TABLE I

Fraction al Distillation of Methyl Esters of Hydrogenated Menhaden Oil

Fraction	°C.	Wt.	Mol. Wt.	I.N.	Composition g.							Above
					C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	C_{24}	C_{26}	C_{26}
Original			300.9	6.9	....	....	....	....	....	....	....	....
1	97-113	14.7	245.2	0.6	13.1	1.6	....	....	....	....	....	
2	113-17	33.7	246.2	0.9	28.7	5.0	....	....	....	....	....	
3	117-34	12.4	268.4	2.6	0.8	11.6	....	....	....	....	....	
4	134-37	135.1	272.2	1.2	....	124.5	10.6	....	....	....	....	
5	137-54	48.1	277.4	3.3	....	35.1	13.0	....	....	....	....	
6	154-56	133.1	296.9	4.8	....	6.1	127.0	....	....	....	....	
7	156-73	15.8	307.1	14.4	....	....	10.7	5.1	....	....	....	
8	173-77	92.9	326.1	9.0	....	....	....	92.9	....	....	....	
9	177-93	16.5	341.3	16.9	....	....	....	7.5	9.0	....	....	
10	193-95	66.5	361.6	0.9	....	....	....	....	48.9	17.6	....	
11	195-215	14.0	367.2	0.9	....	....	....	....	7.3	6.7	....	
Column hold-up		7.0	411.0		....	....	....	....	....		7.0	
Residue		14.6	....		....	....	....	....	....		....	14.6
Totals, grams		604.4			42.6	183.9	161.3	105.5	65.2	24.3	7.0	14.6
Wt. percent					6.9	30.4	26.8	17.5	10.8	4.0	1.2	2.4

esters (2) did not include series above C_{24}, and further since the amounts of C_{24} acids recovered in the saturated and monoethylenic esters were appreciably less than shown in the present work, it is concluded that the C_{24}, C_{26}, and above C_{26} esters include unsaturated esters of these series. Thus it is correct to say that the unsaturated acids of menhaden oil embrace all carbon series from C_{12} to C_{26}, inclusive and probably series still higher.

The writers again wish to express their appreciation to the Procter and Gamble Company, Ivorydale, Ohio, and especially to Dr. A. S. Richardson of that company, for grants which made this work possible through an open fellowship in the Ohio State University Research Foundation.

Summary

Analysis of the fractions obtained as a result of distillation of the methyl esters of the hydrogenated acids of menhaden oil show the amounts indicated (wt. %): C_{14}, 6.9; C_{16}, 30.4; C_{18}, 26.8; C_{20}, 17.5; C_{22}, 10.8; C_{24}, 4.0; C_{26}, 1.2; above C_{26}, 2.4. In addition, about 0.1% of C_{12} acids was demonstrated previously.

REFERENCES

1. Smith, F. A. and Brown, J. B., Oil & Soap, *11*, 277, (1945).
2. Smith, F. A. and Brown, J. B., Oil & Soap, *12*, 321 (1945).
3. Farmer, E. H. and van den Heuvel, F. A., Soc. Chem. Ind., *57*, 24 (1938).

Spectroscopic Changes in Fats During Rancidification[*]

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IN connection with studies that were conducted at the University of Minnesota several years ago concerning natural antioxidants in lards some spectroscopic measurements were made on lards which had been oxidized to various degrees by exposure to air in an oven at 63°C. It was found that in the ultraviolet region there occurred increases in the absorption of light that were approximately proportional to the peroxide values of the samples, at least in the earlier stages of rancidification.

In later measurements, illustrated in Figure I, similar increases in absorption were found in samples oxidized at 100°C. However, in common with the observations of many others who have examined various peroxides spectroscopically, it was observed that the increases in absorption were not due to peroxides. When the peroxide values of the oxidized samples were reduced to a low value by treatment with superheated steam, the absorption increased still further (compare curves 6 and 8), suggesting that the chromophores may result directly from molecular rearrangement, decomposition, and polymerization reactions of the peroxides. Spectrophotometric studies of other oxidized fats have led other investigators to the same general conclusions (1).

The present report is a condensed summary of subsequent spectroscopic studies which were initiated for the purpose of obtaining additional insight on the course and mechanism of the autoxidation reaction in fats, the detailed data of which have been presented elsewhere (2, 3). It must be emphasized from the outset that the measurements to be reported were preliminary in character and that no complete studies, and only tentative interpretations, can be given. The experiments were designed primarily to develop techniques and to suggest lines of attack for the more complete studies that are contemplated.

Experimental

The purest available preparations of oleic, linoleic, and linolenic acids in their common natural isomeric forms, some of their conjugated forms and unnatural stereoisomers, and some of their esters, were oxidized individually by exposure to air at constant temperatures. The temperature selected in each case depended

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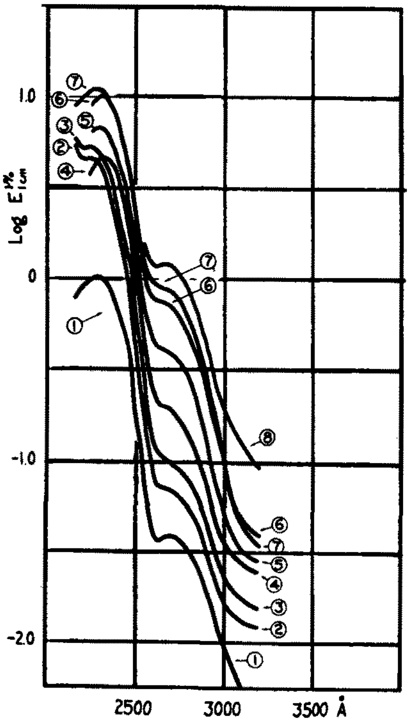

FIG. I. Absorption spectra of lard aerated at 100°C. Measured in diethyl ether.

(1) Fresh lard, peroxide value=0.35 m.e./kg.
(2) (1) aerated 5.3 hours at 100°C., P.V.=18.
(3) (1) aerated 6.5 hours at 100°C., P.V.=30.
(4) (1) aerated 7.0 hours at 100°C., P.V.=70.
(5) (1) aerated 12.8 hours at 100°C., P.V.=128.
(6) (1) aerated 16.3 hours at 100°C., P.V.=188.
(7) (6) steam-blown at 100°C., P.V.=260.
(8) (6) steam-blown at 200°C., P.V.=3.1.

on such properties as the melting point of the material and its susceptibility to oxidation. All of the temperatures used were within the limits 48 and 110°C. The effects of oxidation on the absorption spectra of the samples were determined with a Beckman quartz spectrophotometer.

The purest obtainable specimens of several derivatives of 18-carbon straight chain fatty acids, identical with or closely related to various postulated intermediates in the autoxidation of fats, were also examined spectroscopically before and after constant temperature exposures to air.

In all cases (except triglyceride samples) ethyl alcohol was one of two solvent mediums used in the spectroscopic measurements. Almost from the beginning it was apparent that a second type of medium, alkaline in character, would be particularly valuable in spectroscopic studies of oxidized fats. The principle involved is the well known organic reaction wherein the tautomeric equilibria existing in many types of carbonyl compounds may be radically shifted toward enolic forms by treatment with alkali, which has been used quite frequently in spectroscopic studies of carbonyl compounds.

In certain cases when a carbonyl group is produced by oxidation of a carbon atom near one of the double bonds in an unsaturated fat, one would expect that treatment with alkali would produce conjugated unsaturation, thus yielding a highly chromophoric group. On the other hand, cold alkali is not effective in shifting the bonds in either conjugated or unconjugated polyethenoid systems in fats. The use of an alkaline medium therefore should be useful in obtaining information concerning the constitutions and the quantities of some of the products of autoxidation.

There is evidence that the autoxidation of fats, under some conditions at least, can produce polyethenoid conjugation in which oxygen is not closely associated with the chromophoric groups (4). Properly applied, the technique of using alkaline media may yield insight on the relation between the production of such chromophores and the production of potential or actual chromophores having oxygen closely associated with them since it is possible that the two processes are closely related. The remainder of the discussion will be devoted to a cursory comparison of the preliminary results in the oxidation of a few simple compounds representing mono-, di-, and triethenoid unsaturation without conjugation, particularly individual compounds of the most common unsaturated fatty acids that occur in natural fats. Among such compounds are the ethyl esters of oleic, linoleic, and linolenic acids.

Ethyl Oleate

Figure II illustrates absorption data obtained with variously treated samples of ethyl oleate. The most significant changes brought about by oxidation are found in the regions of 2,300 and 2,750 angstroms, regions where conjugated dienes and trienes absorb most strongly. Unfortunately the very important changes that occur at 2,300 angstroms could not be accurately measured in these samples.

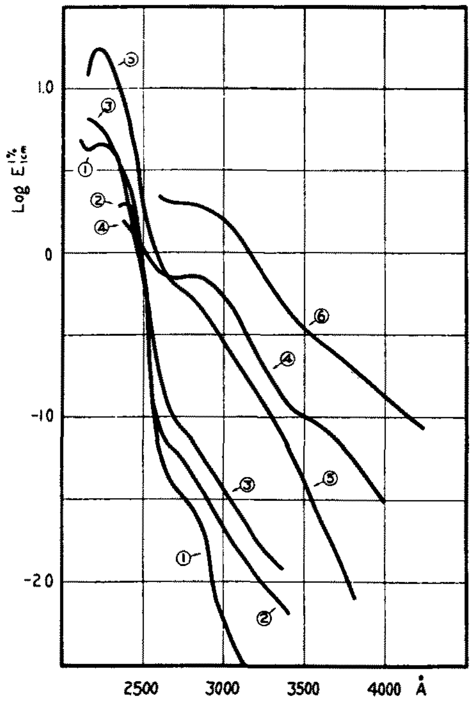

FIG. II. Absorption spectra of variously treated samples of ethyl oleate.

(1) Ethyl oleate in alcohol. P.V.=0.
(2) (1) in KOH medium.
(3) (1) exposed to air 6 days at 68°C., in alcohol, P.V.=725.
(4) (3) in KOH medium.
(5) (3) sealed under vacuum and kept 14 days at 110°C. in alcohol, P.V.=1.6.
(6) (5) in KOH medium.

Curves 1 and 2 are the spectra that were obtained with alcoholic and alkaline solutions of ethyl oleate, respectively. Curves 3 and 4 represent similar measurements made after the ethyl oleate had been oxi-

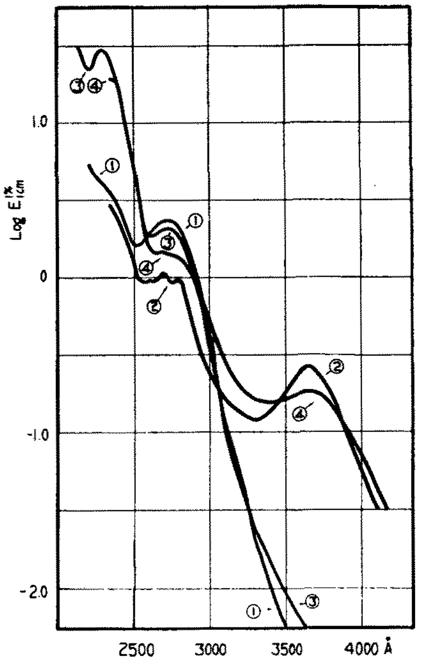

FIG. III. Absorption spectra of variously treated samples of ethyl linoleate.

(1) Ethyl linoleate in alcohol, P.V.=5.6.
(2) (1) in KOH.
(3) (1) exposed to air 70 hours at 63°C., P.V.=211.
(4) (3) in KOH.

dized to a peroxide value of 725 milliequivalents per kilogram by exposure to air for six days at 68°C. After the peroxides in the oxidized sample had been almost completely destroyed by heating in evacuated and sealed tubes for 14 days at 110°C., curves 5 and 6, using alcohol and alkali respectively, were obtained.

Among the conclusions that may be drawn from these curves, and from analogous curves for oleic and elaidic acids are that oxidation under these conditions produces only slight changes in the absorption characteristics of the monoethenoic compounds at 2,750 angstroms in alcohol but produces changes that show up markedly in alkali. Fortunately the relative increases in alkali are most pronounced during the earlier and less variegated stages of the autoxidation, for which the spectroscopic data should be most easily interpreted. As the oxidation proceeds to later stages, the relative increases in absorption brought about by the alkali treatment grow smaller at 2,750 angstroms, indicating that more stable secondary products which cannot be enolized or tautomerized have been formed.

In the case of a sample of oleic acid that was oxidized at 68°C. to a peroxide value of 72.5 and then heated under vacuum at 110°C. for eight days it was found that the absorption at 2,750 angstroms in an alkaline medium corresponded to a maximum conversion of 5% of the peroxides to conjugated trienes, assuming that in such a conversion one mol of peroxide yields one mol of double bonds. Close examination of the data and curves revealed that some of the increased absorption in alkali was probably due to chromophores other than conjugated trienes. After making due allowances for such other chromophores, there remained an increase in absorption at 2,750 angstroms corresponding to a conversion of 2% of the peroxides, conservatively estimated, to conjugated trienes. Since it is reasonable to suppose that there was a considerably greater proportion of conjugated dienes formed from the peroxides, it may be concluded that, under the conditions given, a relatively large portion of the peroxides are converted to oxygen-containing intermediates which by alkali treatment are convertible to tautomeric conjugated polyene derivatives. This is tantamount to saying that much of the peroxides may be converted directly to carbonyl compounds without breaking the carbon chain but does not exclude the addition of oxygen at the double bond followed by cleavage. The latter reaction is known to be favored by higher temperatures (5).

Studies were also made of the spectra of two alpha dihydroxystearic acids, two alpha ketolstearic acids, an alpha diketostearic acid, and an oxidostearic acid, all in pure form, and also after exposures to air at elevated temperatures for varying periods, in some cases (2). An analysis of the spectral curves indicated that none of these compounds nor any products derived from them by exposure to air at elevated temperatures were responsible to any appreciable extent for the increased absorption in oxidized oleates at 2,750 angstroms. The same statement is probably valid at 2,300 angstroms although the data at this wave length are not sufficiently accurate or complete to completely rule out such possibilities. The curves are such that in oxidized oleates the presence of dihydroxystearic and oxidostearic acids is not precluded, the latter type of compound having indeed been shown to be present in oxidized fats (6), but the presence of any alpha ketolstearic and alpha diketostearic acids in more than trace amounts is precluded.

Although these findings are in part negative in character, they eliminate certain types of compounds that have been previously postulated as intermediates in the autoxidation of oleates under these conditions and may be regarded as indirect confirmation of recent observations by others that oxygen also attacks the fatty acid chain at points other than the double bond.

Ethyl Linoleate

Preliminary studies have been made also on ethyl linoleate and several linoleic acids. Figure III illustrates the results obtained with a sample of ethyl linoleate that unfortunately had been oxidized to a slight extent before the experiment began. Curves 1 and 2 are the results of measurements on the original sample in alcohol and alkali, respectively. Curves 3 and 4 are corresponding results obtained after the linoleate had been oxidized to a peroxide value of 211 by exposure to air at 63°C. for 70 hours.

Although satisfactory measurements could not be made in the region of 2,300 angstroms due to the character of the solutions and the limitations of the spectrophotometer, it is apparent that oxidation produces significant changes in the absorption in this region, even in the absence of alkali.

Unlike the case of the monoethenoic compounds there were no significant changes in the absorption in the region of 2,750 angstroms, either in alcohol or in alkali. The reasons for this difference are by no means obvious since on the basis of some previously proposed mechanisms more actual or potential triene conjugation might reasonably be expected to exist in oxidized linoleates than in correspondingly oxidized oleates.

However, a speculative explanation may be given on the basis of the knowledge that the more easily developed linoleate peroxides, that is, the peroxides that have been postulated by Farmer and coworkers to be formed at the methylene carbon between the two ethylene groups (7), are relatively more stable than the oleate peroxides. If one may assume that the linoleate peroxides of this type are still further and relatively easily oxidized at the 8- and 14-carbon positions to give less stable peroxide groupings of the oleate type, it is conceivable that the natural type of linoleate when oxidized under proper conditions would yield only diene conjugation when treated with alkali whereas the oxidized oleate would yield some triene conjugation as well. It must be emphasized that on the basis of the meager evidence this explanation must be regarded at present as highly speculative.

In another experiment with ethyl linoleate conducted under more drastic conditions calculated to destroy the more stable peroxides, the results of which are illustrated in Figure IV, marked changes were found at 2,300 and 2,750 angstroms in alcohol which were not greatly increased in alkali, as shown in curves 3 and 4. Decomposition of the remaining peroxides resulted in additional slight increases, shown in curves 5 and 6. The changes in this study are difficult to interpret since there was a considerable rise in the absorption throughout the whole portion of the spectrum.

In both of the Figures III and IV the most important changes appear to have occurred at 2,300 angstroms, and were not alkali labile, suggesting that a stable diethenoid conjugation had developed.

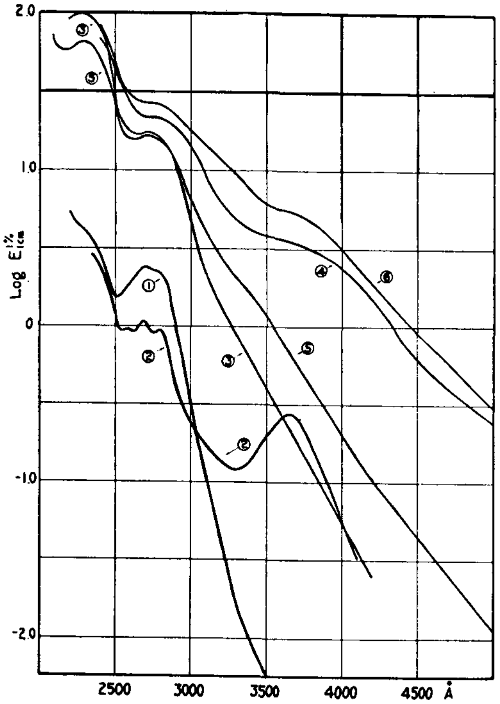

FIG. IV. Spectra of variously treated samples of ethyl linoleate.

(1) Ethyl linoleate in alcohol, P.V.=5.6.
(2) (1) in KOH.
(3) (1) exposed to air 192 hours at 110°C., P.V.=38.3.
(4) (3) in KOH.
(5) (3) sealed under vacuum and kept 312 hours at 110°C., P.V.=0.5.
(6) (5) in KOH.

Ethyl Linolenate

Figure V pictures the spectral changes that occurred in the oxidation of ethyl linolenate. The solid lines represent measurements that were made using alcohol as the solvent medium. The broken lines represent measurements in alkali. The two lowest curves, both labelled number 1, represent the data obtained for fresh ethyl linolenate in alcohol and alkali. The number 2 curves were obtained after the sample had been exposed to air at 63°C. for 60 hours. The number 3 curves were obtained after 138 hours under the same conditions.

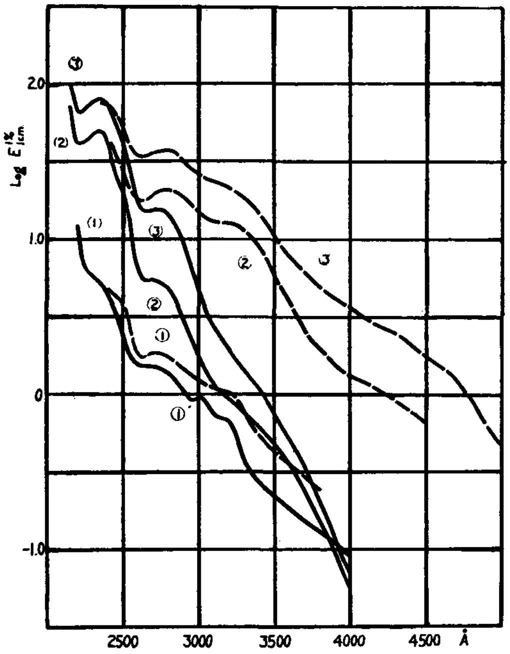

FIG. V. Spectra of variously treated samples of ethyl linolenate.

(1) Ethyl linolenate, unoxidized.
(2) (1) exposed to air 60 hours at 63°C.
(3) (1) exposed to air 138 hours at 63°C.
———————— in alcohol.
– – – – – – – – in KOH.

It is to be observed that the oxidation produces a considerable increase in the absorption at 2,300 angstroms, which, however, is not subject to any marked changes upon the addition of alkali. On the other hand, there are increases in the absorption at 2,750 angstroms which are enhanced by the addition of alkali.

Although the unoxidized linolenate exhibited maxima in the region of 3,100 or 3,200 angstroms, indicating presence of conjugated tetraenes as impurities, the latter disappeared during the oxidation. The two upper curves, however, indicate that alkali treatment of the oxidized compounds produced small amounts of conjugated tetraenes, probably by enolization of oxygen containing chromophores. Whether these tetraenes were derived from those originally present as impurities or from the linolenate is not known.

Summary

Because the measurements that have been described were designed as preliminary studies, a comparison of the spectral characteristics of ethyl oleate, linoleate, and linolenate at this stage can only be made along broad lines. At 2,300 angstroms the measurements for ethyl oleate were too inaccurate or incomplete to permit any comparisons but in the cases of the linoleate and linolenate oxidation produced marked increases in the absorption as measured in alcohol, which were not significantly altered by the addition of alkali.

At 2,750 angstroms the three esters showed marked differences in their behaviors. Quite highly oxidized ethyl oleate showed no appreciable increase in absorption in alcohol but developed a maximum at 2,750 in alkali. The linoleate after oxidation showed no appreciable increases in either alcohol or alkali unless oxidized under more drastic conditions calculated to destroy the more stable peroxides; in the latter case an increased absorption in alcohol was obtained which was not appreciably enhanced in alkali. With the linolenate, oxidation readily produced increases in the absorption in alcohol and a further marked increase occurred when alkali was added. Thus, under the conditions used, oleates and linolenates, to a much greater extent than linoleates, appeared to develop carbonyl derivatives from their peroxides which by the addition of alkali could be enolized to conjugated triene systems.

Supplementary studies indicated that in the cases of the oxidized oleates the increased absorptions at 2,750 could not be attributed to alpha dihydroxystearic or oxidostearic acids, which may, however, have been present. The presence of alpha ketolstearic and alpha diketostearic acids, except in trace amounts, was precluded.

In conclusion, no attempt has been made in the present report to thoroughly analyze the limited spectroscopic data that have been presented. The purpose has been rather to emphasize that there is promise of much knowledge to be gained concerning the course and mechanism of the autoxidation of fats, at least in the early stages, through spectroscopic studies.

REFERENCES

1. Filer, L. J., Mattil, K. F., and Longenecker, H. E., Paper presented at Meeting of American Oil Chemists' Society, New Orleans, May, 1944.
2. Holman, R. T., Lundberg, W. O., Lauer, W. M., and Burr, G. O., J. Am. Chem. Soc. *67*, 1285 (1945).
3. Holman, R. T., Lundberg, W. O., and Burr, G. O., J. Am. Chem. Soc. *67*, 1386, 1390 (1945).
4. Mitchell, J. H., Jr., and Kraybill, H. R., J. Am. Chem. Soc. *64*, 988 (1942).
5. Atherton, D., and Hilditch, T. P., J. Chem. Soc. *1944*, 105.
6. Deatherage, F. E., and Mattill, H. A., J. Ind. Eng. Chem. *31*, 1425 (1939).
7. Farmer, E. H., Bloomfield, G. H., Sudralingam, A., and Sutton, D. A., Trans. Faraday Soc. *98*, 348 (1942).

Modification of Vegetable Oils.

V. Relative Reactivities Toward Hydrogenation of the Mono-, Di-, and Triethenoid Acids in Certain Oils

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THE process of catalytic hydrogenation has long been the most useful available tool for modification of the chemical and physical properties of vegetable oils.

That the diethenoid (linoleic) acids in certain oils are hydrogenated much more readily than the monoethenoid (oleic) acids was perhaps first pointed out by Moore, Richter, and Van Arsdel (9) and has since become a matter of common observation.

The work of Richardson, Knuth, and Milligan (10) and of Hilditch and coworkers (5, 6) on the hydrogenation of marine oils has suggested that, as the number of double bonds in fatty acid chains is increased beyond two, there is not a further comparable increase in the readiness with which they add hydrogen. However, in the past it has not been possible to make any quantitative observations on the ease of hydrogenation of different unsaturated acids in natural fats because of the inadequacy of analytical methods for mixtures of such acids.

Recently a more satisfactory means of estimating certain polyethenoid acids has been provided by the spectral method of analysis of Mitchell, Kraybill, and Zscheile (8). One of the authors and other workers in this laboratory (4) have used the spectral method and other methods to determine the fatty acid composition of a number of series of hydrogenated cottonseed, peanut, soybean, and linseed oils. Selected portions of these data have provided a basis for calculation of the relative readiness with which hydrogen adds to oleic, linoleic, and linolenic acids, and certain isomers of these acids, upon catalytic hydrogenation of the oils.

Hydrogenation and Analysis of the Oils

The methods followed in preparing and analyzing the various oil samples are described in detail elsewhere (4). Of the oils with which the present discussion is concerned, the hydrogenated cottonseed and soybean oils were prepared under "selective" conditions of hydrogenation whereas one of the series of linseed oils was hydrogenated selectively (at 375° F. and 15 p.s.i. hydrogen pressure) and another was hydrogenated relatively nonselectively (at 250° F. and 60 p.s.i.). Hydrogenation was carried out in each case with a dry-reduced, electrolytically precipitated nickel catalyst (1), in the apparatus described previously (3). Each series represented samples withdrawn successively from a single batch of oil.

Analyses of the oils, in terms of their component fatty acids, are given in Table 1. The compositions of the cottonseed oils were calculated from iodine and thiocyanogen values. Briefly, in the case of the soybean and linseed oils, the method of analysis consisted of the following: *(a)* determination of the saturated acids by the Bertram oxidation method, *(b)* determination of linoleic and linolenic acids by the spectral method, and *(c)* estimation of total oleic and isolinoleic acids according to the preceding deter-

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