The Influence of Processing on the Spectral Properties of Vegetable Oils

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IN spite of the importance of absorption spectrophotometry in research on vegetable oils and in their evaluation (24, 27), no systematic study of their complete absorption spectra appears to have been made. The purpose of this report is to present the results of an examination of the absorption spectra from 220 mμ to 720 mμ of typical cottonseed, soybean, peanut, sesame, okraseed, and rice bran oils, with the absorption measurements being made on the crude oils and the processed oils, at successive processing steps of refining, bleaching, and deodorizing.

For purposes of study the ultraviolet and visible spectra of vegetable oils can conveniently be divided into three main regions: 1. the far ultraviolet region from 220 mμ to 320 mμ, 2. the near ultraviolet and the blue to blue-green portion of the visible region from 320 mμ to 500 mμ, and 3. the visible region above the blue-green from 500 mμ to 720 mμ.

Curves have been obtained in isooctane solution with a Beckman Model DU spectrophotometer with readings taken every 2 to 5 millimicrons throughout the entire spectral range covered with additional readings at points of sharp inflection. Nominal band widths, varying from 1.50 mμ at 230 mμ to 1.95 mμ at 320 mμ, were used throughout the far ultraviolet; varying from 2.53 mμ at 320 mμ to 1.97 mμ at 400 mμ throughout the near ultraviolet; and varying from 1.97 mμ at 400 mμ to 5.59 mμ at 720 mμ through the visible spectra.

Absorption Properties Common to All Vegetable Oils

All vegetable oils exhibit absorption spectra which have features in common. In the far ultraviolet region the spectra of a vegetable oil may exhibit one or more of three characteristic maxima, namely, at 232 mμ attributed to diene conjugation, at 268 mμ arising from triene conjugation, and at 316 mμ due to tetraene conjugation. These maxima are superimposed on a smooth or general absorption which increases rapidly towards shorter wavelengths. This is end absorption from the bands attributed to the —COOR and —C=C— groups in the glyceride molecules, which exhibit intense maxima below 200 mμ. The characteristic bands at 232 mμ, 268 mμ, and 316 mμ were originally attributed to preformed conjugation arising from rearrangement to conjugate positions of the double bonds in the naturally occurring linoleic, linolenic, and arachidonic acids, respectively.

O'Connor *et al.* (19) had shown that upon attempts to obtain pure samples of linoleic acid the most troublesome impurity was not diene conjugation arising from isomerization of the linoleic acid, but triene conjugation which could be produced only upon oxidation of this acid. This latter explanation was confirmed when the purification of the linoleic acid under an atmosphere of nitrogen completely eliminated the triene conjugation. Similarly in the purification of linolenic acid these authors found tetraene conjugation the most troublesome impurity. The appearance of triene conjugation in the spectra of many vegetable oils which had been shown by chemical studies to contain no linolenic acid and the rather common occurrence of tetraene conjugation, although arachidonic acid has never been reported as a constituent of these oils from chemical tests, led to some question as to the accuracy of the interpretation of the spectra (12).

Mitchell and Kraybill (16) had shown earlier that blowing oxygen through an oil and following with adsorption on Fuller's earth would produce conjugation of the next higher order, namely, triene from linoleic acid and tetraene from linolenic acid. Swain and Brice (25) have shown how the postulation of Farmer *et al.* (10) of a hydroperoxide followed by a dehydration, as suggested by Mitchell and Kraybill (16), can account for the appearance of a true unsaturated fatty acid of the next higher order of conjugation. This explanation accounts for the ap-

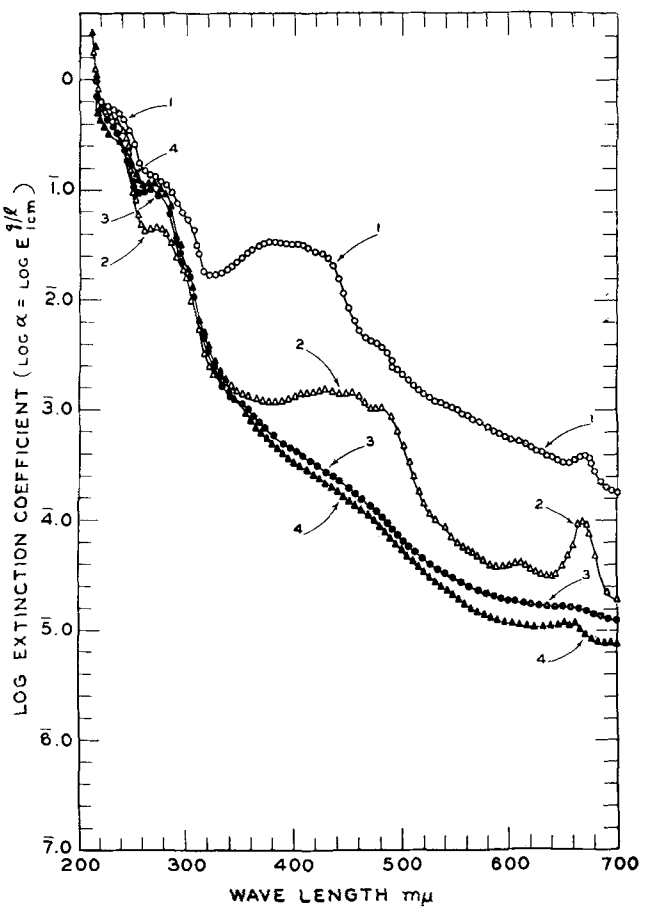

FIG. 1. Absorption spectra of cottonseed oils. 1. Crude; 2. alkali-refined; 3. bleached; 4. deodorized.

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TABLE I

Conditions of Processing Oils

Oil Type	Cottonseed-Hydraulic-pressed	Soybean-Degummed	Peanut-Solvent-Extracted[1]	Sesame-Solvent-Extracted[1]	Okraseed-Solvent-Extracted[1]	Rice bran-Solvent-Extracted[1]
F. F. A., %	0.7	0.6	2.0	1.0	0.4	3.0
Refining conditions[2]						
Lye, °Bé	14	12	16	12	12	16
Excess lye used, %	0.4	0.2	0.5	0.1	0.4	0.5
Refined oil color, 5¼" cell	35 Y 7.6 R	.....	20 Y 1.3 R	35 Y 1.5 R	70 Y 3.0 R	70 Y 7.2 R
Bleaching conditions[3]						
Bleaching earth	AOCS natural	AOCS natural	AOCS natural	"B.C. clay"[4]	"B.C. clay"[4]	AOCS natural
Earth used, %	6	6	6	3	6	6
Temperature, °C	120	120	120	120	120	120
Bleached oil color, 5¼" cell	20 Y 2.7 R	20 Y 1.8 R	6 Y 0.8 R	3 Y 0.5 R	5 Y 0.5 R	15 Y 1.6 R
Deodorization conditions						
Time, hours	2	2.5	1	2	2	1
Temperature, °C	210	200	210	200	200	210

[1] Extracted in pilot plant of this laboratory with commercial hexane.
[2] Cup or kettle refining using conditions of AOCS refining loss methods.
[3] Bleached in open cup or kettle at atmospheric pressure.
[4] This earth is named merely to describe the experimental conditions and does not constitute an endorsement of this product over that of any other manufacturer of similar products.

pearance of the fine structure of preformed conjugation absorption which is indistinguishable from that obtained upon alkali isomerization. These authors propose a test to differentiate between this conjugation formed upon oxidation by the creation of an additional —C=C— group and that produced by isomerization, namely rearrangement of the -C=C- groups already present in the fatty acid molecule.

Recently Swift *et al.* (26) have shown that upon oxidation of cottonseed oil α-, β-unsaturated aldehydes and dienals are obtained by fission of the glyceride molecules. Gibson (11) has postulated conjugation involving an oxygen linkage in a theoretical study of the autoxidation of methyl oleate and linoleate. Preformed conjugation with absorption maxima at 232 mμ, 268 mμ, and 316 mμ would appear therefore to arise from oxidation of oleic, linoleic, and linolenic acids, respectively, probably followed by fission of the acid molecules to yield conjugated systems of the types:

—C=C—C=O —C=C—C=C—C=O

—C=C—C=C—C=C—C=O

which are the absorbing groups. Thus both the fine structure and the broad bands of preformed conjugation are accounted for. In each case the conjugation absorption is due to oxidation of the next lower fatty acid. Diene absorption results from the formation of the hydroperoxide or from oxidation of oleic acid. Triene absorption is obtained from linoleic acid and tetraene absorption from linolenic acid. In oils which have been subjected to considerable autoxidation tetranoic conjugation may arise from oxidation of the conjugated linolenic acid originally formed by oxidation of the linoleic acid. Thus oils which contain only oleic and linoleic acids as unsaturated constituents may exhibit small traces of tetraene conjugation. Measurements of the preformed conjugated constituents are not essentially a measure of the glyceride content of the oil but rather that of oxidation.

Absorption in the near ultraviolet and in the blue to blue-green portion of the visible spectra is responsible for the yellow to brown color of crude vegetable oils. It is obvious therefore that a knowledge of the nature of the chromophores present is of considerable importance to the most economical production of light-colored oils. Very little study has been made to identify the pigments responsible for the absorption in this region. The absorption spectra of all vegetable oils show clearly that the color produced by absorption in this region results from relatively small quantities of a number of pigments rather than to a single pigment. There are moreover considerable differences in the pigments found in the various types of vegetable oils.

The characteristic absorption of vegetable oils in the visible region above the blue-green is attributable to chlorophyll, chlorophyll derivatives, and their degradation products. The absorption of these compounds is discussed later in connection with soybean

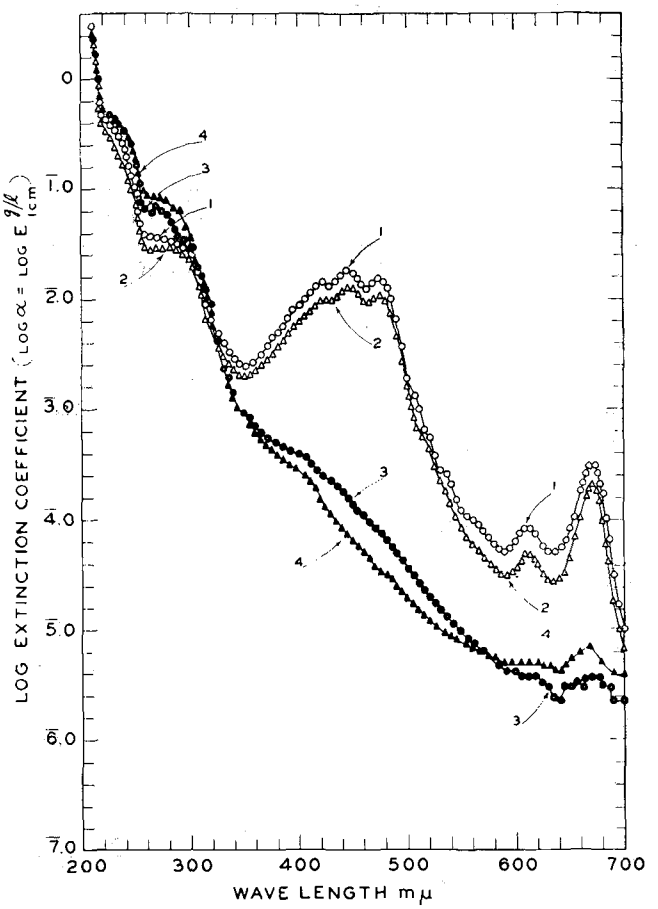

FIG. 2. Absorption spectra of soybean oils. 1. Crude; 2. alkali-refined; 3. bleached; 4. deodorized.

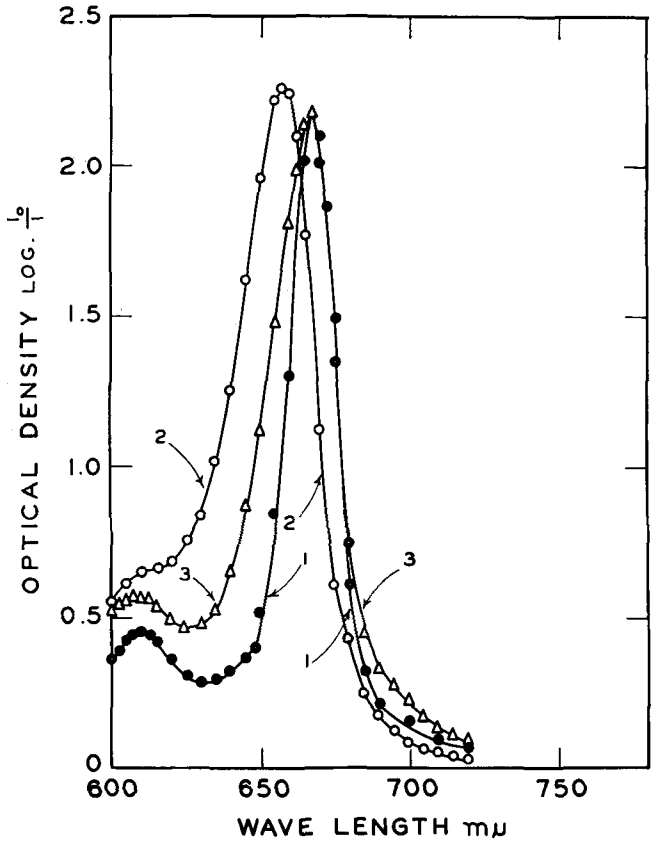

FIG. 3. Comparison of the absorption of 1. crude soybean oil; 2. chlorophyll A; and 3. pheophytin A. (In diethyl ether.)

oil. The red color of "reverted" cottonseed oils is due also to absorption in this region.

Cottonseed Oil

Shown in Figure 1 are the spectra of a crude hydraulic cottonseed oil of commercial origin and of the same oil after alkali refining; after alkali refining and bleaching; and after alkali refining, bleaching, and deodorizing in laboratory equipment. The conditions under which the various oils were processed are shown in Table I.

In the far ultraviolet region the spectrum of the crude oil exhibits the smooth absorption increasing rapidly toward shorter wavelengths which has been attributed to the —C=C— and the —COOR groups in the glycerides. Superimposed on this absorption are two sharp inflections at about 230 mμ and 265 mμ, indicating preformed diene and triene conjugation. There is no evidence of characteristic absorption at 316 mμ. These observations are consistent with the data on cottonseed oil obtained from chemical studies. The inflections at 230 mμ and at 265 mμ may be attributed to oxidation of traces of oleic and linoleic acids. On the basis of chemical evidence cottonseed oil contains no linolenic acid, no oxidation of this compound is possible, no absorption at 316 mμ is expected, and none is observed.

Further insight into the nature of the absorption at 230 mμ and 265 mμ can be obtained by study of the absorption of the processed oils. Examination of the spectrum of the alkali-refined oil reveals a slight decrease in the absorption at 230 mμ and a somewhat greater decrease at 265 mμ. This decrease is a result undoubtedly of the removal, during alkali refining, of gossypol or gossypol-like compounds which exhibit characteristic absorption at these wavelengths (4) and which, unlike most vegetable oil pigments, are removed during the refining process. However while the total absorption at 268 mμ is less, the maxima are more pronounced in the alkali-refined sample than in the crude oil. Two explanations of this observation are possible. Either the removal of the interfering absorption produced by the gossypol-like compounds reveals the characteristic triene conjugation absorption more clearly, or more oxidation products of the linoleic acid have been formed during processing of the oil. Consideration of the absorption spectra of the alkali-refined and bleached, and of the alkali-refined, bleached, and deodorized oils tends to support the latter explanation. In these spectra both the magnitude of absorption and the sharpness of the bands have increased. During each of these last two processes at least triene conjugation has increased, that is, more linoleic acid has been oxidized, and/or more oxidized products have decomposed or undergone further dehydration.

In the near ultraviolet and the blue to blue-green portion of the visible spectrum the absorption of crude cottonseed oil is unique. It bears no resemblance to the absorption of any other vegetable oil. In the absorption spectrum of the crude oil the most surprising observation is the complete lack of any evidence for an absorption band at 360 mμ, the position where gossypol absorbs with a pronounced maximum. The absorption of the crude oil exhibits maxima at 380 mμ and 400 mμ. From these data the conclusion is inescapable that crude cottonseed oil, produced by modern commercial hydraulic press methods, *contains very little or no gossypol.* This absence of a gossypol band is in agreement with the recent observation that an alkaline-extraction method developed for the isolation of gossypol, when applied to crude hydraulic-pressed cottonseed oil, does not isolate sufficient gossypol to give the antimony trichloride reaction product characteristic of gossypol (5). The pigment or pigments responsible for the absorption at 380 mμ and 400 mμ have not been identified. They resemble gossypol in that, unlike most vegetable oil pigments, they are removed during alkali refining.

The alkali-refined cottonseed exhibits an absorption spectrum which is sensibly different from that of the crude oil with maxima at 432 mμ, 455 mμ, and 480 mμ. Two explanations are possible for the appearance of these bands. Either the removal of the gossypol-like pigments whose absorption is considerably greater [3] has uncovered the underlying absorption peaks at the higher wavelengths, or the pigments responsible for these maxima have been formed during the alkali-refining procedure. The absorption curves themselves give some support for the first explanation. The absorption curve of the crude cottonseed oil reveals definite inflections at about 455 mμ and 480 mμ. These are the two maxima exhibited by the alkali-refined sample which would be the least

[3] The statement that the absorption of the gossypol-like pigments at 380 mμ and 400 mμ is greater than the bands which appear (after alkali refining) at 432 mμ, 455 mμ, and 480 mμ must not be interpreted as meaning that the absolute value of the absorption of these gossypol-like pigments is greater than the absolute absorption of the pigments absorbing at higher wavelengths, but merely that the gossypol-like pigments are present in greater concentration in cottonseed oil. As a matter of fact, the absorption of the carotenoids (with which bands at higher wavelengths are to be identified) is about six-fold greater than that of the gossypol, i.e., β-carotene has an extinction coefficient of about 250, while that of gossypol on the same basis is only about 40.

affected by the more intense absorption at 380 mμ and 400 mμ. Additional evidence to support the theory that these bands at 432 mμ, 455 mμ, and 480 mμ are present but almost completely obscured in the crude oil is obtained from the observation that crude oils having high absorption at 380 mμ and 400 mμ yield alkali-refined oils with rather weak absorption at 432 mμ, 455 mμ, and 480 mμ while crude oils having weaker absorption at 380 mμ and 400 mμ yield alkali-refined oils with stronger bands at 432 mμ, 455 mμ, and 480 mμ (5).

Still further support of the theory that these bands are part of the absorption spectra of the crude oil is obtained from the fact that they are the only observable bands which could be attributed to the carotenoids. There is little real evidence for the presence of flavones and anthocyanins in cottonseed oil. Carotenoids have been recognized as a constituent in cottonseed oil for some time (13), and more recently attempts have been made to measure them quantitatively (21, 22). The bands at 432 mμ, 450 mμ, and 480 mμ are in the region of carotenoid absorption. Boatner (4) reported that the carotenoids are not removed during alkali refining of the cottonseed oil. This is in agreement with the absorption shown in Figure 1 and is, as will be seen, also in agreement with the removal of carotenoids from other vegetable oils. Inspection of the absorption curve of the alkali-refined and bleached sample in Figure 1 shows that bleaching completely removes the pigments absorbing in the region of carotenoid absorption.

It can be said tentatively that the absorption of a crude cottonseed oil in the near ultraviolet and the blue to blue-green portions of the visible spectrum

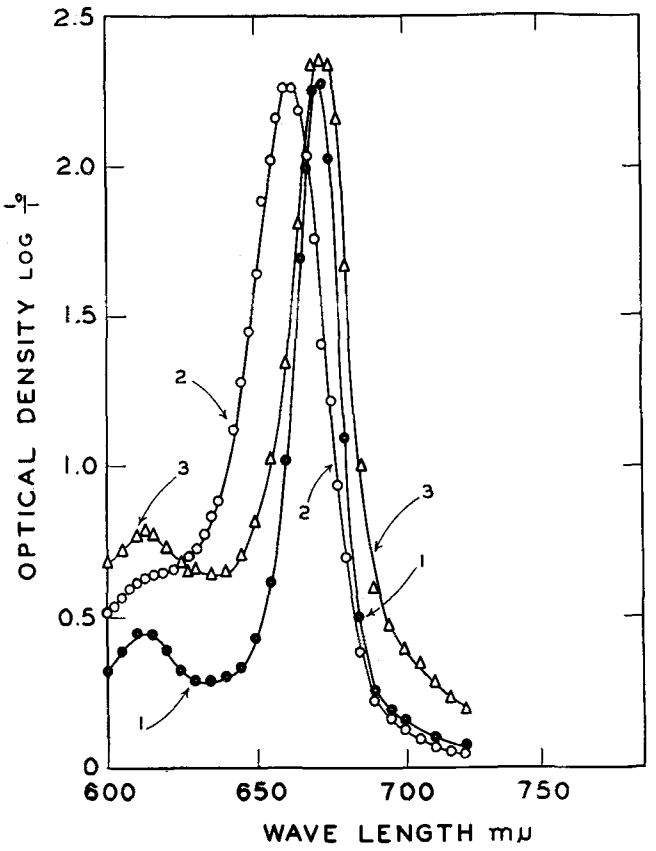

FIG. 4. Comparison of the absorption of 1. crude soybean oil; chlorophyll A; and 3. pheophytin A. (In bleached soybean oil.)

TABLE II

Comparisons of Characteristic Red Absorption of Crude Soybean Oil, Pheophytin A, and Chlorophyll A

	In Diethyl Ether		
	Crude Soybean Oil	Pheophytin A	Chlorophyll A
No. 1. Region of Maximum about 660-675 mμ	M 670	M 670	M 660
No. 2. Region of Maximum about 580-630 mμ	m 588 M 610 m 630	m 580 M 610 m 625	No Band

	In Soybean Oil (Refined Bleached)		
	Crude Soybean Oil	Pheophytin A	Chlorophyll A
No. 1. Region of Maximum about 660-675 mμ	M 672	M 672	M 662
No. 2. Region of Maximum about 580-630 mμ	m 590 M 612 m 630	m 590 M 612 m 630	No Band

M = maximum.
m = minimum.
All values are wavelengths in millimicrons.

results from two groups of pigments: one a group of gossypol-like pigments, but probably not including gossypol itself, which absorb with maxima at 380 mμ and at 400 mμ, and which, like gossypol, are removed by alkali refining; and a group of pigments with absorption maxima at 432 mμ, 455 mμ, and 480 mμ, which are probably carotenoids and like carotenoids are unaffected by refining, but completely removed by bleaching. The residual color of the finally processed cottonseed oil is attributable to traces of these pigments not removed during the processing and to further oxidation of the compounds responsible for the oxidation-conjugation absorption found in the far ultra-violet region.

In the visible portion of the spectra above 500 mμ the crude cottonseed oil exhibits an absorption maximum at 670 mμ. This absorption becomes more pronounced upon alkali refining because undoubtedly the interfering yellow pigments are removed, and a weaker band, at 610 mμ, appears. Both of these bands are almost completely removed by bleaching. Deodorizing has little further effect. The bands at 610 mμ and 670 mμ are commonly referred to as chlorophyll A absorption bands despite the fact that the positions of their maxima are located somewhat greater than 10 mμ toward higher wavelengths than the known bands of chlorophyll A. Actually, as evidence to be presented later will show, the bands at 610 mμ and at 670 mμ in most vegetable oils arise from absorption *not of chlorophyll but of pheophytin*, a degradation product of chlorophyll.

Soybean Oil

The ultraviolet and visible absorption spectra of a crude degummed soybean oil of commercial origin and of the same oil after the various processing steps in the laboratory are shown in Figure 2. Superimposed on the smooth or general absorption of the —C=C— and —COOR groups is the characteristic absorption in the far ultraviolet region consisting of an inflection at about 230 mμ, maxima at about 260 mμ to 270 mμ, and a plateau or shoulder at about 320 mμ. They can be attributed to oxidized oleic, linoleic, and linolenic acids. Alkali refining has little

or no effect on the absorption. Bleaching and deodorizing however somewhat increase the intensities of the characteristic bands, particularly those about 265 mμ, indicating formation of some increased oxidation products of the linoleic acid, in particular, during these processes.

Markley and Goss (15) reported that the pigments responsible for the color of soybeans consist of four groups, namely, the red-yellow carotenoids, the yellow isoflavone glycosides, the blue-purple anthocyanins, and the chlorophylls. The absorption of the first two of these types would occur in the near ultraviolet and the blue to blue-green portion of the visible spectrum. Inspection of the spectrum of the crude oil in this region shows maxima at 420 mμ, 445 mμ, and 475 mμ. Most flavone pigments exhibit characteristic absorption below 400 mμ. However a few of the hydroxy-, methoxy-, or oxy-flavones have their most intense bands just above 400 mμ. The maximum just above 400 mμ observed in the absorption of the crude soybean oil could therefore be attributed to the trihydroxyisoflavone compounds which have been reported to have been isolated from soybeans (18, 20, 28). However some of the carotenoids also have absorption bands at 420 mμ. Absorption spectra alone cannot reliably identify the pigments responsible for the yellow-brown color of crude soybean oil. The bands at 445 mμ and 475 mμ are probably attributable to carotenoids. They are in close agreement with the two maxima of β-carotene. Alkali refining removes only a small proportion of these pigments, and there is no evidence for any change in their nature during this process. Bleaching however removes them quantitatively. Deodorizing has very little further effect.

In the visible region above 500 mμ two distinct maxima, at 610 mμ and at 670 mμ, are observed in the absorption of the crude oil. These bands are found also in the spectra of other vegetable oils and have been attributed to chlorophyll (3). However the position of maximum red absorption of chlorophyll has been found to be at about 660 mμ for component A or 640 mμ for component B (30). Hence in the oils there is a strong bathochromic shift of from 12 to 30 mμ. This shift is too great to be a solvent effect. Evans and Gillam (9) and Woodward (29) have shown that shifts accompanying changes in solvents do not exceed 7 mμ. Detwiler *et al.* (8) have pointed out that the positions of observed absorption maxima in the visible red spectra of soybean oil are in better agreement with the absorption of pheophytin A than with chlorophyll A.

To test the theory that the band at 670 mμ could not be that of chlorophyll with a bathochromic effect resulting from the solvent, *i.e.*, the oil, a sample of pure chlorophyll was dissolved in diethyl ether and also in a bleached soybean oil which showed no characteristic absorption in this region and the absorption of each solution was determined. Comparison of the curves of chlorophyll A in diethyl ether and in the bleached soybean oil (Figures 3 and 4) shows that the maxima in the red are at 660 mμ and 662 mμ, respectively. The bathochromic effect therefore does not result from the solvent. A similar comparison of the crude soybean oil in the diethyl ether and in the bleached soybean oil reveals bands at 670 mμ in diethyl ether and at 672 mμ in the soybean oil. Hence the bathochromic shift resulting from change in solvent from diethyl ether to bleached soybean oil would appear to be about 2 mμ.

A pronounced bathochromic effect is produced when chlorophyll undergoes decomposition by the loss of magnesium to form pheophytin. The positions for the red absorption of pheophytin are 670 mμ for component A and 660 mμ for component B (30). Pheophytin can readily be prepared from chlorophyll by the addition of a small quantity of mineral acid. The spectra of samples of pheophytin prepared in this manner were obtained when dissolved in the diethyl ether and in the bleached soybean oil. Comparisons of these absorption curves with the curves for the crude soybean oil in these two solvents conclusively show that the red absorption results from pheophytin. Because crude soybean oil has a rather strong absorption below 520 mμ as a result of the presence of carotenes and/or carotenoids, comparisons of the absorption due to chlorophyll and its derivatives which occur in vegetable oils only in small quantities must be made above this wavelength.

If the curves for the crude soybean oil in either the diethyl ether or in the bleached soybean oil are moved along the ordinate (which is in terms of the relative optical densities, so that such adjustment represents the physical process of changing the concentrations of the solution), it will be seen that they superimpose exactly upon the curves for the pheophytin in the respective solvents. Furthermore the curves for the pure chlorophyll have many points of considerable differences. A comparison of these curves at two points in particular (Table II) emphasizes the similarities between the absorption of the crude soybean oil and the absorption of pheophytin, and the dissimilarities between the absorption of the oil and chlorophyll. The conclusion is thus inescapable that the red absorption in vegetable oils at about 610 mμ and about 670 mμ is a result of the presence of *pheophytin A* and not *chlorophyll A*.

Alkali refining has very little effect on the bands of pheophytin A. Bleaching greatly reduces their intensity but does not effect their complete removal. Deodorizing has no significant effect upon the absorption.

In the visible spectra above 500 mμ, other than the bands now attributed to pheophytin A, the absorption of the crude soybean oil exhibits a series of inflections at about 500 mμ, 515 mμ and 535 mμ. This absorption would, if sufficiently intense, result in a red color. It may be caused by traces of the anthocyanin pigments reported to be constituents of the soybean (1, 14). Anthocyanins exhibit major absorption bands between 480 mμ and 550 mμ (17). They are unaffected by refining but are completely removed by bleaching and are not further affected by deodorizing.

Peanut Oil

The spectra of commercial hydraulic peanut oils (Figure 5) exhibit absorption in the far ultraviolet region which closely resembles that of cottonseed. There are pronounced inflections about 232 mμ and maxima about 265 mμ which can be attributed to oxidized oleic and linoleic acids. There is no indication of characteristic absorption about 316 mμ. The spectrophotometric curve is therefore also in agreement with chemical data on peanut oil in indicating the presence of no linolenic acid. Processing has little effect upon the characteristic absorption in this region although at 268 mμ there is some indication of forma-

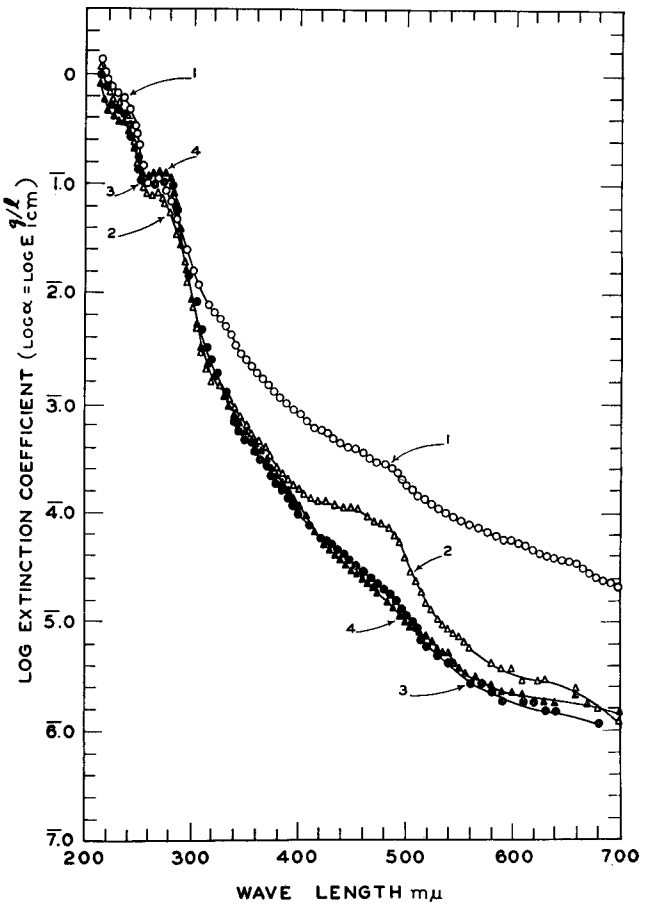

FIG. 5. Absorption spectra of peanut oils. 1. Crude; 2. alkali-refined; 3. bleached; 4. deodorized.

tion of additional oxidation products of the linoleic acid.

In the near ultraviolet and the blue to blue-green portions of the visible spectra the absorption curve of the crude peanut oil is unexpectedly lacking in characteristic absorption. The most obvious explanation for the smooth absorption exhibited is either that the pigments which absorb in this region are present in small concentrations or there are several pigments absorbing in the region so that the characteristic bands exhibited by each are hidden by the overlapping of others. This explanation is somewhat strengthened by the appearance of characteristic absorption in the spectrum of the alkali-refined sample. In this sample the intensities of the absorption have decreased, but there are indications of absorption at about 420 mμ, 450 mμ, and 470 mμ. These bands are completely removed by the bleaching process. Thus, like cottonseed oil, peanut oil appears to contain two types of pigments which absorb in this region, one of which is removed by alkali refining, the other only by the bleaching process. The bands removed by the alkali refining probably arise from pigments related to those which produce the red color of peanut oils. As will be shown later, they act similarly to the pigments which show absorption in the visible spectra above 500 mμ. The pigments whose absorptions are unaffected by the alkali refining but which are removed by bleaching have not been identified although they undoubtedly include carotenoids which are known constituents of peanut oil. Deodorizing has no further effect upon the absorption in this region.

In the region of the visible spectra above 500 mμ the smooth absorption persists with a uniformity of intensity which indicates that it does not result entirely from end absorption from the bands below 500 mμ. Not much is known regarding pigments which are responsible for this absorption. The absorption is almost completely removed by alkali refining, and further processing has little or no effect. In this manner these pigments resemble those postulated in the region below 500 mμ. Peanut oil is the only oil encountered which exhibits no characteristic absorption attributed to chlorophyll, pheophytin, or other chlorophyll derivatives.

Sesame Oil

The spectra of a pilot-plant hexane-extracted sesame oil are reproduced in Figure 6. In the far ultraviolet the crude oil exhibits two maxima, a weak band at 232-234 mμ and a pronounced band at 287-288 mμ, superimposed on the smooth general absorption. These bands are produced by sesamin and probably by sesamol or sesamolin. They account for the unusual appearance of the far ultraviolet absorption of this oil. These bands completely mask any conjugated absorption resulting from oxidation. In the well-known direct spectrophotometric method for quantitative measurement of the unsaturated glycerides by alkali isomerization (24) there is some disagreement as to whether the oil should be measured spectrophotometrically before the alkali treatment and this measured absorption subtracted

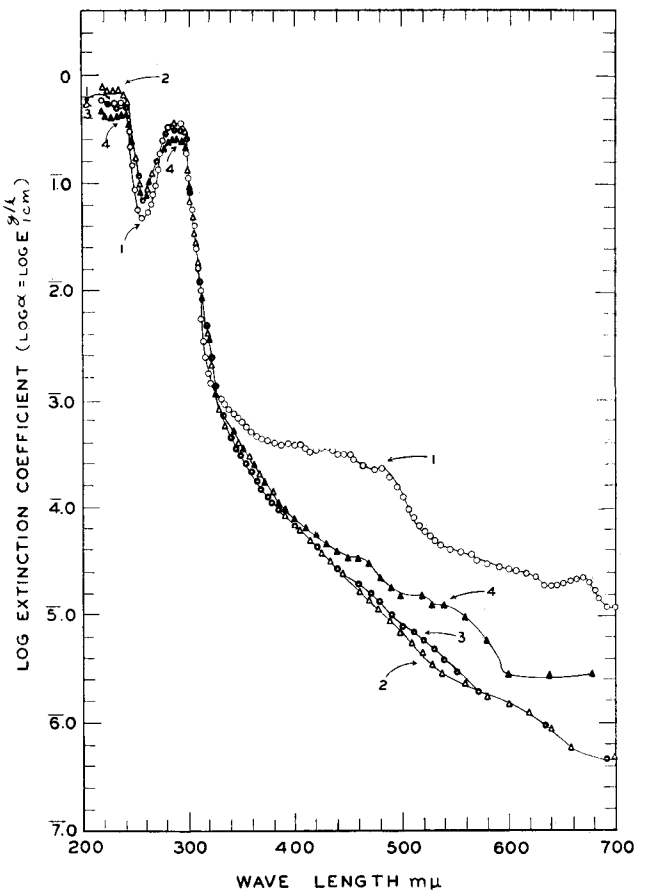

FIG. 6. Absorption spectra of sesame oils. 1. Crude; 2. alkali-refined; 3. bleached; 4. deodorized.

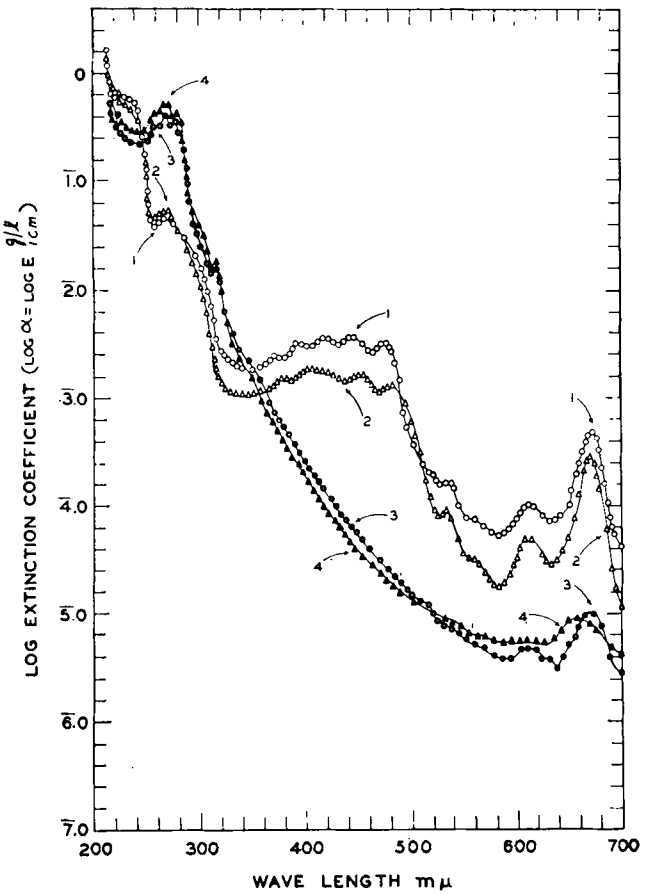

FIG. 7. Absorption spectra of okraseed oil. 1. Crude; 2. alkali-refined; 3. bleached; 4. deodorized.

from that obtained after the isomerization. The spectrum of sesame oil offers an excellent illustration where such a correction for absorption, at either 234 mμ or at 268 mμ, not produced by the alkali isomerization, is essential to precise measurements. Without such a correction the intense absorption of the sesamin would result in somewhat high results. This characteristic absorption of sesamin and sesamol or sesamolin might, with further study, be made the basis for a direct spectrophotometric estimation of these components in sesame oil or in mixtures suspected of containing sesame oil. The absorption of these constituents of sesame oil appears to be unaffected by any of the processing steps.

In the region of the near ultraviolet and the visible to 500 mμ the crude sesame sample has a long plateau extending from about 350 to 480 mμ. This absorption is probably attributable to small traces of carotenoids. It is almost completely removed by the alkali-refining process. In this respect the carotenoids of sesame oil, if this tentative identification is correct, appear to act differently toward alkali refining than the oils discussed previously. However while alkali-refining did not completely remove the carotenoids of cottonseed or soybean oils, it did usually result in their partial removal. Their complete removal from sesame oil can be explained on the basis that the amounts in the oil are small. Crude sesame oil is light-colored. Bleaching has no further effect on the absorption, as could be predicted, in view of the complete removal of the pigments in the previous step. Deodorizing however results in a slight increase in absorption.

In the visible portion of the spectra above 500 mμ there appears in the absorption of the crude sesame oil only a weak band attributed to pheophytin A. This band is completely removed by the refining step, and again the bleaching has no effect upon the absorption. Examination of Figure 6 indicates that deodorizing produced a slight increase in the absorption throughout this region. However the values of the extinction coefficients are so small that the observed effect is almost negligible. A similar, though less, increase in absorption in this region was observed following deodorization of another refined and bleached sesame oil.

Okraseed Oil

The spectrum of crude okraseed oil, extracted with hexane in a pilot plant, shown in Figure 7, exhibits an inflection at the region of diene conjugation and a maximum at the triene region. Absorption at these wavelengths is unaffected by the alkali refining. Bleaching however reduces the absorption at 232 mμ, indicating removal of some unidentified material. But at the triene region the bleaching process causes an increased absorption characteristic of triene conjugation, obviously caused by formation of increased amounts of oxidation products of the linoleic acid. Deodorizing effects no further change in the far ultraviolet absorption. The bleaching process also reveals a band with maximum at 316 mμ, which does not have the characteristic shape of tetraene conju-

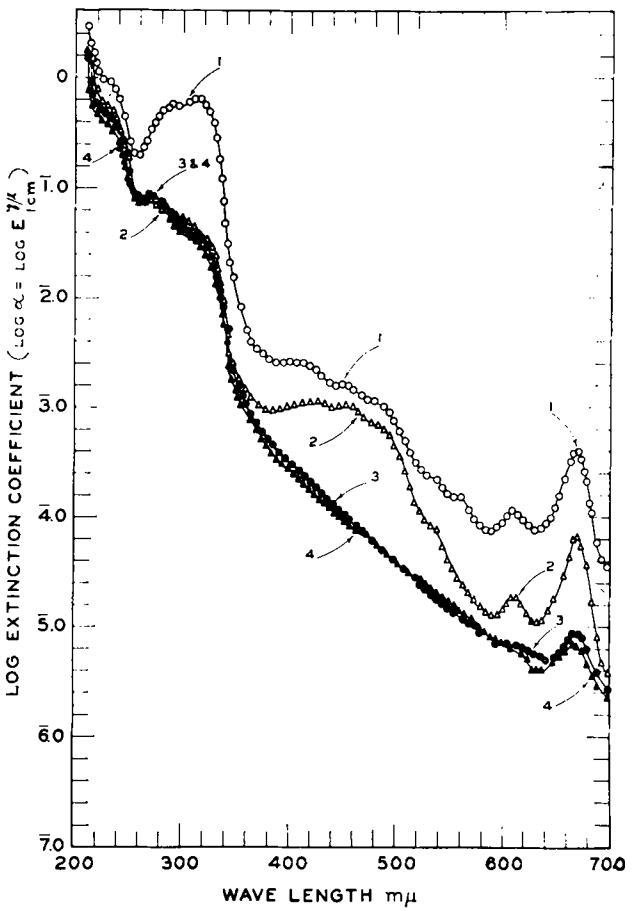

FIG. 8. Absorption spectra of rice bran oil. 1. Crude; 2. alkali-refined; 3. bleached; 4. deodorized.

tion. The lack of evidence for linolenic acid in this okraseed oil, which has not been subjected to severe autoxidation, would be against the explanation for the appearance of this band as oxidized linolenic acid. However, as the absorption spectra reveal, it is caused by some change occurring during bleaching for there is no evidence of this maximum before the bleaching process.

In the near ultraviolet and the blue region of visible absorption the spectrum of crude okraseed oil exhibits a series of intense bands having maxima from 370 mμ to 480 mμ. The pigments of okraseed oil responsible for this absorption have not been identified. The absorption is in the general region of the absorption of carotenoids, flavones, or isoflavones. The absorption is only slightly decreased, and the appearance of the bands is unchanged by alkali refining. Bleaching however apparently removes these pigments quantitatively. Deodorizing has no further effect upon the absorption.

In the visible region above 500 mμ the characteristic spectra of pheophytin A at 535 mμ, 610 mμ, and 670 mμ are very strong in the crude oil and are reduced only very slightly by the alkali-refining process. The intensity of these bands is decreased considerably but they are not completely removed by bleaching. Deodorizing has no further effect. The final product therefore still shows these bands of pheophytin and can be expected to possess a slightly greenish color attributed to this red absorption.

Rice Bran Oil

The far ultraviolet absorption of crude rice bran oil, also extracted in a pilot plant with hexane, shown in Figure 8, is characterized by a broad band with two maxima at 290 mμ and 314 mμ. The identity of the material responsible for this absorption is unknown although it is probably the result of some of the naphthalene derivatives which have been reported as present in crude rice bran oil (23). Naphthalene derivatives exhibit major bands at about 285 mμ and 320 mμ (2). The absorption is completely removed by the alkali-refining step, and the usual triene conjugation absorption from oxidized linoleic acid is revealed. This absorption is not affected by either the bleaching or the deodorizing.

In the near ultraviolet and blue to blue-green portion of the visible region of the spectra, crude rice bran oil exhibits a series of bands all of low magnitude with maxima from 400 mμ to 480 mμ. These bands, arising from unidentified pigments are only slightly decreased by the alkali-refining procedure without any apparent change in shape. The decrease in intensity is probably the result of removal of the underlying end absorption from the bands in the far ultraviolet with maxima at 290 mμ and 314 mμ. Bleaching completely removes the bands between 400 mμ and 480 mμ, and deodorizing has no further effect upon the absorption. Studies of the pigments responsible for the absorption in this region require of necessity some method of concentrating the absorbing material or materials.

Figure 9 shows the characteristic absorption of a fraction separated by molecular distillation. In Table III the wavelengths of the the various maxima are given along with these same data expressed on the frequency scale. Column 3 of this table shows the differences in frequency between the series of

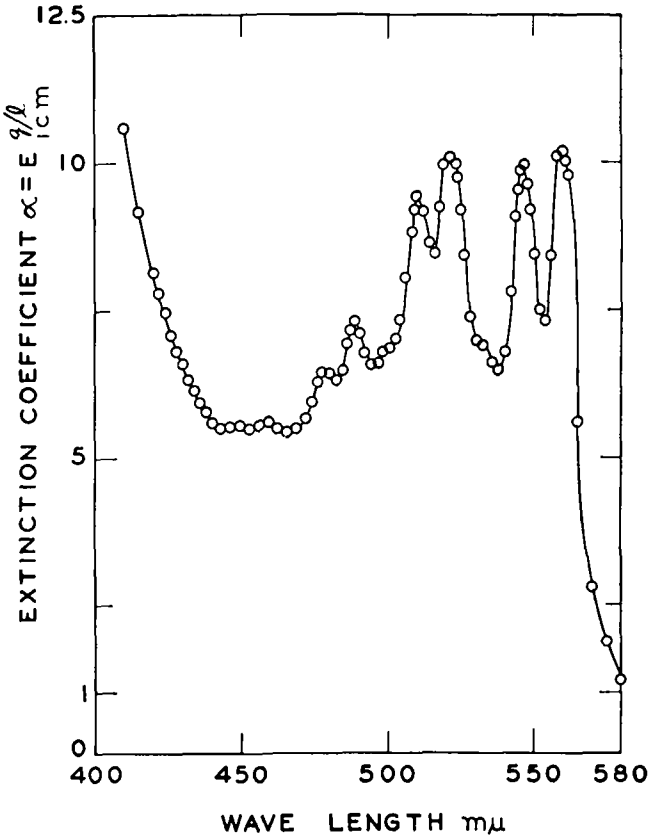

FIG. 9. Absorption of a fraction from molecular distillation of rice bran oil. (In isooctane.)

bands, and column 4 shows that these frequencies are successive multiples of the constant difference as if the observed and measured bands were overtones of some simpler fundamental. While there is no theoretical significance to this type of analysis on a frequency scale, which was originally proposed and applied to the study of azo dyes by Brode (6. 7), such empirical relationships can be of considerable assistance in identifying and distinguishing certain types of bands in attempts to separate and identify the pigments responsible for them.

In the region above 500 mμ the characteristic bands of pheophytin A at 535 mμ, 610 mμ, and 670 mμ are clearly exhibited. The intensities of these bands are reduced by alkali refining, further reduced by bleaching, but neither of these processes is sufficient completely to remove this red absorption. To obtain a colorless rice bran oil, as in the case of okraseed oil, stronger adsorbents than are usually employed are required.

TABLE III

Analyses of Bands in the Absorption Spectra of a Molecular Distillate of Crude Rice Bran Oil

	Wave Length mμ	Frequency f	Δf	Calculated Band
				12.8 ×
a	478.5	627.0	.....	49 = 627.2
b	488.5	614.1	12.8	48 = 614.4
c	500.0	601.2	12.9	47 = 601.6
d	510.0	588.2	13.0	46 = 588.8
e	521.5	575.3	12.9	45 = 576.0
f	533.5	562.3	13.0	44 = 563.2
g	546.5	549.4	12.9	43 = 550.4
h	559.0	536.6	12.8	42 = 537.6

Average Δf = 12.8 ~ Fundamental Frequency 12.8.
This is equivalent to an absorption in the infrared at 2.34 μ.

Summary

A detailed study has been made of the absorption of the six edible vegetable oils, namely, cottonseed, soybean, peanut, sesame, okraseed, and rice bran, throughout the entire ultraviolet and visible spectra from 220 mμ to 720 mμ. The study has included the crude oil; the alkali-refined oil; the alkali-refined and bleached oil; and the alkali-refined, bleached, and deodorized oil.

In the far ultraviolet region from 220 mμ it has been shown of vegetable oils in general that:

1. The assumption that conjugated absorption arises from oxidation affords an interpretation of the characteristic absorption which is in all cases completely in accord with chemical data;

2. Processing has little effect upon the conjugation absorption although it appears to cause some further oxidation or further dehydration or decomposition of oxidation products, particularly of linoleic acid;

3. Bands other than those resulting from the oxidized glycerides do occur. These bands constitute an interference with the direct spectrophotometric estimation of the unsaturated glycerides by measurement of their alkali-produced absorption; their appearance, arising from natural constituents other than the glycerides, is an argument for subtracting from the measured alkali-produced absorption the absorption of the oils before this isomerization treatment; and

4. The characteristic absorption of components other than the glycerides might with further study be made the basis for their quantitative determination in the vegetable oils.

In the region of the spectra between 320 mμ and 500 mμ, embracing the entire near ultraviolet and the blue to blue-green portions of the visible, it has been shown of vegetable oils that:

1. Many vegetable oils contain two entirely different groups of pigments, one of which is removed by alkali refining, the other only by the bleaching, a fact which accounts for the considerable difference noted in the appearance of the spectra of some crude and alkali-refined oils;

2. In spite of the obvious importance of a knowledge of the pigments responsible for absorption in this region to the most economical production of a light-colored oil, little is known regarding the exact nature of the pigments responsible for this absorption;

3. In general, the observable absorption can be accounted for only upon the assumption that more than one, probably several, pigments are responsible, which furthermore differ considerably in the various oils; and

4. A method of frequency analysis has been used in connection with rice bran oil to illustrate how this technique might be helpful in locating or identifying the various pigments, especially upon attempts to concentrate them for detailed study.

In the visible portion of the spectra above 500 mμ it has been shown that:

1. With the exception of some indications of traces of the anthocyanins, all characteristic absorption is attributable to pheophytin A and not to chlorophyll A as commonly supposed; and

2. All oils studied have exhibited clear evidence for pheophytin A, with the exception of the peanut which appears to have no absorption attributable to chlorophyll or chlorophyll derivatives.

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