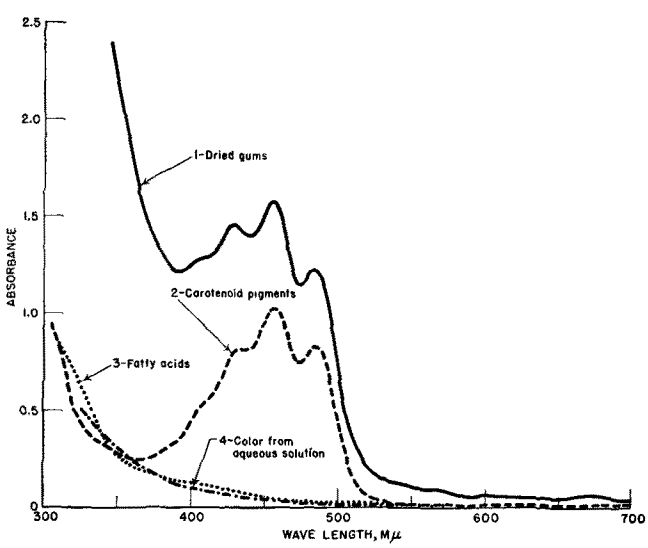

FIG. 2. Absorption spectra of dry gums compared with carotenoid pigments, fatty acids, and aqueous solution in Cary recording spectrophotometer. All samples are on basis of 5-g. gums/100 ml. solvent and 1-cm. cell length.

[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 phosphatidyl choline.

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solution. This latter material was obtained by neutralizing the solution after removal of the fatty acids and lyophilizing an aliquot. The salts remaining were extracted with alcohol to obtain the solution shown in the figure. Since the sum of Curves 2, 3, and 4 does not equal Curve 1 and since recovery of carotenoids is quantitative, it must be concluded that in part brown substances are destroyed by saponification.

No specific absorption coefficients for carotenoids in carbon tetrachloride solutions were found in the literature. However, by the use of the value of 255 for lutein in alcohol given by Zscheile (18), an approximate value of about 8 mg. of carotenoids per 100 g. of lecithin is obtained.

A 10-ml. aliquot of the carotenoid pigment solution described above was chromatographed on a 1-cm. I.D. magnesium oxide column (Westvaco 2642 Magnesia and Hyflo Super Cel 1:1[4]) using 1,2-dichloroethane for the developing solvent as described by Strain (12). This dichloroethane had been pretreated with MgO to remove acid (15) and redistilled. A poor separation of the pigments was obtained due to interference by other unsaponifiable materials in the solution. However this interfering material was removed by partitioning the residue from 40 ml. of the pigment solution between petroleum ether and 90% methanol, and extracting the petroleum ether an additional three times with 90% methanol. The pigments dissolved in 90% methanol were transferred back to ether by adding an equal volume of ether and water to the methanol solution. After the ether solution was washed free of residual methanol and dried over sodium sulfate, ether was removed by evaporation. The residue was dissolved in dichloroethane and chromatographed in four equal aliquots in 1-cm. MgO columns. Then the pigments separated into clearly defined bands as diagrammed in Figure 3.

[4] The mention of products does not imply endorsement or recommendation by the Department of Agriculture over other products of a similar nature not mentioned.

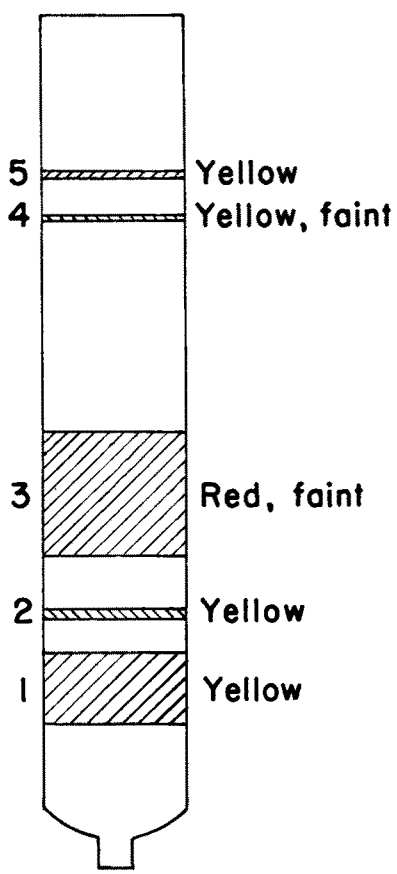

FIG. 3. Pattern of bands formed by chromatographing carotenoid pigments from lecithin on MgO with dichloroethane as solvent.

The pattern of bands is similar in appearance to that found by Strain for leaf xanthophylls (13, 14). In the leaf-xanthophyll mixture the pigment corresponding in position to band 1 is lutein. After being extracted from the column with ether, this pigment was therefore compared with lutein from spinach. Mixed chromatograms were run on MgO columns, using both dichloroethane and 1% ethanol in benzene as developing solvents. The two pigments behaved identically and formed a single band. Also the absorption spectrum of the pigment in ethanol is the same as that of spinach lutein.

The lutein found in band 1 makes up the greater part of the carotenoids. A chromatogram similar to that in Figure 3 was prepared, using 1% ethanol in benzene as the developing agent. The bands were removed from the MgO with ether. A comparison of the absorption spectrum of the solution from band 1 with that of the combined other bands indicated that the pigments are 75% lutein.

The other carotenoid pigments isolated from the column were obtained in amounts too small for identification. In leaf xanthophylls isolutein is found in a position similar to band 2. However the pigment from band 2 is not isolutein. Its absorption spectrum is displaced approximately 2.5 mμ toward the red from that of spinach isolutein. Also it does not give a blue color with hydrochloric acid (14) as does isolutein.

Both the position of band 3 on the column and its red color suggest that it is zeaxanthin. This pigment, as well as spinach zeaxanthin, seemed to be easily destroyed, and only small amounts were recovered from the column. When it was readsorbed, a single red band was formed together with a faint yellow band which moved ahead of it.

Bands 4 and 5 were extracted from MgO with ether and alcohol. Both gave blue colors with hydrochloric acid. On rechromatographing on magnesium oxide with dichloroethane as developing solvent, band 4 remained a single band, but band 5 separated into two bands. These unidentified bands, no doubt, include some of the pigments found by Nakamura (7).

β-Carotene was not found in the chromatographic investigation. However, because of its nutritional importance as a vitamin A precursor, its possible presence was also investigated by means of a countercurrent distribution. The carotenoid pigments from 25 ml. of the ether solution were distributed between 90% methanol and hexane, using a 24-tube distribution with 8 ml. of each solvent in each tube. From the data given by White and Zscheile (16) xanthophylls are very methanol-soluble and carotene very hexane-soluble in this solvent combination. Nearly all the color was found to be present in tubes 0 through 12 and thus to have partition coefficients characteristic of the xanthophylls. The upper layers of tubes 18 through 24, which contain the hexane-soluble carotene, were combined, evaporated, and made to 5 ml. in hexane. Based on the absorbance of this solution the gums contain not more than 0.05 mg. carotene per 100 g. of gums. A comparison of the density of this solu-

tion with that of the original pigment solution shows that the hexane-soluble fraction, presumably carotene, makes up only 0.5% of the total carotenoids. Thus the carotenoids in this sample differ completely from those reported by Obata (8).

In order to determine the fate of carotene upon degumming oil a sample of crude oil was saponified as described for lecithin above, and the pigments were submitted to a 24-tube countercurrent distribution. The solvent combination used in this experiment was hexane, 98% methanol, which according to the data of Curl (4) is most suitable for separation of carotene from dihydroxy carotenoids. The resulting fractions were diluted to 25 ml. with acetone and the absorbance read at 446 mμ, the absorption peak in this solvent mixture. Results are shown in Figure 4. In this

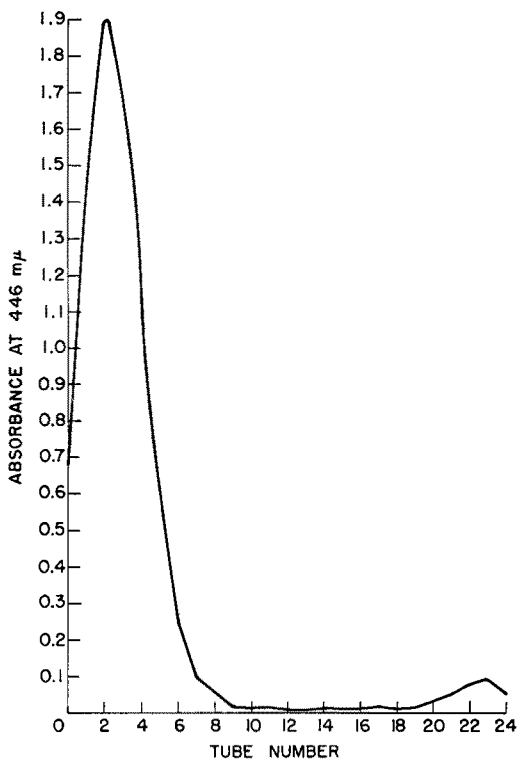

FIG. 4. Countercurrent distribution of carotenoid pigments from soybean oil between 98% methanol and hexane.

case 94.5% of the color is found in tubes 0 through 9, the xanthophyll region. Only 4.1% of the color is found in the carotene region in tubes 18 through 24. Thus the xanthophylls are also the predominant carotenoid pigments in the oil. However the relative amount of carotene is much greater in the oil than in lecithin. Thus it appears that, in the degumming of the crude oil, xanthophylls are selectively removed with the phosphatides.

Brown Pigments

It is well known that lecithin darkens on excessive heating, and it is to be expected that this is associated with the development of the brown color. As can be seen from Figure 1, a certain amount of this brown color is removed by bleaching but not to the extent that carotenoid color is removed. Figure 5 compares the absorption of the dried gums shown in Figure 1

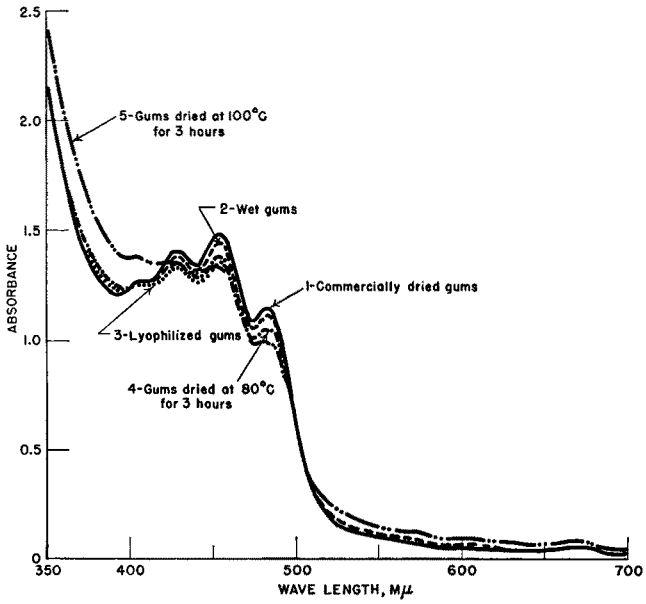

FIG. 5. Absorption spectra of wet gums compared with gums dried under different conditions. All samples contain 5 g./100 ml. CCl_4 on dry weight basis and are measured in Cary recording spectrophotometer (1-cm. cell).

with a sample of commercial wet gums, with a sample of the wet gums lyophilized from a benzene slurry, and with samples dried under vacuum at 80°C. and 100°C. for 3 hours. A large amount of brown color is already present in the commercial wet gums. This is not increased in the sample dried under commercial conditions or in the sample lyophilized or dried at 80° in the laboratory. However heating at 100° causes a further increase in color.

Light-colored and unheated gums were obtained from soybean oil miscella (20% oil in hexane by weight) by evaporating at room temperature under vacuum. The crude oil was stirred with 3% of water at room temperature and the gums were removed by centrifuging. A solution of the gums in carbon tetrachloride (5 g./100 ml. on a dry weight basis) was

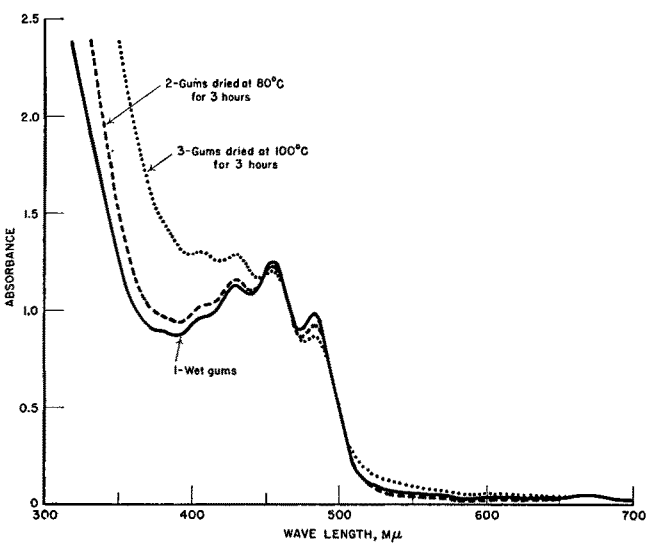

FIG. 6. Absorption spectra of gums produced in laboratory from 20% hexane miscella. Solutions in CCl_4 (5 g./100 ml.) are measured in Cary recording spectrophotometer (1-cm. cell).

very turbid but became clear on passage through a mixture of filter cell and sodium sulfate. The absorption of this solution is shown in Figure 6.

Also shown in Figure 6 are the absorptions of samples of these wet gums dried at 80° and 100°. The results agree with those of Figure 5 in that little additional color is produced in the lecithin by drying up to at least 80°; however heating at 100° causes additional darkening. The low absorption at the minimum of 390 mμ in Curve 1 compared to that of the comcercial wet gums shown in Figure 5, Curve 2, indicates that in the latter sample a large amount of brown color has already been formed before the gums are removed from the oil. This color is probably formed in the oil during the solvent stripping operation.

The brown color bodies of soybean lecithin have many of the characteristics of aldehyde-amine reaction products, such as are formed in a variety of food products. The absorption curve for the brown color is of the same shape as that found, for example, in egg lipides (1). As seen in Figure 2, a large part of the brown color is lost during saponification; however, if the curve for the aqueous solution is extended into the ultraviolet, a slight maximum at 270 mμ typical of an aldehyde-amine reaction product is found. The fluorescence of the lecithin under ultraviolet light, which increases with increasing brown color (11), is also typical of an aldehyde-amine reaction product.

The probable source of the amino group is the ethanolamine of the phosphatides. Sugars might be the source of the aldehyde group. However a sample of lecithin from which free sugars had been removed by Folch's (3) procedure darkened on heating in the same way as ordinary lecithin. Other sources of aldehyde may be the bound sugars remaining with the phosphatides, or possibly the acetal phosphatides recently reported by Lovern (6) to be present in soybean lecithin. It was also considered that the development of aldehyde amine brown color might be associated with the presence of unsaturated fatty acids in the lecithin with the oxidized acids furnishing aldehyde groups. A brown color might also be formed by a reaction similar to the yellowing of drying oils. However a sample of lecithin hydrogenated from an iodine value of 95 to 16 increased in color on heating even more rapidly than other samples.

Summary

The color of soybean lecithin is due to carotenoids, brown pigments, and occasionally porphyrins. In the water-washing of crude oil xanthophylls are preferentially removed with the gums, and carotene is practically absent in lecithin. Lutein is the principal carotenoid, comprising about three-quarters of the carotenoids in lecithin. Hydrogen peroxide bleaching destroys all the color to some extent, but by far the greater effect is on the carotenoids.

The brown color is very likely an aldehyde amine reaction product. It is largely formed by heating of the oil during the solvent-stripping operation. It is not increased by drying the gums under vacuum for 3 hours at 80°C. but it is increased on heating at 100° C. under the same conditions. The formation of the brown color is not prevented by removal of free sugars or by hydrogenation of the lecithin.

Acknowledgment

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REFERENCES

1. Dutton, H. J., and Edwards, B. G., Ind. Eng. Chem., Anal. Ed., *18*, 38 (1946).
2. Dutton, H. J., and Edwards, B. G., Ind. Eng. Chem., *37*, 1123 (1945).
3. Folch, J., Ascoli, I., Lees, M., Meath, J. A., and LeBaron, F. N., J. Biol. Chem., *191*, 833 (1951).
4. Curl, A. L., J. Ag. and Food Chem., *1*, 456 (1953).
5. Lantzing, J. C., and Van Veen, A. G., Geneeskund. Tijdschr. Nederland.-Indië, *77*, 2777 (1937); C.A. *32*, 1763 (1938).
6. Lovern, J. A., Nature. *169*, 969 (1952).
7. Nakamura, M., and Tomita, S., J. Soc. Chem. Ind. Japan, *43*, 246B, 249B, 267B, 268B (1940); C.A. *35*, 1655, 1656 (1941).
8. Obata, Y., Nukata, K., and Zama, K., J. Agr. Chem. Soc. Japan, *24*, 29 (1950); C.A. *46*, 11295 (1952).
9. Sherman, W. C., and Salmon, W. D., Food Research, *4*, 371 (1939).
10. Sherman, W. C., Food Research, *5*, 13 (1940).
11. Scholfield, C. R., and Dutton, H. J., in preparation for J. Am. Oil Chemists' Soc.
12. Strain, H. H., "Chromatographic Adsorption Analysis," p. 68, New York, Interscience Publishers Inc., 1942.
13. Strain, H. H., *ibid.*, p. 144.
14. Strain, H. H., "Leaf Xanthophylls," Carnegie Institute of Washington Pub. 490, 147 pages, 1938.
15. Strain, H. H., J. Am. Chem. Soc., *70*, 1672 (1948).
16. White, J. W. Jr., and Zscheile, F. P., J. Am. Chem. Soc., *64*, 1440 (1942).
17. Wittcoff, H., "The Phosphatides," p. 495, New York, Reinhold Publishing Corp., 1951.
18. Zscheile, F. P., White, J. W. Jr., Beadle, B. W., and Roach, J. R., Plant Physiol., *17*, 331 (1942).

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The Application of Fatty Chemicals to Flotation

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THE fatty acids and their derivatives are versatile tools of modern industry. They present such a wide variety of applications that few industries do not utilize them directly or indirectly. One has only to mention the soap, paint, petroleum, and rubber industries to bring to mind a few of their well-known uses. However, in addition to such familiar applications of fatty acids, there are a number which are equally vital and important but which are less well known to those engaged in fatty acid production. Among these is the art of flotation.

The Flotation Process

Currently, the major industrial application (the one which we are describing) is in the concentration of the valuable minerals in ore deposits. Here, flotation refers specifically to the art and science of separating mineral particles from each other in a liquid pulp by means of air bubbles. This basic principle of the flotation process is simple, but its application involves numerous complexities.

How does one go about floating minerals that normally sink in water? This is done in a water medium