Gas-Liquid Partition Chromatography and Ultraviolet Absorption of Oils Before and After Hydrogenation

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Summary

Analyses of various natural fats and oils have been compared by G.L.P.C. and ultraviolet spectrophotometry. Good agreement was found for linoleic acid in unhydrogenated samples. The ultraviolet method gave lower results for linolenic acid. For hydrogenated oils the ultraviolet method gave lower results for both linoleic and linolenic acids in most instances where an appreciable amount of these components was present.

It is known that the ultraviolet method measures only the naturally occurring isomers of the unsaturated fatty acids while the G.L.P.C. analysis includes all the isomers. This would account for the difference in responce between unhydrogenated and hydrogenated oils by the two methods of analysis.

THE American Oil Chemists' Society Official Method Cd 7-58 (6) for polyunsaturated acids by ultraviolet absorption spectrophotometry does not apply to a variety of fats and oils. As stated in the method it is not applicable, or is applicable only with specific precautions, to modified drying oils, hydrogenated oils, or other fats containing *trans* isomers of the unsaturated fatty acids; to fish oils, or similar fats containing acids more highly unsaturated than pentaenoic; to crude oils, or unusual samples containing pigments whose absorption undergoes changes during the alkali procedure, or to fats and oils containing large quantities of preformed conjugated fatty acids. In addition, this method allows the determination of saturated and monounsaturated acids only from iodine values and polyunsaturated values. Errors in these determinations would result in errors in the saturated and monounsaturated acids.

The science of gas-liquid partition chromatography (G.L.P.C.) introduced by James and Martin (4) only a few years ago has advanced so tremendously during the past few years that it is now being used routinely for fatty acid analysis of fats and oils in many laboratories. Materials volatile at the column temperature are distributed between a moving gas phase and a stationary liquid phase. Different constituents move through the column at different rates because of differences in partition coefficients. Using a suitable detection system the concentration of eluate in the effluent gas is plotted against time. Polyunsaturated, monounsaturated, and saturated acids can easily be separated both as to chain length and degree of unsaturation. A quantitative determination of each component is readily obtained.

Craig and Murty (2) reported on the comparison of the ultraviolet method with G.L.P.C. for unhydrogenated vegetable oils. The ultraviolet method in all cases gave higher results for linoleic acid than did G.L.P.C. Oils analyzed were corn, sunflower, soybean, linseed, olive, and peanut.

Herb *et al.* (3), on the other hand, found a good correlation between the ultraviolet method and G.L.P.C. for linoleic and linolenic acids in unhydrogenated lard, and in olive, sesame, and safflower oils.

In this study a comparison of the two methods of analysis for polyunsaturated fatty acids was carried out using oils hydrogenated by different methods. Analyses of unhydrogenated fats and oils are included to illustrate the difference between hydrogenated and unhydrogenated oils, and to supplement the information given by Craig and Murty and Herb *et al.* and therefore help to resolve the courtadictory conclusions reached by these two groups.

Experimental

The G.L.P.C. studies were performed using a Barber-Colman Model 10 gas chromatograph. An ionization-type detector cell containing approximately 56 microcuries of radium-226 was used with an ionization voltage of 900 volts. Separate heating and thermostating were employed for the flash heater at the injector block, the column, and the cell. The following conditions were employed: column temperature 180° C.; detector cell 210°C.; flash heater 235°C. A 2-millivolt 10-inch strip chart recorder having a chart speed of 20 inches per hour with detector signal attenuation from 1×10^{-5} to 1×10^{-9} amperes full scale was used. All experimental conditions were maintained constant throughout the analysis. Samples of methyl esters of fatty acids were dissolved in normal hexane to make a 1% solution from which a 2-microliter sample was applied to the column using a microsyringe. Quantitative results using the ionization detector depend upon the proper choice of the linearizing resistor and the use of a small sample size. It was found that the use of a 10,000 megohm resistor inserted into the detector circuit in series with the cell resulted in good quantitation of a standard mixture of methyl esters of the common fatty acids. Also it was found necessary to use a small sample not exceeding about 0.06 microliters of methyl ester.

A Pyrex glass column 6.5 ft. long with 0.25 in. inside diameter was employed. The packing used was 60–100 mesh Chromosorb W coated with 20% W/W succinic acid-diethylene glycol polyester. The argon carrier gas pressure was 20 p.s.i. with an outlet flow of 155 ml. per minute as measured with a soap bubble meter.

The ultraviolet analyses were performed using a Beckman DU spectrophotometer and following the A.O.C.S. Method Cd 7-58 (6) where isomerization is carried out with 6.6% KOH for 25 min. at 180°C. It has been our experience that on some samples this method will give erratic results when the linolenate values are appreciable. On such samples the linoleate values were high, and the linolenate values were low, in relation to the iodine value and normal expectancy.

Cottonseed and soybean oils were hardened both selectively and nonselectively. In selective hardening the polyunsaturated fatty acids of the oils are

hydrogenated to oleic acid before an appreciable amount of the oleic acid goes to stearic acid. There is an excellent review of this subject (1).

The method of Merker (5) was used for nonselective hydrogenation. The oil charge of 3,000 grams was placed in a hydrogenator. Approximately 3 ml. of a 10% aqueous citric acid solution were added to the oil charge and the mixture agitated for several minutes. An absorbent composition containing 0.1% carbon and 0.1% diatomaceous earth was added to the acidified oil and the mixture heated to 135°C. after evacuation of the reaction vessel. About 0.1% nickel catalyst was added and the charge heated to about 180 to 200°C. The hydrogenation was carried out at a pressure of 20 p.s.i. The reaction was stopped after 30 min. at which time the refractive index of the oil indicated that it was at a level of hydrogenation equivalent to that of its selectively hydrogenated counterpart.

Results and Discussion

The gas chromatograph was standardized, using known pure fatty acid methyl esters and mixtures (Table I). Quantitative results on the standards

TABLE I

G.L.P.C. Analysis of a Known Mixture of Methyl Esters of Fatty Acids

	% Fatty Acid				
	C16:0	C18:0	C18:1	C18:2	C18:3
Known	20.0	20.0	20.0	20.0	20.0
G.L.P.C.[a]	20.1	19.8	20.2	20.2	19.7

[a] Average of 3 runs.

were calculated by the triangulation method and agreed well with the known composition. The methyl esters of commercial fats and oils were obtained by refluxing with an excess of methanol in the presence of sodium hydroxide, and their composition was determined in a similar manner. In Table II are shown the values for polyunsaturated fatty acid composition of unhydrogenated fats and oils by G.L.P.C. and by ultraviolet analysis, together with iodine values. The linoleate values are nearly the same by the two methods. In safflower oil the difference approaches 5% of the value, but here the total linoleic acid is considerably higher than in most oils. On the other hand the linolenate values are definitely lower by ultraviolet on the oils containing an appreciable quantity of linolenic acid. Small amounts of arachidonic acid were not accurately determined by G.L.P.C.

Polyunsaturated fatty acid compositions of hydrogenated fats and oils by G.L.P.C. and ultraviolet analysis are shown in Table III together with iodine values. The linoleate values again are practically the same by the two methods for hydrogenated lard and tallow, but are lower for hydrogenated soybean, cottonseed, and linseed oils when determined by the ultraviolet method. Hydrogenated linseed oil, the only sample run having an appreciable amount of linolenate, had a lower value of linolenate when determined by the ultraviolet method.

It is known that the ultraviolet method for polyunsaturated fatty acids is primarily sensitive to the natural isomers of those acids while the G.L.P.C. method determines all of the isomers. Thus both methods yield the same results for unhydrogenated oils which contain only the natural isomers of fatty acid. Hydrogenation of oils however does produc isomerization of the unsaturated acids. It is this formation of isomers during hydrogenation which could account for the difference in analysis between the two methods for any hydrogenated oils. The lard and tallows were hydrogenated to such a small extent however that the differences in analysis between the methods were too small to be considered with any degree of reliability.

There are the usual differences between high and low selectivity of hydrogenation of vegetable oils, specifically in that the polyunsaturated acids are greater and oleic acid lesser in concentration for low selectivity than for high. These differences are more pronounced for the soybean oil than for the cottonseed oil since the soybean oil has been subjected to relatively more hydrogenation than has the cottonseed oil.

There seems to be little difference between high and low selectivity of hydrogenation as far as positional isomerization of fatty acids is concerned. The

TABLE II

Polyunsaturated Fatty Acid Composition of Unhydrogenated Fats and Oils by G.L.P.C. and Ultraviolet Analyses

	Iodine value (Wijs)	% Fatty Acids						
		G.L.P.C.				U.V.		
		C18:0	C18:1	C18:2	C18:3	C18:2	C18:3	C20:4
Lard[a]	65.3	13.6	47.6	11.2	0.4	11.7	0.3	0.26
Tallow	42.2	23.0	39.1	1.7	0.2	2.1	0.3	0.04
Soybean	129.4	4.5	26.5	50.4	8.2	49.8	5.2	0.02
Cottonseed	104.4	4.2	17.8	52.8	0.0	53.5	0.0	0.00
Linseed	184.6	4.1	20.2	15.6	54.2	16.0	46.4	0.00
Safflower	146.2	3.1	13.2	76.1	0.0	72.4	0.0	0.00

[a] Trace of C20:4 found by G.L.P.C. Method.

TABLE III

Polyunsaturated Fatty Acid Composition of Hydrogenated Fats and Oils by G.L.P.C. and Ultraviolet Analyses

	Iodine value (Wijs)	% Fatty Acids					
		G.L.P.C.				U.V.	
		C18:0	C18:1	C18:2	C18:3	C18:2	C19:3
Lard	58.9	14.2	50.6	5.5	tr	5.7	0.2
Tallow	39.8	21.4	40.7	1.1	tr	1.4	0.2
Soybean (high selectivity)	80.3	7.9	72.8	7.1	0.0	4.3	0.1
Soybean (low selectivity)	78.6	15.2	57.7	15.4	0.7	9.9	0.9
Cottonseed (high selectivity)	78.0	3.8	55.1	14.1	0.0	11.0	0.1
Cottonseed (low selectivity)	74.4	10.8	44.9	16.6	0.0	13.0	0.1
Linseed	147.4	3.8	35.6	26.9	27.1	16.9	17.3

extent of geometric isomerization was not determined.

It should be pointed out that the ultraviolet method has one function that is not readily done by G.L.P.C. The presence of lard and tallow in shortenings is sometimes detectable only by the arachidonic acid content of the shortening. The quantity of acid involved is usually less than 0.3%. This amount is readily detected by the ultraviolet method, but is not easily determined quantitatively by G.L.P.C.

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Properties of Oil Extracted from Cottonseed with Acetone-Hexane-Water Solvent Mixture[1]

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Slightly more neutral oil was obtained on the exhaustive extraction of raw cottonseed meats with the acetone-hexane-water (AHW) solvent mixture than was obtained under the same conditions with commercial hexane. Although most of the gossypol originally present in the seed is extracted with the oil when the AHW solvent mixture is used, the crude oils refined and bleached to yield oils of excellent colors. Methods of recovery of the solvent from the mixed solvent miscellas are reported, together with the refining and bleaching data for the recovered oils.

IT WAS REPORTED previously (1,2) that the extraction of raw cottonseed flakes with a solvent mixture composed of commercial hexane (boiling range 67–71°C.) 44%, acetone 53%, and water 3% yields a cottonseed meal product that is rich in available lysine (lysine with the epsilon amino groups free) as compared with cottonseed meals presently produced by conventional methods, and that is low in both "free" and "total" gossypol. The fraction of the meal nitrogen that is soluble in 0.02N aqueous NaOH is also high. Moreover the meals prepared through the use of the solvent mixture are of high nutritive quality (3).

The crude cottonseed oils obtained through the use of this solvent mixture contain the major portion of the gossypol originally present in the seed, together with other cottonseed constituents that are not normally present in oils obtained through the use of commercial hexane. It is shown in this paper that, although the crude oils obtained through the use of this solvent contain relatively large concentrations of gossypol, they may be refined and bleached to yield prime oils.

Experimental

Methods of Analysis. Free fatty acids and neutral oil contents were determined by AOCS methods (4). Color values for the refined and bleached oils were determined by the color index (area method) of Pons, Kuck, and Frampton (5). Methods of analysis for composition of the recovered solvent mixture were as follows:

Water. Twenty-five ml. of sample were pipetted into a 100-ml. A.S.T.M. oil tube graduated in 0.1 ml. steps from 0 to 3.0 ml. in the lower stem. The tube was then filled to the 100-ml. mark with with commercial hexane (petroleum ether, boiling range 67–71°C.), stoppered, and the contents mixed. After centrifuging for 5 min. at 1,000 r.p.m. the volume of the lower layer was read to obtain the tube reading. Volume per cent of water in the sample was calculated, using the following equation:

$$\text{Vol. \% of } H_2O = 4(0.56T + 0.10)$$

where T is the tube reading in ml.

Hexane. An 8-ml. sample was pipetted into a 50% (9 g.) Babcock cream bottle. Distilled water was added until the bottle was filled to the 40-ml. mark. The bottle was stoppered and the contents were mixed until all of the acetone was extracted from the hexane by the water. After centrifuging for 5 min. at 1,000 r.p.m. the scale was read between the menisci. This reading was multiplied by the factor 1.25 to obtain volume per cent of hexane.

Acetone. Volume per cent of acetone was obtained by subtracting the sum of volume percentages of hexane and water obtained by the above procedures from 100.0.

When the above determinations were made in duplicate the averaged results were found, by analyses of known mixtures, to be accurate to three significant figures. Other analytical methods used will be described later in the text.

AHW Mixed Solvent vs. Commercial Hexane in Extraction of Cottonseed Meats. The purpose of the extraction tests was to determine the comparative efficiencies of the AHW mixed solvent with commercial hexane in extraction of oil from cottonseed meats. It was also desired to compare these with the official AOCS method for determination of oil content of cottonseed meats.

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