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## Application of the Line-Width Method to the Spectrochemical Analysis of Oils, Fats, and Related Substances

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IN a recent paper (3) the present authors described a method for the preparation of the ash of oils and fats together with a method of spectrochemical analysis of the ash for traces of certain metallic elements having sensitivity of about 2 to 4 parts of individual metals per million parts of the fat or oil. However, a sensitivity of about 1 part of a metallic trace element in 10 million parts of a sample is essential in studies on the stability of oils and fats. The present communication describes a shortened and improved procedure for the preparation of ash and the application of the newly proposed line-width method (2) for the evaluation of the spectrograms. Data are presented from critical studies of the sensitivity, precision, and accuracy of the procedure for the quantitative determination of copper, iron, manganese, nickel, and tin in various types of vegetable oils and fats with a sensitivity of about 1 part in 10 million.

### Preparation of Ash

Wet washing methods, involving the use of mineral acids and chemical glassware, were investigated and proved unsatisfactory. The recently adopted tentative method of the American Oil Chemists' Society for the determination of ash in oils and fats (1), in which the oil is heated and then ignited on the surface, was studied in some detail. As shown in Table I, use of this method for ashing and subsequent determination of the trace metals by spectrochemical analysis showed consistently low results in comparison with a more adequate ashing procedure.

The ashing procedure previously proposed (3) was reinvestigated for the purpose of simplification and increasing the sensitivity. Through the use of the line-width method (2) of evaluation of spectrograms the incorporation of internal standards into the ash is not required. By the elimination of this step the over-all time of ashing can be reduced from about 72 to about 48 hours with the need of less personal attention.

The most obvious means of increasing the sensitivity is to increase the size of the sample and at the same time reduce the amount of the magnesium ni-

trate used to perform the triple function of ashing-aid, carrier, and spectroscopic buffer. The ratio of 2.5 grams of oil to 1.25 grams of magnesium nitrate has been changed to 16.67 grams of oil and 0.50 gram of magnesium nitrate, providing approximately a twenty-fold increase in this ratio, and permitting the quantitative determination to a sensitivity of about 1 part of metal in 10 million parts of sample.

The abbreviated ashing procedure follows. A sample of exactly 16.67 grams of the oil or fat is accurately weighed into a 100-ml. Vycor dish (90 mm. across the top) and 0.50 gram of magnesium nitrate in alcoholic solution [2 ml. of a solution of 250 grams  $Mg(NO_3)_2 \cdot 6H_2O$  per liter of 95%  $C_2H_5OH$ ] is added. The dish is covered with an inverted short-stemmed Pyrex funnel, whose maximum diameter is less than the maximum diameter of the dish. The sample is heated on a hot plate and the temperature gradually and cautiously raised until the maximum temperature of the hot plate, approximately 300°C., is attained, and the heating is continued overnight. The charred sample is then ashed in a muffle furnace, with an initial temperature of 225° and increasing in increments of 25° at 30-minute intervals until a temperature of 450° is reached. The samples are held at this temperature in the muffle furnace overnight and then cooled and quantitatively transferred to a small mortar with the aid of a camel's hair brush. The ash is finely ground and very thoroughly mixed and is then ready for the spectrochemical analysis.

### Spectrochemical Analysis by the Line-Width Method

The spectrograms were prepared exactly as described in the earlier paper (3) except that a step-sector was no longer required. All the precautions previously discussed in detail—such as complete absence even of traces of the elements to be determined from all reagents, from glassware and from the carbon rods after their preparation and handling, and very careful mixing of the prepared ash—must be maintained if accurate results are to be obtained. Fig. 1 shows the spectrograms of a number of vegetable oils photographed on a single plate for evaluation by the line-width method. As the step-sector,

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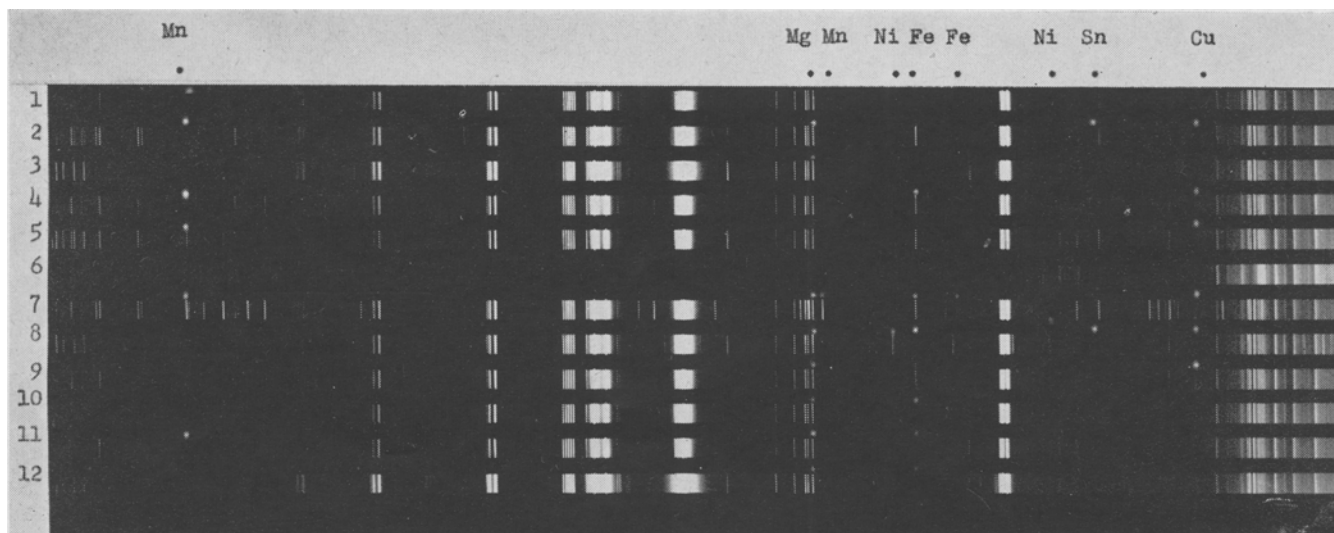


FIG. 1. Spectrochemical Vegetable Oil Analysis. A spectrographic Plate Showing Spectrograms of Typical Vegetable Oils.

- |                              |                                 |
|------------------------------|---------------------------------|
| 1. Calabash seed oil (crude) | 7. Rice bran oil (crude)        |
| 2. Cottonseed oil (crude)    | 8. Rice bran oil (hydrogenated) |
| 3. Cottonseed oil (refined)  | 9. Sesame oil (crude)           |
| 4. Okra seed oil (crude)     | 10. Shortening (commercial)     |
| 5. Peanut oil (crude)        | 11. Soybean oil (crude)         |
| 6. Pure carbon rod           | 12. Soybean oil (bleached)      |

which necessitates a longer line, is not required, 12 spectra can be photographed on a single plate.

The line-width method of quantitative spectrochemical analysis has been described by Pierre Coheur (2). It is based on the principle that if the physical and instrumental factors which normally affect line width, such as natural width, Doppler effect, Stark effect, pressure broadening, self-absorption effects, size of source, path of the light beam through the spectrograph, diffraction by the slit, aberrations of the optical parts, diffusion of light in the photographic emulsion, etc., are all maintained rigorously constant, the physical width of any line will remain constant. Consequently, if the physical width of a major line of the spectrogram—for example, a magnesium line in the spectrogram of our magnesium oxide-carbonate buffered ash—is measured in microns at a density corresponding to the maximum density of a line of the element to be determined, the resulting width will be dependent only upon the concen-

tration of this element. This principle is shown schematically in Fig. 2.

In the present work Coheur's method of obtaining line widths has been modified to take advantage of the automatic recording microphotometer. The spectrogram is densitometered as usual through the selected line of the major element, i.e., magnesium, and through all of the selected lines of the elements to be determined. From these profiles the widths in millimeters can be read directly, thus eliminating the need for a wave length comparator. Fig. 2 is a reproduction of a portion of such an actual microphotometer tracing, showing the magnesium 2942.11 Å. line and the manganese 2949.20 Å. line for two different concentrations of manganese.

Working, or calibration, curves were prepared by ashing samples of a molecularly distilled oil, shown to be free of metallic impurities (3), containing a series of added amounts of the elements to be determined. The spectrograms and their microphotometric

TABLE I  
Spectrochemical—Vegetable Oil Analysis Comparison of Ashing Techniques

Sample	Determination of Trace Elements—Parts Per Million											
	Copper			Iron			Manganese			Nickel		
	Av. Conc. P. P. M. Ashing Technique No. 1	Av. Conc. P. P. M. Ashing Technique No. 2	Av. Conc. P. P. M. Ashing Technique No. 3	Av. Conc. P. P. M. Ashing Technique No. 1	Av. Conc. P. P. M. Ashing Technique No. 2	Av. Conc. P. P. M. Ashing Technique No. 3	Av. Conc. P. P. M. Ashing Technique No. 1	Av. Conc. P. P. M. Ashing Technique No. 2	Av. Conc. P. P. M. Ashing Technique No. 3	Av. Conc. P. P. M. Ashing Technique No. 1	Av. Conc. P. P. M. Ashing Technique No. 2	Av. Conc. P. P. M. Ashing Technique No. 3
Crude peanut oil from Texas.....	0.20	< 0.1	0.13	4.25	1.48	2.70	0.66	0.33	0.51			
Refined, bleached cottonseed oil.....	< 0.1	0	0									
Refined, bleached cottonseed oil hydrogenated, rerefined.....										6.73	3.42	5.23
Crude cottonseed oil from Arkansas.....							0.36	0.26	0.27			
Refined, bleached, and hydrogenated cottonseed oil with added hard fat, rerefined and rebleached.....				0.36	0.20	0.16						

Ashing Technique No. 1—As recommended in this communication.  
Ashing Technique No. 2—Method of A.O.C.S. Committee on Analysis of Commercial Fats and Oils,  $Mg(NO_3)_2$  added after ashing, *Journal of the American Oil Chemists' Society*, XXIV, 76 (1947).

Ashing Technique No. 3—Method of A.O.C.S. Committee on Analysis of Commercial Fats and Oils,  $Mg(NO_3)_2$  added before ashing, *Journal of the American Oil Chemists' Society*, XXIV, 76 (1947).

Values given for concentrations found are averages for at least four independent determinations by each ashing technique.

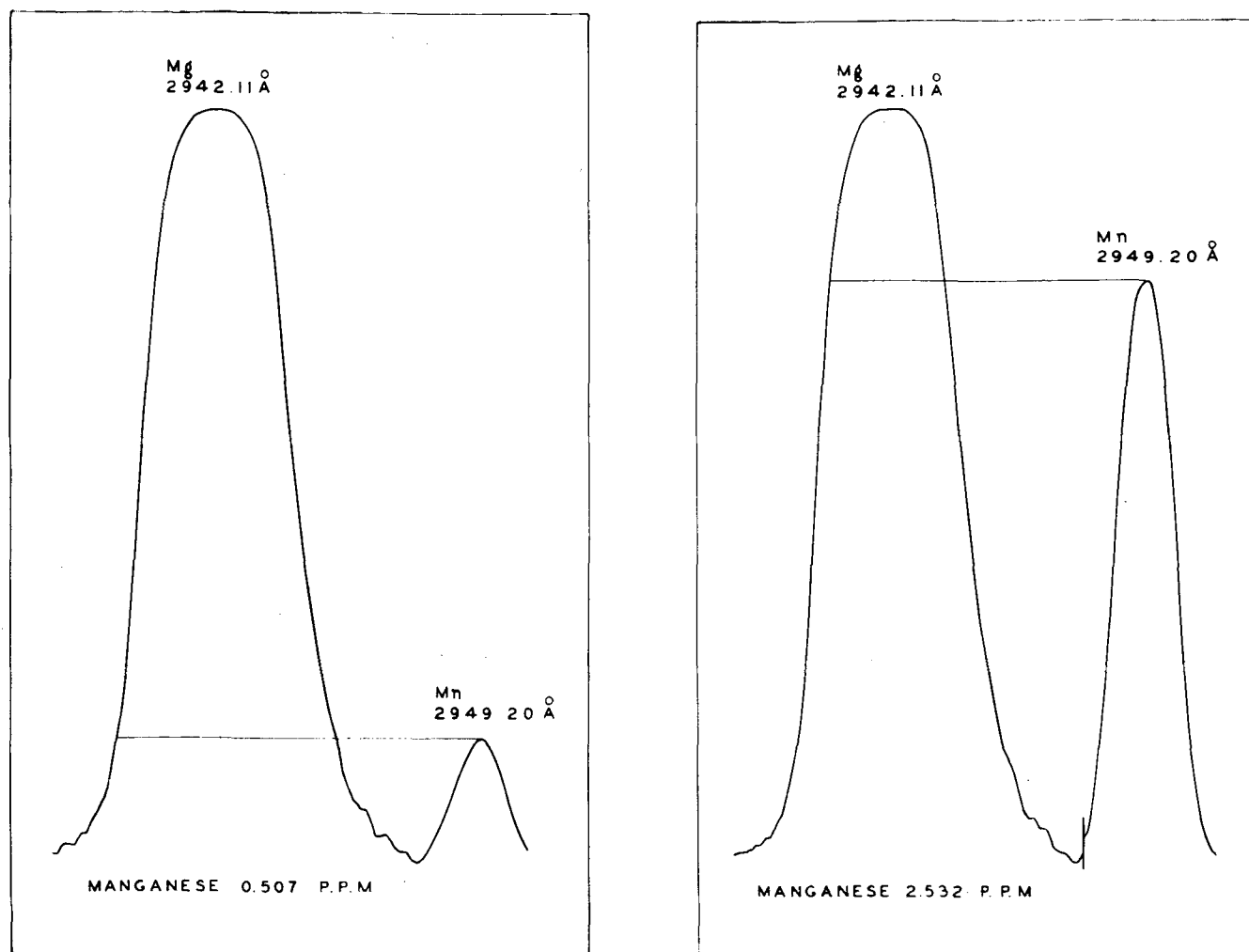


FIG. 2. Spectrochemical—Vegetable Oil Analysis. The Photometric Line-Width Method.

tracings were obtained and from the latter the widths of the profiles of the selected magnesium lines were measured in millimeters. These widths for a series of concentrations of the elements were plotted against the logarithms of the known concentrations and the smooth curves shown in Figs. 3 and 4 were obtained. Fig. 3 shows the working curves for the five elements studied at the lowest concentration range, that were usually encountered in actual vegetable oil analyses. Fig. 4 shows a series of curves for copper covering the concentration range from 0.1 to 4,000 parts per million. Similar curves have been prepared for each of the other four elements. The lines selected for the analyses with their accompanying selected magnesium line and the concentration range covered by each are given in Table II. The most probable interferences in the various determinations listed in the table will be discussed later.

Presumably the lower range might be extended by further increasing the amount of sample ashed with the 0.50 gram of magnesium nitrate buffer, if there is any practical value in precise measurements below a concentration of 1 part of the trace elements in 10 million parts of vegetable oil. The practical lower limit is reached on one hand when the amount of oil to be ashed is too great to permit sufficiently rapid ashing for routine analysis and on the other where the amount of magnesium nitrate ashed is too low to

TABLE II  
Spectrochemical—Vegetable Oil Analysis. Spectrum Lines Selected for Various Analyses

Element	Line Identification Angstrom Units	Mg. Line Angstrom Units	Range With 16.67 g. Sample P. P. M.	Range With 2.5 g. Sample P. P. M.	Most Probable Interferences Angstrom Units
Cu	2961.16 Cu I	2942.11		400-4000	
Cu	3273.96 Cu I	3336.68	1 - 30	10- 600	
Cu	3273.96 Cu I	2942.11	0.1- 1		
Fe	3017.63 Fe I	3336.68		400-4000	3017.88 Pt 3017.57 Cr I
Fe	3020.64 Fe I	3336.68	0.1- 5.0		3020.67 Cr I
Fe	3057.45 Fe I	3336.68	4.0-150	60-2400	3057.64 Ni I
Mn	2576.10 Mn II	3336.68	0.1- 1.5		
Mn	2949.20 Mn	3336.68	0.5- 12		
Mn	3054.36 Mn	3336.68	12 -260	200-4000	3054.32 Ni I
Sn	2571.59 Sn	3336.68	10 -240	160-4000	
Sn	3175.02 Sn I	3336.68	0.3- 15		
Ni	2943.91 Ni I	3336.68	10 -240	160-4000	
Ni	3002.49 Ni I	3336.68	0.3- 40		3002.27 Pt I

provide enough ash to obtain a satisfactory spectrogram. There is no upper limit. A ten-fold increase from 4,000 parts per million (that is, 0.4%) to 4% and a second similar ten-fold increase to 40% can readily be attained either by decreasing the amount of vegetable oil and increasing the amount of magnesium nitrate buffer or by selecting weaker spectrum lines for the analysis, or by both. The practical upper limit is at the value where the usual chemical methods can provide the analysis with a greater accuracy than the spectrochemical methods, a concentration of about 4%.

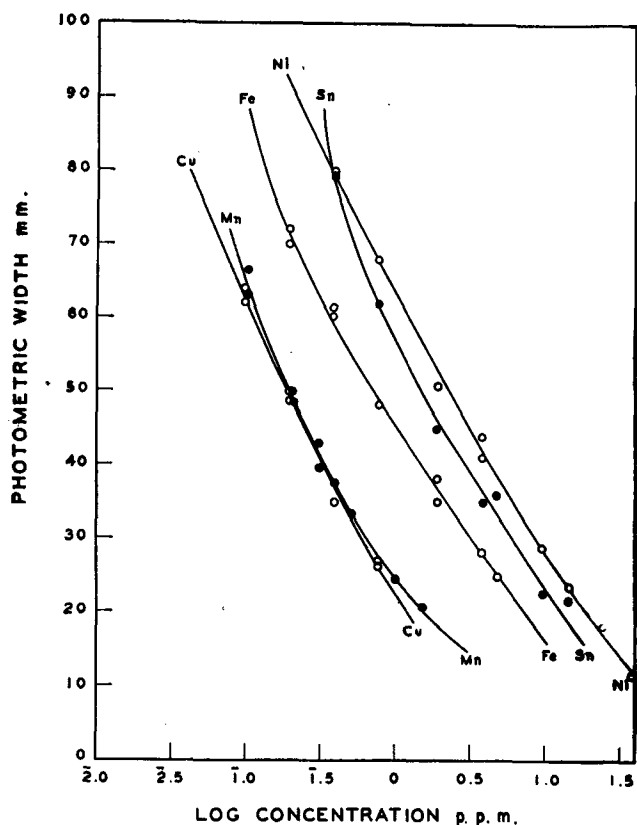


FIG. 3. Spectrochemical—Vegetable Oil Analysis. Working Curves, Lowest Concentrations.

Working directions for the preparation and evaluation of the spectrograms follow. Exactly 5 mg. of the sample ash are weighed into the electrode crater and the sample is completely consumed by arcing in a direct-current arc at 235 v., 20 a., for exactly 90 seconds. The excited sample is photographed on Eastman Spectrum Analysis No. 1 plates, using a large Littrow spectrograph set to photograph the ultraviolet region from 2500 to 3500 Å. Any spectrograph which will cover this range with equal or higher dispersion could, of course, be used. The processed plates are densitometered with a recording microdensitometer through the selected magnesium lines and all the selected lines of the elements to be determined. From measurements of the width of these magnesium lines at the maximum densities of each of the lines of the elements being determined, by use of the previously prepared working curves, the concentrations of the elements in parts per million are obtained.

#### Results and Discussion

The results obtained from at least 2 to 4 completely independent analyses of 30 samples of vegetable oils and fats are listed in Table III. The table gives the mean value obtained for each element indicated, the number of analyses made for each element, and the average per cent deviation of the individual values from the mean. When the amount of element found is of the order of 0.1 or 0.2 of a part per million in the oil, the deviation from the mean will be seen to be of the order of 10 to 15% of the amount found. If the concentration is of the order of 1 to 5 parts per million, a deviation of only from 3 to 9% is encountered. This deviation is based on the average value found for the particular element. For exam-

ple, in the analysis of a crude peanut oil from Texas, 16 determinations were made for copper with a mean value of 0.20 parts per million and an average deviation of 16%. This means that the expected value of copper in this sample upon subsequent analyses should be within the range of 0.17 to 0.23 parts per million. Similarly, the average of the two values found for the iron content of the crude cottonseed oil from Mississippi of 3.02 with deviation of 6.6% (which is about the average deviation for this concentration range) implies that the values for this element in this sample lie within the rather close range of 2.9 to 3.2 parts per million.

In Table IV are given the results obtained by applying the method to the analysis of oils of known metal concentration. These samples were prepared by adding to metal-free molecularly distilled oil, traces of each of the five elements studied in varying amounts, covering and extending beyond the concentrations usually found in vegetable oil samples. Approximately 20 analyses have been made for each element. The results indicate that the accuracy with which these metals can be determined in vegetable oils, within the concentration range from 0.1 to about 250 parts per million, lies between the mean values of 3.93% for manganese to 8.02% for tin.

As further tests of the accuracy of the procedure, additional amounts of the metals found to be present were added to oils selected from those listed in Table III, and these samples were reanalyzed. Results of these recovery tests are given in Table V. From the results of the tests for precision described earlier, variations of twice the 10 to 15% for the lowest concentrations, or of the 3 to 9% for the concentrations

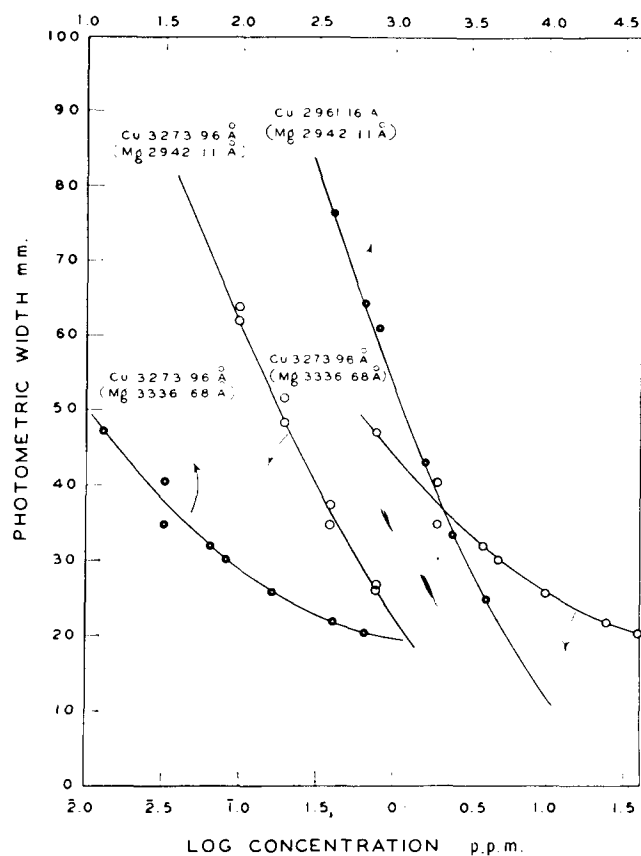


FIG. 4. Spectrochemical—Vegetable Oil Analysis. Working Curves for Copper from 0.1 to 4000 p.p.m.



above 1 part per million, must be expected as statistical extremes. The values in Table V show that the recoveries obtained were well within these limits.

The unexpected result from the data in the table is that, in nearly every case, the recovery is low. In attempts to locate the sources of error, tests were made not only by repeatedly analyzing the oils but also by multiple examinations of the same ash and multiple evaluations of the same spectrograms. These tests indicate that over half of the total variation was incurred in the ashing step. Repeated analysis of the same ash resulted in average deviations of a magnitude less than half that encountered for the whole procedure. Repeated evaluations of the same spectrogram showed that this step contributed nothing significant to the total variation. This last test constitutes a verification of the satisfactory performance of the line-width method for the evaluation of spectrum lines. These results coupled with the usual low values obtained in recovery tests indicate that, even with the slow and careful ashing technique employed, very small losses may occur during ashing.

In Table II are listed the spectrum lines most likely to interfere with the determinations as described, using an instrument having a dispersion

in the ultraviolet region from 2500 to 3500 Å. of from 2 to 8 Å. per millimeter. The most serious interference is that of the chromium line at 3020.67 Å. with the most persistent iron line at 3020.64 Å. In the presence of any considerable amount of chromium this means that the sensitivity for which iron could be determined would be somewhat less than 1 part in 10 million. Actually, chromium has not been found in the vegetable oils analyzed. However, if it should be present in quantities sufficient to interfere with the determination of small traces of iron as described, substitution of the iron line at 3021.07 Å. could be made with no great loss of sensitivity.

Chromium also interferes with the iron line at 3017.63 Å. and nickel interferes with both iron at 3057.45 Å. and with manganese at 3054.36 Å., but in these cases the interferences are with the weaker lines of the element being determined and if sufficient chromium or nickel is present to cause interferences, substitutions of other lines can readily be made.

Platinum interferes with the most sensitive line of nickel and, if small traces of this element are to be obtained, the use of platinum dishes and spatulas must be avoided. Platinum also interferes with the

TABLE IV  
Spectrochemical—Vegetable Oil Analysis. Analyses of Known Samples

Line	Amount in Sample	Amount Found Spectrochemically	Difference
$\lambda$ in Å.	p.p.m.	p.p.m.	Per cent
Copper			
2961.16	24.0	25.0	4.17
3273.96*	9.60	8.80	8.33
3273.96*	4.80	4.40	8.33
3273.96*	3.84	4.04	5.21
3273.96*	0.768	0.759	1.17
2961.16	144.0	148.0	2.78
2961.16	24.0	25.0	4.17
2961.16	38.4	36.7	4.43
3273.96	0.096	0.100	4.17
3273.96*	9.60	8.87	7.60
3273.96*	4.80	4.06	15.40
3273.96*	0.768	0.851	10.80
2961.16	240.0	238.9	0.46
Average			5.92
Iron			
3057.45	3.84	4.10	6.77
3057.45	4.80	4.82	0.42
3020.64	0.768	0.713	7.16
3020.64	3.84	4.04	5.20
3020.64	4.80	5.32	10.80
3057.45	4.80	4.74	1.25
3020.64	4.80	4.64	3.33
3057.45	38.4	39.5	2.86
3057.45	24.0	21.8	9.17
3057.45	96.0	98.5	2.60
3017.63	96.0	94.7	1.35
3057.45	24.0	22.0	8.33
3057.45	38.4	39.8	3.65
3017.63	24.0	22.3	7.08
3017.63	38.4	40.4	5.21
3017.63	24.0	24.0	0.00
Average			4.70
Tin			
2571.59	48.0	44.5	7.29
3175.02	0.384	0.397	12.20
3175.02	1.92	1.92	0.00
3175.02	9.60	10.19	6.15
3175.02	14.40	12.19	15.30
2571.59	14.40	12.25	14.90
2571.59	192.0	181.2	5.63
2571.59	240.0	265.0	10.40
2571.59	192.0	173.8	9.48
3175.02	0.384	0.387	0.78
3175.02	0.768	0.721	6.12
Average			8.02

Line	Amount in Sample	Amount Found Spectrochemically	Difference
$\lambda$ in Å.	p.p.m.	p.p.m.	Per cent
Manganese			
2576.10	0.507	0.508	0.20
2576.10	1.02	1.10	7.84
2949.20	1.02	1.10	7.84
2949.20	7.62	7.47	1.97
2949.20	12.66	12.59	0.55
3054.36	12.66	12.98	2.53
3054.36	26.28	26.19	0.34
3054.36	26.34	27.43	4.14
3054.36	52.68	48.21	8.49
2949.20	0.507	0.507	0.00
3054.36	210.6	216.8	2.94
3054.36	263.4	251.3	4.59
2949.20	1.014	1.014	0.00
2949.20	12.66	11.17	11.77
2576.10	0.101	0.101	0.00
2576.10	1.518	1.406	7.38
2949.20	1.518	1.507	0.72
2576.10	0.507	0.455	10.26
2576.10	1.014	9.73	4.04
2576.10	1.518	1.439	5.20
3054.36	26.34	26.80	1.75
Average			3.93
Nickel			
2943.91	144.0	142.3	1.18
2943.91	192.0	205.7	7.14
2943.91	14.40	15.04	4.44
2943.91	24.00	24.50	2.08
2943.91	38.40	41.5	8.07
2943.91	48.00	46.0	4.17
2943.91	192.0	188.9	1.61
3002.49	9.60	10.00	4.17
3002.49	14.40	14.90	3.47
3002.49	24.00	22.19	7.54
3002.49	38.40	42.9	11.70
2943.91	14.4	15.7	9.03
2943.91	24.0	23.9	4.17
2943.91	192.0	208.0	8.33
3002.49	0.384	0.370	3.65
3002.49	3.84	4.20	9.38
3002.49	9.60	10.92	13.80
3002.49	14.40	16.34	13.50
2943.91	192.0	215.3	12.10
3002.49	0.384	0.461	4.43
3002.49	9.60	8.87	7.60
Average			6.74

\* For these determinations of copper the reference line was Mg. 3336.68 Å. For all other determinations the Mg 2942.06 Å. line was used. All values within the ruled black lines were obtained from the same spectrographic plate.

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 ac-

curacy 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.

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## 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.

### 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

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