weak 3017.63 Å. iron line, and the same precaution is necessary.

Though no attempt was made to correlate metal content with processing procedure, presence of hydrogenation catalyst, etc., it is obvious from an inspection of Table III that the results obtained by the method would readily permit such studies. Most vegetable oils examined contained a very small trace of copper, usually less than 1 part in 10 million, probably about 0.03 to 0.04 part per million. In some samples as much as 0.1 to 0.3 part per million were found. There is some indication that these larger quantities were acquired during extraction. Similarly, tin is seldom found except in very small traces; and again, when present, it would appear to have been picked up from metal parts of the equipment during extraction or from metal containers during storage. Iron is frequently present in concentrations of a few parts per million, and manganese is often found in concentrations of a few tenths of a part per million. Nickel is found only in the hydrogenated oils where this metal has been used as a catalyst. It is, apparently, removed quantitatively if the hydrogenated products are rebleached. In general, bleaching, as would be expected by the use of an adsorbent, decreases the trace metallic content.

Correlations of the metal content of the oils and their stability are beyond the scope of this paper. Use of a method capable of determining the metal content of an oil to the sensitivity and with the accuracy indicated should, however, make such studies readily possible. The method described has been applied to a few fats, such as commercial shortening and lards. It is readily applicable to such materials and can, in fact, be used for any organic material having a low ash content.

Summary

The application of the line-width method to the spectrochemical analysis of oils and fats in conjunction with the use of an improved ashing technique has been shown to permit the quantitative determination of copper, iron, manganese, nickel, and tin in quantities as low as 1 part in 10 million.

The procedure has been critically examined by precision tests on commercial and experimental samples, by accuracy tests on synthetic samples, and by recovery tests.

Results of actual analysis of 30 vegetable oils and fats indicate the use of the procedure as a research tool.

The procedure may be used for the trace element analysis of organic material low in ash content.

REFERENCES

1. American Oil Chemists' Society, Official and Tentative Methods, 1946. Method Ca 11-46.
2. Coheur, Pierre. "A Method of Quantitative Spectrochemical Analysis Based on Line Widths." J. Opt. Soc. Am., *36*, 498 (1946).
3. O'Connor, R. T., Heinzelman, D. C., and Jefferson, M. E. "Preparation of Ash and Spectrochemical Determination of Traces of Metallic Elements in Oils, Fats, and Related Substances." J. Am. Oil Chem. Soc., *XXIV*, 185-189 (1947).

Ultraviolet Spectrophotometric Characteristics of Unhydrogenated Fish Oils*

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Introduction

WITH the exception of those of marine origin no fatty oils of commercial importance contain appreciable amounts of polyunsaturated acids with more than three double bonds. Beadle *et al.* (1) have shown that lard is characterized by the presence of up to about 0.6% of glycerides of the 20 carbon atom, tetraene acid known as arachidonic. The easy detection of this acid by ultraviolet spectroanalysis has been proposed as a means for the qualitative identification of lard in admixture with hydrogenated vegetable shortenings.

Several cases of suspected contamination, or adulteration, of commercial fat stocks have been brought to our attention during the period of shortage of such materials incident to and following World War II. The usual evidence, based upon odor and abnormal characteristics, such as high iodine value and positive insoluble bromides test, is not entirely reliable without confirmation, especially in the case of low grade stocks. Further tests, of a nature such as to exclude other possible contaminants, were sought in an examination of typical commercial fish oils.

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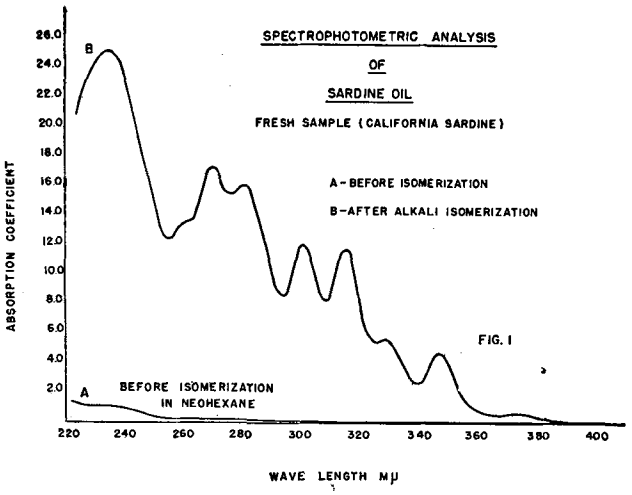
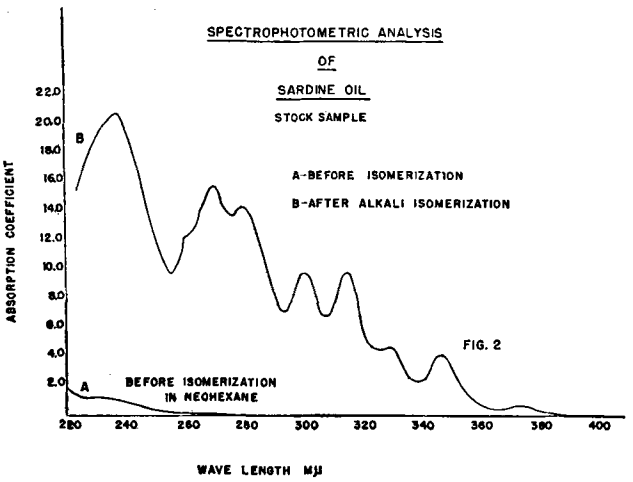
Experimental

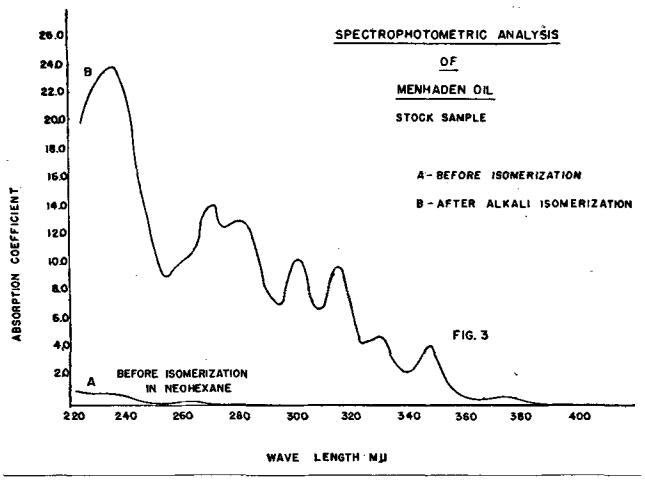
Samples of menhaden and sardine oils were procured from stocks in storage. A second sample of fresh sardine oil was obtained from a reliable source in California.

These oils were subjected to ultraviolet spectroanalysis by the methods prescribed by Brice, Swain, Schaeffer, and Ault (2). The only deviation from these excellent methods was the precaution of protecting the samples from oxidation during the alkali isomerization process by use of a blanket of purified nitrogen. This procedure has been found to improve the ultraviolet transparency of both blanks and samples. This permits utilization of a very narrow slit opening with improved detail in the absorption curves obtained.

The absorption curves of these oils are shown in Figures 1, 2, and 3. The curves marked "A" were obtained on solutions of the samples in neohexane and indicate only small amounts of conjugated constituents, nearly all of which are in the diene region. The curves marked "B" represent absorption after alkali isomerization to effect a considerable degree of conjugation. Typical absorption maxima may be noted for conjugated acids containing two, three, and







four double bonds, with additional maxima at about 346 and 375 millimicrons. While it cannot be stated with assurance, these latter maxima may indicate the presence of small quantities of acids with five, and possibly even six, double bonds.

Apparent composition was calculated in terms of the three polyunsaturated acids investigated by Brice *et al.* (Table 1). No claim is made for the accuracy of these values as indicative of the true composition of these oils. On the contrary, it is our opinion that the huge apparent arachidonic content, from 37 to 44%, is quite fictitious, being a result of the absorption induced in the conjugated tetraene region by the pentaene acid known as clupanodonic, whose constitution and presence in marine oils has been fairly

TABLE I

Apparent Composition
Calculated by the method proposed by Brice, Swain, *et al.*

	Sardine Oil (Fresh California)	Sardine Oil (Stock Sample)	Menhaden Oil (Stock Sample)	Linseed Oil (Crude)
Before Alkali Isomerization				
% Conjugated diene.....	0.87	0.96	0.67	0.24
% Conjugated triene.....	0.01	0.00	0.01	0.00
% Conjugated tetraene....	0.00	0.00	0.01	0.00
After Alkali Isomerization				
% Linoleic acid.....	15.3	9.52	15.4	17.0
% Linolenic acid.....	0.00	0.00	0.00	47.4
% Arachidonic acid.....	44.1	37.2	38.0	0.05

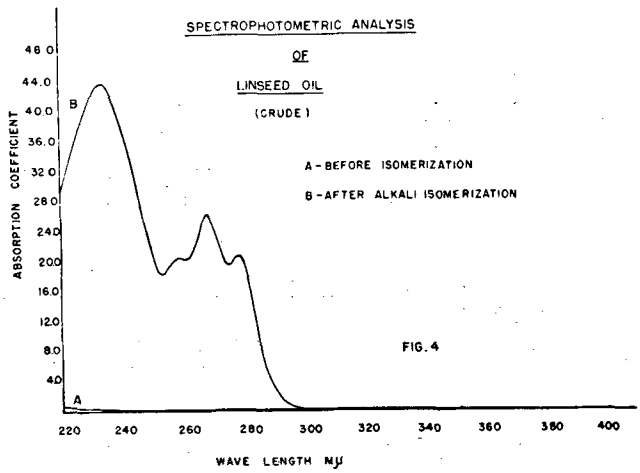
well established and is generally accepted (3, 4). The absence of apparent linolenic acid is undoubtedly the result of the enormous deduction from conjugated triene absorption to correct for the effect of conjugated tetraene and higher unsaturation. The correction to be applied is uncertain, and the calculations employed are unquestionably incorrect for such oils.

A drop of about 6% in apparent arachidonic acid content was noted in an analysis of the fresh California sardine oil after storage for one year in a refrigerator. It is well known that oxidation causes such an effect in ultraviolet absorption after alkali isomerization. For this reason the oils from storage tanks are believed to exhibit subnormal apparent arachidonic content. Consequently a value of about 40% apparent arachidonic may be taken as a fairly typical value for unhydrogenated fish oils. Applying this as a factor to a commercial oil or fat suspected of contamination or adulteration, it can be seen that the following formula affords a ready calculation of the amount of fish oil which may be present:

$$\% \text{ Unhydrogenated Fish Oil} = 2.5\ (\% \text{ apparent arachidonic acid} - 0.5)$$

The correction of 0.5% is included to compensate for the arachidonic normally present in unhydrogenated lard. This is a generous allowance, purposely set at close to the upper limit.

Fish oils and their oleines are not infrequent adulterants of raw linseed oil. The odors and ordinary characteristics of these oils are so similar that the presence of either is easily masked by the other. The absorption curve of a normal raw linseed oil is shown in Figure 4 and its composition in terms of polyunsaturated acids in the last column of Table 1. Even in such a difficult case the detection and estimation



of fish oil adulteration is fairly easy by use of the formula, with the 0.5 correction omitted.

Summary

Typical ultraviolet spectrophotometric absorption curves are presented for unhydrogenated fish oils of commercial importance, before and after alkali isomerization. These curves show the usual maxima for isomerized polyunsaturated fatty acids, including five, and possibly six, double bonds. Tetraene absorption is sufficient to obliterate that of trienes completely. Such a method of analysis affords an easy means of detecting unhydrogenated fish oil contamination in the ordinary vegetable and animal oils and fats as well as a test for the presence of fish oils in admixture with raw linseed oil.

Acknowledgment

The authors wish to express their appreciation for the assistance and encouragement given by Procter Thomson in this work.

REFERENCES

1. Beadle, Kraybill, and Stricker. Oil & Soap *22*, 50-51 (1945).
2. Brice, Swain, Schaeffer, and Ault. Oil & Soap *22*, 219-24 (1945).
3. "Protective and Decorative Coatings" (edited by Mattiello). Chapter 2 (by J. S. Long), "Drying Oils," esp. pp. 6 and 7 (1945).
4. "Fatty Acids." K. S. Markley, Chapter 2, pp. 35 and 36 (1947).

The Identification of the Fatty Acids of the Fat From a North American Black Bear[1]

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Introduction

RECENTLY Rasmussen, Morgal, and Miller (1) reported the physical and chemical constants of the fat of a North American black bear. Their values were within the range of those found for other species (2-7). They ascribed the differences of the various constants of the fats as due to nature of the fat in the ration consumed during the fattening processes. Their analyses showed the bear fat to contain 10.4% of the saturated fatty acids and 79.4% of unsaturated fatty acids. In view of the high percentage of the latter substance the study of bear fat was continued. The present paper deals with the identification of the various higher fatty acids of bear fat.

Materials

The bear fat used in this study was rendered on December 22, 1945, from adipose tissue taken from the shoulders, neck, and sides of a 4-year old North American male black bear weighing 287 pounds. The bear was killed near Adam's trail in Alger County, Michigan, on November 17, 1945. The fat was removed immediately after the bear was killed and then kept in the frozen state until it was rendered. This was accomplished by placing the fat in a kettle which was set into a container of boiling water and then removing the non-fatty material by straining through a cloth. The fat was a white semisolid at room temperature and had a slight lard odor.

The following constants were observed: melting point 22°C.; refractive index at 20°C. 1.4689, 40°C. 1.4613; specific gravity 100/15 .8776; saponification number 197.6; iodine number (Wijs) 96.0; thiocyanogen number 75.2; per cent free fatty acid (oleic) 6.3; per cent saturated acid 19.6 and per cent unsaturated acid 73.4.

The methods employed for these analyses are those found in the Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists (8) and and Vegetable Fats and Oils by Jamieson (9).

Experimental

Preparation of the Fatty Acids.

The bear fat (532 gm.) was saponified by refluxing for 4 hours with one liter of 98% ethanol containing 130 gm. of potassium hydroxide. After saponification the solution was diluted with 4 liters of distilled water and extracted with Skelly B for 6 hours in a continuous liquid-liquid extraction unit. The ethanolic-aqueous alkali solution was acidified with 188 ml. of concentrated hydrochloric acid and the solution extracted with diethyl ether for 6 hours. The ether was removed in vacuo at 16-mm. pressure and 35°C. The crude fatty acids were dried in vacuo at 4-mm. pressure and 60°C. for 4 hours. A yield of 510 gm. of fatty acid was obtained.

Preparation of the Methyl Esters of the Fatty Acids.

The fatty acids (510 gm.) were converted to the methyl esters by refluxing 4 to 6 hours with 1 liter of absolute methanol containing 5% sulfuric acid (by weight). The methanol was then removed in vacuo and the residual solution was diluted with water and neutralized with 10% sodium carbonate. The fatty acids were extracted with ether and the ether solution was washed with water to remove the last traces of sodium carbonate. The ether was removed in vacuo and the esters dried at 6-mm. pressure and 60°C. for 8 hours. A yield of 491.8 gm. of crude methyl esters was obtained.

Fractionation of the Methyl Esters of the Fatty Acids.

a) Description of Distilling Column. A four-foot Stedman column, having an internal diameter of 25 mm., was used for this study. It was provided with an electrical heating jacket to permit the column to be heated. The one liter distilling flask was heated by a Glas-Col electrical heater. The temperature of both the flask and column was regulated by means of an A. C. variable transformer. A D. M. Smith still head with an enclosed thermometer was used at the top of the column. The reflux ratio was regulated by a

[1] Published with the approval of the Director of the Michigan Agricultural Experiment Station as Journal article No. 970 (new series).