The Stability of Vegetable Oils

I. The Spectral Transmittance of Soybean Oils

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IN THE course of an investigation of the stability of soybean oil by the methylene blue method[2] it became imperative to obtain information relative to the absorption spectra of these oils in order to arrive at an understanding of the mechanism and energy relations of the oil-methylene blue system. A search of the literature revealed that although a considerable number of cottonseed oils have been examined with respect to their spectral transmittance and colorimetric properties, very little attention has been accorded soybean oil from this point of view.

Priest (1) examined two refined soybean oils and published the spectral-transmissive curve for one of them with the notation that "the two samples differ but slightly." Wesson (2) reported data for a soybean oil which indicated that although the oil was qualitatively similar to those examined by Priest, it was quite dissimilar quantitatively with respect to its spectral transmittance. McNichols (3) published data covering five soybean oils which apparently included the two oils previously examined by Priest and the one examined by Wesson (2). Except for the statement by Priest (1) that the soybean oils which he examined were refined, no information is available concerning the methods of processing, chemical composition, and origin of the oils. Nevertheless, it was concluded by both Priest and McNicholas that the spectral-transmissive properties of soybean oil are essentially the same as those of cottonseed oil. However, the results of the present examination of soybean oils representing a number of varieties, sources, and processing treatments indicate that all of these factors influence, in some cases very markedly, the spectral-transmissive properties of these oils. For the most part, these properties have been found to be dissimilar in certain respects to those observed by previous workers.

It may be surmised from the date of the work of Priest (1920) and Wesson (1922) that the oils which they examined were of Manchurian origin, produced by hydraulic or wedge pressing, and that, if alkali-refined the operation was carried out by the batch or kettle process. These assumptions are made in view of the facts that little oil from American-grown soybeans was available at that time, that Manchurian oil was then, and still is, produced principally by hydraulic pressing, and that the present centrifugal method of alkali-refining had not been developed. As will become evident later, the divergences in the spectral-transmissive properties of the soybean oils reported here, as compared with those reported by previous workers, can be readily understood in the light of the effect on these properties of differences in methods of processing. Eighty-four oils were examined; the spectral-transmissive curves of 35 representative samples are reproduced and discussed in this communication.

Apparatus and Method

Examination of the oils was made visually by a single observer using a Schmidt-Haensch spectrophotometer of the Lummer-Brodhun type which is constructed with the optical system shown in Figure 1. The photometric field at C was observed through the eye-piece E and was balanced by adjusting the width of the slit at B. The eye-piece and its collimator were pivoted to permit rotation and consequent change of color of the field. A spectral range of 15 to 20 Å was admitted by the eye-piece at one setting. Observations were made as frequently as the character of the oil warranted, generally at intervals of 5 mμ. The coarse scale and vernier at S were calibrated with respect to wave length on an average of twice a week by means of a mercury arc. Two 40-watt tungsten filament bulbs at A and B were used as light sources.

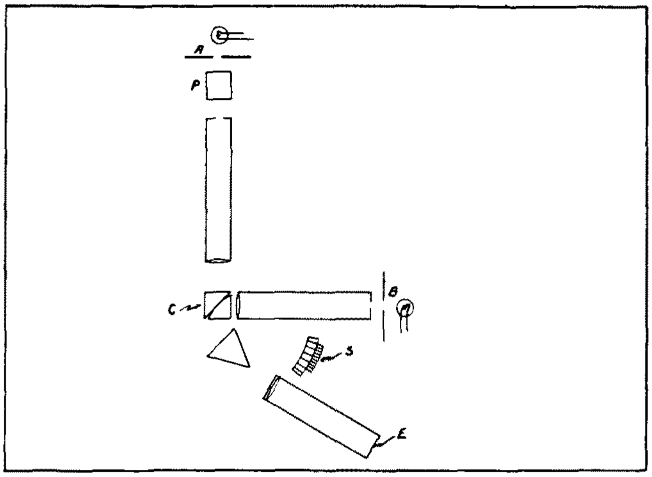

FIG. 1

The cell shown at P was made of pyrex glass and had a width corresponding to an oil thickness of 1 inch[3] between faces. In order to obtain as nearly as possible the same loss of light by reflection at the glass-oil interfaces, glycerol was used as the standardizing medium for 100 percent transmission. The ratio of the width of the slit at B with oil in the cell, compared to its width with glycerol in the cell, is designated as the transmittance of the oil for the wave length corresponding to a given setting of the scale.

Measurements were most difficult to make in the violet end of the spectrum (420 to 440 mμ) and in the extreme red (700 to 720 mμ). The experimental error in these regions was of the order of 1 part in 30. From 440 to 700 mμ the settings were quite easily made with an experimental error of less than 1 part in 100.

Crude Soybean Oils

The spectral-transmissive properties of 19 crude soybean oils were examined in the apparatus by the method previously described, and the curves representing the data thus obtained for 13 of them are reproduced here. For purposes of comparison 1 hydraulic-pressed oil and 5 expeller-pressed oils are reproduced in Figure 2, while

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[1] A cooperative organization participated in by the Bureaus of Agricultural Chemistry and Engineering and Plant Industry of the U. S. Department of Agriculture and the Agricultural Experiment Stations of the North Central States of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin.

[2] The results of an investigation of the methylene blue-oil system will appear shortly.

[3] McNicholas used a cell thickness of 5¼ inches for purposes of comparison with the official Lovibond color values.

similar curves for 5 solvent-extracted oils are reproduced in Figure 3. Examination of these curves indicates that all of the pressed oils (Figure 2), although differing appreciably in some respects, are similar in exhibiting well-defined absorption maxima in the region of 675 mμ. All but 1 of these oils (Curve 6, Figure 2) exhibit a less intense absorption maximum at about 610 mμ. These maxima correspond to the principal orange-red absorption bands exhibited by chlorophyll *a* derived from leaves and green tissues in general (6). Because of the broad width of the violet absorption bands of chlorophyll and carotenoids, coupled with the possibility that both may be present in large quantities in certain oils, it was not possible to distinguish between chlorophylls *a* and *b*, and the carotenoids in this region. The onsets of absorptions of the various pigments are so close to one another in the violet region that the differences between them are not greater than the shift in the absorption bands resulting from their solution in the oil. Therefore, the fact that crude oils are opaque in the region below 500 mμ does not afford a clue to the identity of the pigments which are present.

It might be noted at this point that McNicholas (4) stated in connection with the oils which he examined that: "The most significant difference herein exhibited between cottonseed oils and the rape, *soybean*, sesame, and olive oils is the relatively higher transmittance of the latter group near 580 mμ as compared with that near 630 mμ." In the crude soybean oils reported here, as well as others examined by the authors, high transmittance was observed in the region of 580 mμ, but the transmittance in the region of 630 to 640 mμ was almost invariably higher.

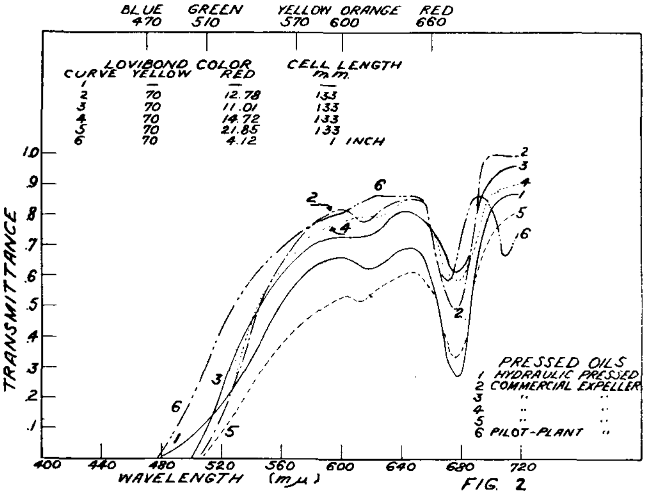

FIG. 2

The crude soybean oil represented by Curve 6, Figure 2, was produced in a half commercial-size or pilot-plant expeller, and it is to be noted that it differs from all the other pressed oils which were examined by the absence of a maximum absorption in the region of 610 mμ and the presence of a third, and rather strong, absorption maximum in the extreme red region at 710 mμ. A similar maximum was observed in the case of two commercial, solvent-extracted oils (Curves 11 and 31). The absorption band at 710 mμ for these oils was readily observable and cannot be attributed to experimental error. To the knowledge of the authors, its presence has never been reported for any vegetable oil, and it is not known to what substance such an absorption can be attributed.

Curve 1, Figure 2, which was obtained with an oil produced by hydraulic pressing, exhibits two quite clearly defined chlorophyll absorption bands, and it is almost identical with those reported by McNicholas for the soybean oils which he examined. This similarity in spectral-transmissivity strengthens the assumption that McNicholas worked with soybean oils produced by hydraulic pressing.

Unlike the pressed oils, the solvent-extracted oils exhibit considerable differences in spectral-transmissivity both in comparison with one another and in comparison with pressed oils generally. The sharp absorption maxima in the region of 670 mμ observed in the pressed oils are either shifted or poorly defined, and may even be entirely absent (Curves 7 and 9) in the solvent-extracted oils. A sharp absorption maximum at 610 mμ is observed in only one solvent-extracted oil (Curve 8) which was derived from a wild variety of soybean (7). Curve 11 was obtained with a solvent-extracted, commercial crude oil and is unusual in exhibiting five definite absorption maxima at about 535, 575, 625, 680, and 710 mμ. In contrast to this sample, oils represented by Curves 7 and 9, which represent laboratory-scale extracted crudes, show no definite absorption maxima throughout the entire spectral range.

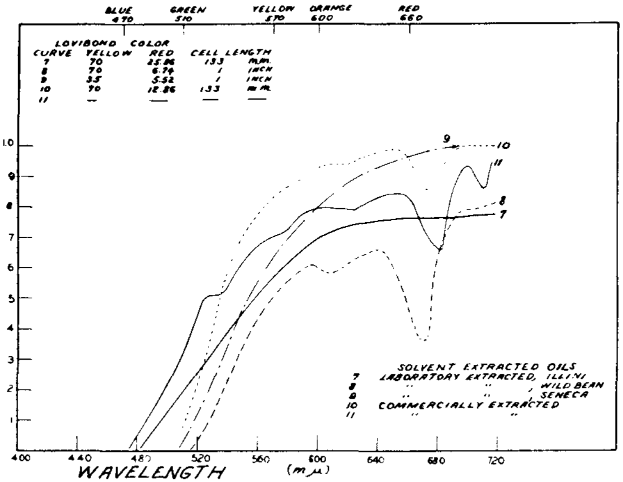

FIG. 3

Effect of Refining Treatments on the Spectral Transmittance of Soybean Oils

Preliminary observations indicated that laboratory refining operations exerted a marked effect on the spectral-transmissive properties of soybean oil, and it seemed desirable to determine the manner in which these properties varied under conditions of commercial operation. In order to follow the effect of the various refining operations on the spectral-transmissive properties of soybean oil, it was essential that the same batch of oil be followed through the refinery by means of samples withdrawn at each stage of the process. A number of processors supplied samples representing the various steps in their refining process.

It was found from an examination of the samples submitted that most processing practices pertaining to the production of edible oil may be divided into four general groups insofar as these practices affect the spectral-transmissive properties of the oil. These four groups are illustrated by the curves reproduced in Figures 4, 5, 6, and 7, which are characteristic of processors A, B, C, and D. Each of these groups of curves will be discussed separately so far as their specific characteristics are concerned, and this discussion will be followed by comments relative to the characteristics common to all of them. It should be borne in mind throughout this discussion that oil refinery practices

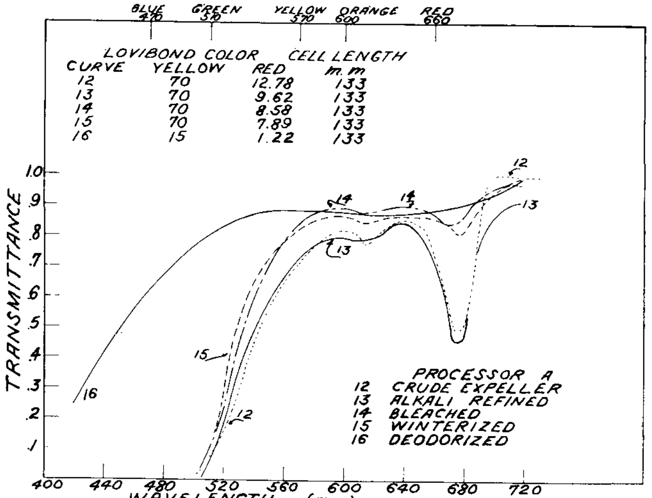

FIG. 4

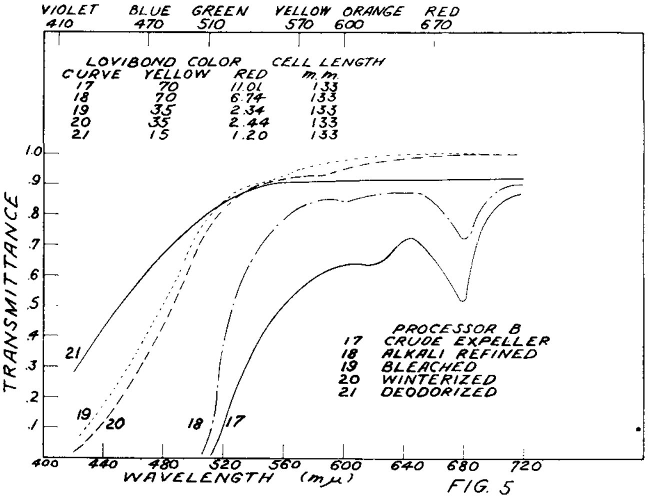

FIG. 5

are not invariable but are subject to change from time to time, and consequently the oils examined here merely reflect the refinery practice in vogue at the time these particular samples were withdrawn.

Processor A. Curves 12 to 16, inclusive, Figure 4, were obtained with soybean oils representing the various processing steps from crude to completely refined product as carried out by Processor A. Curve 12 represents an expeller-produced crude soybean oil and Curve 13 the same oil after alkali refining by the centrifugal process. Except for the slightly greater transmittance of the crude oil in the region above 690 mμ, these two curves are nearly identical, indicating that the treatment with alkali has removed very little pigment. This observation is quite contrary to the evidence afforded by the Lovibond color readings which might be presumed to indicate a considerable removal of red pigment.

Examination of Curve 14, representing the oil after bleaching, reveals the fact that the two chlorophyll bands at 670 and 610 mμ are still discernible, but very weak, indicating the nearly complete removal of this pigment during the bleaching operation. However, very little carotenoid pigment has been removed as is evident from the general opacity of the oil below 500 mμ. Curve 15 is, in general, similar to Curve 14, indicating that winterization has removed little or no coloring matter from the oil. This result is to be expected in view of the manner in which this process is normally carried out.

Curve 16 which represents the oil after deodorization is quite unlike those from any previous stage of the processing. No evidence of the presence of chlorophyll can be observed, and the greatly increased transmittance below 500 mμ indicates that extensive removal or destruction of the carotenoids has occurred during deodorization. The change in Lovibond color from 70Y/7.89 R to 15Y/1.22 R is compatible with the change exhibited in the spectral-transmissive properties as measured spectrophotometrically.

Processor B. Curves 17 to 21, inclusive (Figure 5), were obtained with a series of oils covering all operating stages from the crude to the completely refined product as carried out by Processor B. The processing steps are the same in both order and number as those of Processor A, and the color finally obtained in the finished product, whether measured spectrophotometrically or in Lovibond units, is the same; nevertheless, the individual operations were apparently performed quite differently as is evident from an inspection of the curves corresponding to each stage of refining.

Curve 17 was obtained with expeller-pressed crude oil and Curve 18 with the same oil after alkali refining by the centrifugal method. The refining operation in this instance has resulted in a considerably higher transmittance, especially in the green-red region between 520 and 690 mμ (Curve 18) following the removal or destruction of the chlorophyll and some other pigment or group of pigments which McNicholas (5) refers to as the "brown group" as distinguished from the yellow or carotenoid group of pigments. It should be noted that despite the lower Lovibond color value of the crude oil, 70Y/11.01 R, in comparison to the crude oil of Processor A, 70Y/12.78 R, the former oil exhibits a much lower spectral transmittance and, hence, contains a greater proportion of natural pigment.

Curve 19 represents the oil after bleaching, during which operation the last trace of the chlorophyll appears to have been removed, as well as considerable carotenoid pigment, as is indicated by the general increase in transmittance extending into the violet region at 420 mμ. Winterization, as has been seen previously, has little effect on the color of the oil, which fact is again evident from the similarity of Curves 19 and 20.

Curve 21, which represents the oil after deodorization, is characterized by increased spectral transmittance in the blue-green region between 420 and 510 mμ, and a decreased transmittance in the yellow-red region between 560 and 720 mμ. This change in transmissive properties results from a further destruction or removal of carotenoids on the one hand, and the formation of colored degradation products on the other, during the prolonged treatment at the high temperature obtaining in the deodorizer.

Processor C. Curves 22 to 25, Figure 6, were obtained with a series of soybean oils refined according to the procedure followed by Processor C, who, it is to be noted, does not winterize the oil at any stage. Alkali refining (Curve 23) by the centrifugal method results in a slight increase in spectral transmittance throughout the green-red region between 500 and 720 mμ, corresponding to a partial removal of green and brown pigments. The extent of the removal of the natural pigments during alkali refining as carried out by Processor C, is intermediate between that of Processor A who removes practically no pigment at this stage and that of Processor B who removes a considerable portion of the total pigment at this stage.

Bleaching (Curve 24) as practiced by Processor C results in the nearly complete removal of the chlorophyll and practically all of the brown pigments, as well as a considerable amount of the carotenoids, as is indicated by the greater transmittance in the blue-green

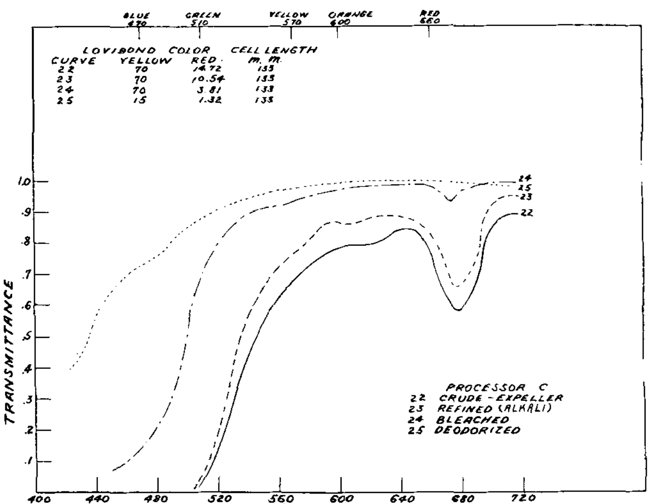

FIG. 6

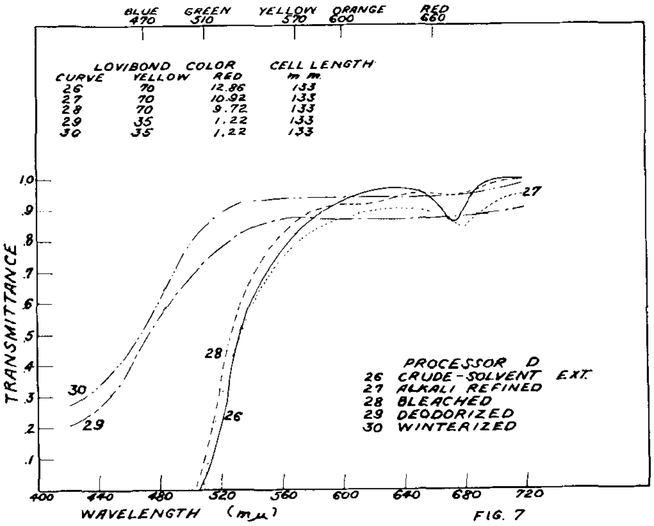

FIG. 7

region between 450 and 520 mμ. The removal of additional color during bleaching by Processor C is again intermediate between that accomplished by Processor A who removes but little pigment by this operation, and that of Processor B who removes the major portion of these materials at this stage. As in the case with Processor A, extensive destruction of the carotenoids and the complete removal of all chlorophyll occurs during deodorization as is indicated by Curve 25. This oil, it is to be noted, is more transmittant in the blue-violet region than any of the other oils examined, and the inflection of curve in the region of 480 mμ probably corresponds to one of the characteristic maxima of a- and β-carotenes which occur in this region.

Processor D. Curves 26 to 30, Figure 7, represent a group of oils processed somewhat differently from those previously discussed. First, it should be noted that the crude oil in this case was produced by solvent extraction, and despite the fact that the Lovibond color reading is the highest, 70Y/12.86 R, of all the crude oils of this group, it is spectrophotometrically the least absorbent throughout the spectral range from 500 to 720 mμ. From about 565 mμ well into the red region, the crude oil is from 80 to 100 percent transmittant. No trace of the chlorophyll band at 610 mμ is observable, and only a slight absorption is evident in the region of 670 mμ.

The second difference in processing between this series and those previously examined is the reversal of the winterization and deodorization steps, the former of which represents the last step in the processing.

Despite the apparent improvement in color as indicated by the change in the Lovibond color values, alkali refining appears to have resulted in slight darkening of the oil and a slight shift of the absorption band in the region of 670 mμ (Curve 27). Bleaching (Curve 28) removed practically all of the chlorophyll and resulted in a slightly greater transmittance throughout the green to red region.

Deodorization (Curve 29), as in all the previously examined oils, resulted in considerable destruction or removal of yellow carotenoid pigments which is evident from the marked increase in transmittance in the region 420 to 500 mμ. The increased transmittance in the blue-green region is partly offset by a decreased transmittance in the yellow-red region resulting from the formation of colored degradation products during deodorization.

Winterization (Curve 30) resulted in the further removal of pigmented substances by virtue of the absorbing power of the small amount of earth used in the filter press by the processor.

The spectral transmittance curves of all of the completely refined soybean oils which have been examined are characterized by high transmittance, namely, 90 to 100 percent, in the region above 550 mμ and a more or less rapid decrease in transmittance in the blue-green region below this value. So far as the spectrophotometric evidence is concerned, chlorophyll appears to have been completely removed, together with most of the brown group pigments. The absorption in the blue-green region, which is responsible for the light yellow color of these oils, is owing principally to the incompletely removed carotenoids.

The similarity of the spectral transmittance of the finished oils would seem to indicate that, given the same or relatively the same crude oil, different processors using the same general refining technique obtain, although in different stages, a completely refined oil of the same general color quality. It would appear, therefore, that the method of attaining the final result is immaterial to the oil processor, and this observation is true so far as the color quality is concerned. However, it has been determined that, although the final color quality may be the same, irrespective of the method of attaining it, a similar statement can not be made regarding the organoleptic quality of the finished oil. Each of the foregoing methods of refining leads to finished oils of different stability as determined by the methylene blue method. This subject will be discussed in a subsequent paper of this series. For the present it will suffice to say that the stability of the four final oils, which were received and examined within 24 to 48 hours after processing, ranged from very slight to excellent. It may also be stated that the variable stability of these oils can scarcely be related to the presence of chlorophyll as has been predicated by Coe (8), since spectrophotometric evidence indicates this substance to be absent from all completely refined oils which have been examined. Actually, the crude oils containing the maximum content of chlorophyll are exceedingly stable with respect to rancidification and become more unstable as the chlorophyll is progressively removed.

Spectral Transmittance of Soybean Oils as Affected by Laboratory Refining Processes

In Figure 8 there are reproduced a series of four curves, Nos. 31 to 34, inclusive, which represent an extracted oil before and after laboratory refining by one of the processors. The crude oil (Curve 31) is from

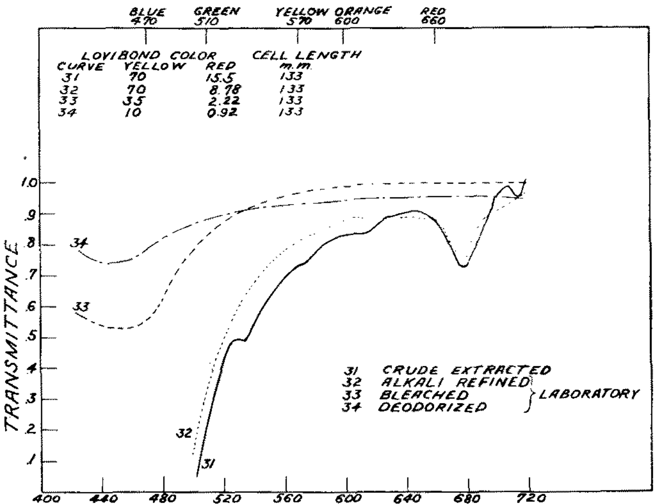

FIG. 8

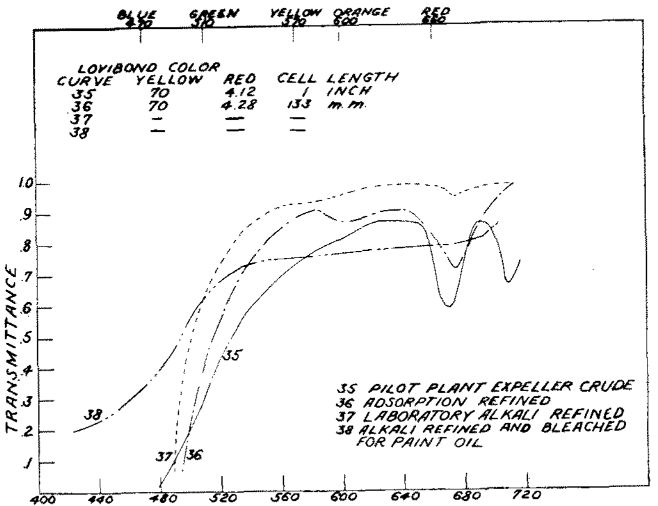

FIG. 9

the same processor as that represented by Curve 11, and shows the same absorption maxima at 535, 575, and 710 mμ in addition to those normally observed between 610 and 620mμ, and between 670 and 680 mμ. Following alkali refining (Curve 32), all of the absorption maxima except the band at 680 mμ have disappeared, but otherwise little effect on the absorption properties is evident despite the very marked reduction in Lovibond red reading from 15.5 to 8.78. Bleaching resulted in the removal of practically all of the color substances which were responsible for the absorption in the yellow to far red region (550 to 720 mμ), and also considerable carotenoid pigment as is indicated by the greater transmittance in the blue region. Deodorization, as was observed in the commercially processed oils, resulted in additional destruction or removal of carotenoid pigments with the simultaneous formation of pigmented degradation products which decreased the transmittance in the yellow-red region. To the unaided eye this oil appeared to be nearly water-white when viewed by transmitted light in a 2-inch thick layer, and it had an extremely low Lovibond reading, 10Y/0.92 R, in a 133 mm. cell.

In Figure 9, Curve 35 is identical with Curve 6 of Figure 2, and represents a pilot plant, expeller-pressed, crude oil which is characterized by the unsual absorption at 710 mμ. Curve 36 was obtained with the oil after absorption refining with hydrated aluminum silicate according to the method of Kraybill (9). Comparison of these two curves with those of Processors B and C (Curves 17 to 18, and Curves 22 to 23) indicates that the extent of the removal of color by absorption refining is comparable to that obtained in commercial alkali refining.

The same oil when refined by the official A.O.C.S. refining loss method gave Curve 37. The latter curve approaches in appearance that of a completely refined oil and indicates that the alkali refining has been exceedingly drastic and has resulted in considerable destruction of the natural pigments, and no doubt, of the natural antioxidant. The truth of the latter assumption has been repeatedly verified by the observation that soybean oils when refined according to the official refining loss procedure, followed by bleaching and deodorization, result in products of little or no stability as measured by the methylene blue method.

Curve 38 was obtained with a commercially refined soybean oil produced and sold for use in the drying oil industry. Since oils of this type are not ordinarily deodorized, the process of alkali refining and bleaching is carried out so as to produce as light an oil as possible, without regard to the effect on the stability of the product. In fact, the complete removal, rather than the preservation, of the natural antioxidant is desirable in order to improve the drying and film-forming properties of the oil.

Comparison of Normal and So-Called "Green" Soybean Oils

Early in the 1939-40 crushing season there were received at the mills soybeans whose seedcoats and a considerable portion, if not the whole, of the cotyledons were colored green. The oil produced from these beans possessed a green color which could not be exactly matched in the Lovibond color system with the use of red and yellow glasses. It is probable that a better match could have been obtained by the use of a red absorbing filter (a blue glass for instance) in connection with the usual red and yellow Lovibond glasses.

Two expeller-pressed soybean oils produced at the same mill from soybeans representing 1938 and 1939 crops were examined spectrophotometrically and were found to have the spectral-transmissive properties shown in Figure 10, Curves 39 and 41, respectively. The considerably greater concentration of chlorophyll in the 1939 oil compared with the 1938 oil is evident from the decreased transmittance in the region of 675 and 620 mμ. In the 1939 oil only about 3 percent of light is transmitted in the region of 675 mμ compared

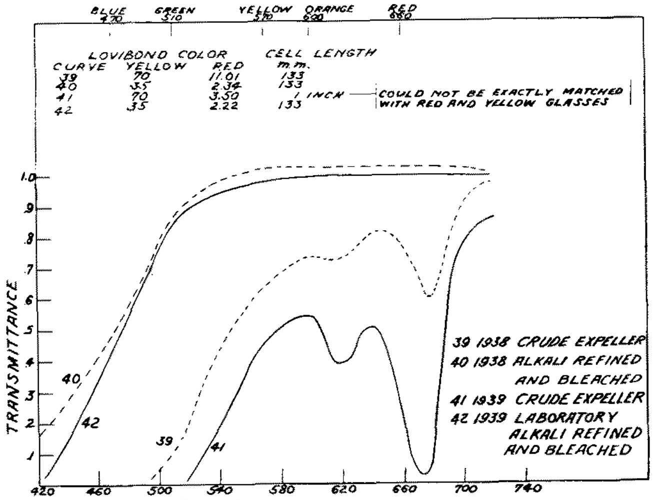

FIG. 10

to 60 percent in the 1938 oil. It is also to be noted that transmittance in the 1939 oil is cut off at about 520 mμ compared with 490 mμ for the 1938 oil. It is evident that the 1939 oil actually contained more red, as well as considerably more green, pigment despite the low Lovibond red reading.

Both oils when refined and bleached yielded products of approximately the same spectral-transmissive properties (Curves 40 and 42).

In order to demonstrate the masking effect produced by chlorophyll on the Lovibond color reading of an oil, a small amount of chlorophyll was added to a normal soybean oil whose Lovibond color reading was 70 yellow and 4.56 red. With the same yellow glass, the Lovibond red color reading was reduced to 3.46. When twice as much chlorophyll was added, the red color was further reduced to 1.63. This observation clearly indicates the fact that green oils in general will give abnormally low red values when the official Lovibond color method is used.

Acknowledgments

The authors wish to express their most sincere thanks and appreciation to the individuals and organizations who cooperated in furnishing samples of oils for this investigation. They also wish to thank Professors F. W. Loomis and G. M. Almy of the Department of Physics, University of Illinois, for their advice and the use of their spectrophotometric equipment; and to Mr. P. Krauczunas of the Soybean Laboratory for the Lovibond color readings reported in this communication.

Summary

Eighty-four samples of soybean oils, representing various types of processing and different refining procedures, have been examined spectrophotometrically and the spectral-transmissive curves for 35 of them are reproduced in this communication. The spectral-transmissive properties of these oils have been discussed with particular reference to the effect which variations in processing have on these properties.

In the light of the results obtained in these studies it is apparent that: (1) The spectral-transmissive properties of soybean oils are influenced by the method used in removing the oil from the bean as well as by subsequent processing procedures; (2) the Lovibond color readings give at best only approximate information concerning the spectral color of an oil; and (3) the carotenoids are removed during refining operations, the greatest loss usually occurring during the deodorizing treatment. Chlorophyll, which is present in various concentrations in the crude oil, is completely absent from the finished oil so far as spectrophotometric evidence is concerned.

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(5) McNicholas, H. J. Idem, 103.

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Report of Soap Stock Committee-1939-40

DURING the past year the committee worked on a method for determining neutral fat in soap stock. Methods in use in several laboratories as well as published methods were reviewed. A modification of the Wesson method was approved for study by the committee.

Proposed Method for Neutral Fat in Soap Stock

Weigh out from a weighing bottle 8-10 grams of a well mixed sample and transfer to an extraction cylinder. Dissolve in 125 ml. of 50% alcohol, made up from redistilled alcohol. Add 50 ml. of petroleum ether (see specifications) and shake until a homogeneous solution is formed. Cool to 20-25°C. and add 10 ml. of a 14% solution of potassium hydroxide and shake until thoroughly mixed (avoid violent shaking). Add 25 ml. of 50% alcohol and shake until thoroughly mixed. Allow to settle until two layers are formed. Do not allow the petroleum ether solution of oil to remain in contact with the alcohol-alkali layer for longer than 30 minutes. Draw off the petroleum ether layer as closely as possible with a glass siphon into a 500 ml. separatory funnel. Make at least four more extractions, using 50 ml. of petroleum ether for each extraction.

Wash the combined extracts in a separatory funnel with three 25 ml. portions of 10% alcohol, or until neutral to phenolphtalein. Shake vigorously each time. Transfer the petroleum ether extract to a tared Soxhlet flask. Evaporate off the petroleum ether and heat in an oven at 105°C. to constant weight. Weigh as neutral oil and unsaponifiable matter.

Results using proposed method for neutral fat in soap stock. Each sample was sent to only part of the committee.

Sample	Barrow	Heider	Long	Marmor	Reese	Watkins	Average
*1. Cottonseed			15.01		15.23	15.39	15.21
*2. Cottonseed			0.43		0.66	0.75	0.61
*3. Cottonseed	17.41	17.64		17.69		17.88	17.66
*4. Soya Bean	5.80	5.63		5.24		5.72	5.60
5. Corn	18.76	18.24		18.80		18.15	18.49
6. Soya Bean			16.31		16.36	17.55	16.74
7. Cottonseed	8.83	9.38		8.45		9.15	8.95
8. Cottonseed			14.04		14.80	14.15	14.33

* Sample dissolved in 80 ml. of 50% alcohol. Combined extracts in separatory funnel washed with three 25 ml. portions of 10% alcohol.

Recommendations

The Committee recommends (1) tentative adoption of the above method for neutral fat in soap stock; (2) that the tentative official method for total fatty acids of soap stock or acidulated soap stock from copra and palm kernel oils be adopted as official; (3) that the Soap Stock Committee be discontinued.

E. R. Barrow
George Heider
C. P. Long
R. A. Marmor
W. J. Reese
W. T. Watkins, Chairman.