Rapid Determination of Free Fatty Acids in Vegetable Oils by Gas Liquid Chromatography

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ABSTRACT

Determination of the free fatty acids in small quantities of vegetable oil is accomplished by gas liquid chromatography. The free fatty acids are isolated from a hexane solution of the vegetable oil into an aqueous solution of trimethylphenylammonium hydroxide (TMPH). Due to the alkalinity of TMPH, the free fatty acids readily partition into this aqueous phase. Injection of the free fatty acid-TMPH salts into a gas chromatograph results in pyrolytic methylation of the free fatty acid salts – yielding the methyl esters. Excellent results were obtained when this new procedure was used on neutral lipid oils containing known amounts of free fatty acids and compared with the results obtained by a modified BF_3/MeOH esterification procedure. When compared to the AOCS titration procedure, this new procedure gave comparable results. This new procedure has advantages over the AOCS procedure: it is more sensitive and gives quantitative results for individual free fatty acids. This new procedure also has several advantages over the modified BF_3/MeOH esterification procedure: it is easily and more rapidly performed, there is no deposition of glyceride on the column when the sample is injected, and because there is quantitative recovery, the new procedure is more sensitive and can be used on oils with a low weight percentage of free fatty acids.

INTRODUCTION

The free fatty acid content of vegetable oils is used as an index of the oil quality by commercial oil refiners. The free fatty acid content of oilseeds is also of interest in physiological studies. There are many published procedures for the determination of free fatty acids in oils; the most numerous being titration and spectrophotometric procedures. The AOCS official procedure for the determination of free fatty acids in a sample of crude or refined vegetable oil is a titration procedure (1).

Of the many procedures developed for the determination of free fatty acids in vegetable oils, there are only three noted in the literature which measure the individual free fatty acids: separation of underivatized free fatty acids on gas chromatographic columns (2); a BF_3/MeOH esterification procedure (3); and a new procedure using BSTFA that measures not only the free fatty acids but the mono-, di- and triglycerides as well (4). Gas chromatographic columns which separate underivatized fatty acids are impractical due to the shortened column life resulting from repeated injections of vegetable oil onto the column. A new method developed by Chapman methylates the free fatty acids by a modified BF_3/MeOH esterification procedure using methylurea to reduce triacylglyceride transesterification of the free fatty acids. The presence of methylurea, however, reduces the yield of methyl esters to ca. 18%. Also, because there is no partition of the methyl esters from the triglycerides before column injection, all goes onto the column, thereby reducing column life.

We have developed a new procedure which measures individual free fatty acids in vegetable oils. This new procedure is rapid, simple, highly sensitive, and obtains quantitative results with all vegetable oils tested. Results are obtained in ca. 20 min. As shown previously (5,6), free fatty acids can be extracted from an organic solvent with a small volume of an aqueous quaternary ammonium solution. Using this principle, the underivatized free fatty acids are partitioned from a vegetable oil/hexane mixture into aqueous trimethylphenylammonium hydroxide (TMPH). The lower phase is washed with ethyl ether and hexane successively to remove traces of glyceride. Upon injection of an aliquot of the lower aqueous phase into the gas chromatograph's hot injection port, pyrolytic methylation of the free fatty acid salts occurs, allowing separation of the acids as methyl esters. The column life is not affected since the triglycerides and diglycerides were partitioned from the free fatty acids.

The procedure recently published by D'Alonzo et al. (4) using BSTFA to form trimethylsilyl (TMS) ethers of the mono- and diglycerides and the TMS esters of free fatty acids uses the gas chromatograph to separate not only the free fatty acids but the mono-, di- and triglycerides in a single gas liquid chromatographic run. This procedure is excellent if you want to get data for all the components in vegetable oil. Due to the high temperatures required to burn off the triglycerides and the need for minimal column bleed during programming, the liquid phases used do not fully separate the mono- and polyunsaturated fatty acids.

MATERIALS AND METHODS

Vegetable oils used in this study were obtained from local food markets and drug stores. The soybean oil was IGA brand (IGA, Inc., Chicago, IL) partially hydrogenated soybean oil. Mazola (Best Foods, Englewood Cliffs, NJ) was the brand corn oil used. Safflower oil was from Hollywood Health Foods (Los Angeles, CA) and the cottonseed oil was obtained through Lannet Co. (Philadelphia, PA). All reagents used were the best grades available.

TMPH, 1 M, was prepared as described previously (6) by mixing 2.6 g of trimethylphenylammonium iodide (Eastman Kodak Chemical Co., Rochester, NY) and 2.6 g silver oxide (Fisher Scientific Co., Fair Lawn, NJ) with 10 mL of distilled water in a stoppered centrifuge tube. After vigorous mixing by hand and on a vortex mixer to achieve a uniform suspension, the preparation was centrifuged and one drop of the clear supernatant solution was diluted with 10 drops of distilled water and tested for halide with one drop of 0.1 M silver nitrate in 6 M nitric acid. If a positive halide test resulted, the mixing and centrifugation steps were repeated until a negative halide test was achieved. The TMPH solution was stored in a stoppered test tube at 4 C in the dark.

Methyl acetate (certified, Fisher Scientific Co.) was shaken in a stoppered test tube with granular anhydrous sodium carbonate (Mallincrodt Chemical Works, St. Louis, MO) to remove any water or free acetic acid and was stored at room temperature over the sodium carbonate. Occasional shaking of the mixture is recommended to keep it acid free.

The titration procedure used was the AOCS Official Method Ca 5a-40 (1). The modified BF_3/MeOH esterification procedure used was as described by Chapman (4).

In our new procedure, to a 15-mL conical centrifuge tube with a ground glass stopper, add 100-300 mg of vegetable oil with no more than 5% free fatty acid. Bring the

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volume to 5 mL with hexane containing 90 μg of heptadecanoic acid as the internal standard. Mix to yield a uniform solution. Add 50 μL 1.0 M TMPH and mix on a vortex mixer for 10-15 sec, then centrifuge at 400-600 X g for 1 min. Remove and discard the hexane phase which contains most of the vegetable oil. To remove the traces of glyceride left, wash the aqueous phase with 5.0 mL ethyl ether for 10-15 sec on a vortex mixer. After centrifugation, remove the ether from the opaque viscous lower phase. Add 5 mL of hexane, vortex 10-15 sec, centrifuge 400-600 X g for 1 min and the lower phase will be clear and ready for injection into the gas chromatograph. When removing the upper hexane and ether phases, it is more important that you do not disturb the lower phase or any interface than it is to remove all of the upper phase. Prewet the injection syringe with pretreated methyl acetate, draw up 1-5 μL of the TMPH extract followed by 1 μL of methyl acetate. Pump the plunger of the syringe a few times to mix the contents before injecting the sample into the chromatograph.

Gas liquid chromatographic analyses were performed on a Perkin-Elmer Sigma I gas chromatographic system (Perkin-Elmer Corp., Norwalk, CT) equipped with flame ionization detectors. The 6 ft X 1/4 in. (4 mm id) coiled glass column was packed with pretested 10% Silar 10C on Gas Chrom Q, 100-120 mesh (Applied Science Laboratories, State College, PA). The injector was held at 280 C, the detector was held at 250 C and the carrier gas was nitrogen at 50 mL/min. The column oven was maintained at 165 C. Peak areas were measured by the Perkin-Elmer Sigma I system.

Recovery studies were performed on neutral lipid oils to which mixtures of free fatty acids had been added. Neutral lipid oils of soybean and safflower seed oils were prepared by silicic acid (Biosil A, 100-120 mesh, Bio-Rad Laboratories, Richmond, CA) column chromatography. The silicic acid was washed several times with chloroform to remove fines by decantation prior to packing the column. Vegetable oil (1-2 g) was applied to the 1.5 X 12.0 cm column and eluted with chloroform. The initial 50 mL of eluent was collected and the solvent evaporated under reduced pressure by rotoevaporation. This fraction contained neutral lipid oil which was 75-80% of the weight of the vegetable oil applied to the column. This neutral lipid oil was free of free fatty acids as determined by the new procedure and by two-solvent, one-dimensional thin layer chromatography; development to 14 cm with isopropyl ether; acetic acid (144:6, v/v), dried and followed by ethyl ether/hexane/acetic acid (15:135:1.5, v/v/v) to the top of the plate.

Two hundred mg of the neutral lipid oil obtained from safflower seed oil or soybean oil was combined with 2.5 mg of a free fatty acid mixture prepared from palmitic, palmitoleic, stearic, oleic and linoleic acids (Supelco, Inc., Bellefonte, PA) in hexane. Hexane was added to a final volume of 5 mL and the new procedure used to recover the free fatty acids. A control sample was prepared in the same way but without the presence of a neutral lipid oil. Other samples were prepared by the modified BF_3/MeOH esterification procedure for comparison.

RESULTS AND DISCUSSION

The data from the recovery experiments are given in Tables I and II. These tables show the composition and percentage of the free fatty acid mixture being unchanged after recovery by the new procedure. A comparison with the modified BF_3/MeOH procedure (Table II) shows slightly better agreement of the new procedure with the control.

TABLE I

Free Fatty Acids Recovered from Neutral Lipid Oils as Determined by GLC

	Composition (%)	
	Mixture added	Mixture recovered
Neutral safflower seed oil		
16:0	4.2	4.2 ± 0.04
16:1	48.3	48.1 ± 0.40
18:0	2.9	2.8 ± 0.06
18:1	4.0	4.0 ± 0.06
18:2	40.6	40.8 ± 0.49
Neutral soybean oil		
16:0	29.2	28.7 ± 0.12
16:1	23.5	23.2 ± 0.19
18:0	13.7	13.4 ± 0.16
18:1	3.0	3.9 ± 0.23
18:2	30.2	29.9 ± 0.23

TABLE II

Recovery of Free Fatty Acids from Neutral Soybeal Oil, Comparison Between the New and the Modified BF_3/MeOH Procedures

	Composition (%)		
	Mixture added	Mixture recovered	
		New	BF_3/MeOH
16:0	28.9	28.7	22.0
16:1	22.7	22.6	22.0
18:0	13.7	13.8	19.0
18:1	4.5	5.1	9.3
18:2	29.6	29.3	22.3

Yield of the free fatty acid methyl esters by the new procedure approached 100%, whereas the modified BF_3/MeOH procedure has a yield of ca. 18%.

Comparison of the total amount of free fatty acid obtained by the AOCS titration procedure and the new procedure show that there is good agreement between the two procedures for the safflower seed oil (0.033 ± 0.0017 as oleic percent and 0.028 ± 0.0021 as weight percent by AOCS and the new procedure, respectively), and corn oil (0.024 ± 0.0080 as oleic percent and 0.029 ± 0.0010 as weight percent by AOCS and the new procedure, respectively). Cottonseed oil gave 0.150 ± 0.0050% free fatty acid as weight percent. A confident titration point for the cottonseed oil could not be obtained, despite several attempts. The reason for this is unknown.

Table III shows the data obtained using the new method for percent composition and weight percent of free fatty acids. The weight percent data were calculated using heptadecanoic acid as the internal standard.

Shorter chain free fatty acids, 12:0 and 14:0 were also recovered and observed in the chromatograms using the new procedure, but were not quantitated. If desired, these shorter chain fatty acids could be included in the analysis.

It is necessary to complete the extraction promptly once the TMPH has been added. The alkalinity of the TMPH is sufficient to cause hydrolysis of the glyceride fatty acids at room temperature. If the extraction is halted after the first centrifugation and allowed to stand overnight, an increase in the amount of "free" fatty acid content will be observed.

TABLE III

Free Fatty Acid Composition in Vegetable Oils as Determined by the New Procedure (mean ± SD n = 6)

	Composition of free fatty acids (%)			
	Safflower seed oil	Soybean oil	Corn oil	Cottonseed oil
16:0	8.6 ± 0.18	17.8 ± 0.58	12.8 ± 0.28	25.2 ± 0.19
18:0	4.5 ± 0.22	18.5 ± 0.51	3.7 ± 0.19	3.4 ± 0.06
18:1	12.6 ± 0.12	43.0 ± 0.85	25.1 ± 0.18	18.6 ± 0.10
18:2	72.2 ± 0.50	20.6 ± 1.43	56.7 ± 0.35	52.9 ± 0.21
		Wt % ($\times 10^{-4}$)		
16.0	23.9 ± 1.40	16.6 ± 1.06	36.8 ± 1.60	365.5 ± 4.00
18:0	12.6 ± 2.70	17.3 ± 0.88	10.8 ± 0.80	49.7 ± 1.30
18:1	35.3 ± 2.70	40.2 ± 1.88	72.3 ± 2.80	270.6 ± 4.10
18:2	201.9 ± 15.90	19.2 ± 1.07	163.4 ± 6.00	769.3 ± 12.00
Total	279.3 ± 20.50	93.5 ± 3.45	288.0 ± 10.9	1455.1 ± 20.20

It is best to prepare the sample and do the chromatographic analysis in the same day. This prevents analysis of any glyceride fatty acids that may be made from traces of glyceride left on the walls or at the interface.

This new procedure for the determination of free fatty acids in vegetable oils overcomes difficulties presented by other procedures in the literature. The new procedure is rapid, simple, highly sensitive and gives quantitative results for the individual fatty acids as well as a measure of the total amount of free fatty acid. Furthermore, the partitioning of the free fatty acids from the vegetable oil allows repeated injections into a gas chromatograph without destruction of the column by glyceride contamination. This new procedure should be useful in determining the free fatty acid content in commercial in-process oils and in oilseeds.

ACKNOWLEDGMENT

This work was supported by the Veterans Administration.

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[Received May 28, 1982]

A Quantitative Study of the Pathways Involved in the Formation of Radiolysis Products in Ethyl Palmitate

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ABSTRACT

The various compounds produced by irradiation in ethyl palmitate have been determined by direct mass spectrometry and gas chromatography mass spectrometry. Postulated mechanisms for their formation have been confirmed by a comparison of product yields, and a material balance is shown between the G-values for products and their putative precursors. The total product yield is seen to be nearly equal to the amount of radiation absorbed. Three new radiolysis products, viz. ethyl α-ethylpalmitate, ethyl hexadecen-2-oate and butyl palmitate are reported.

INTRODUCTION

The various compounds formed in meats (1-12), triglycerides (2,4,5,9,12-21), fatty acids and fatty acid esters (4,9, 12,16-18, 20-25) have been well characterized by qualitative analysis employing mainly mass spectrometry (MS). Quantitative methods have also been devised (1,3,9,12,26-29) allowing determination of the relative amounts of these components, and the reaction pathways have been adduced or proposed (16,17,19,30).

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Since a variety of compounds are formed in varying amounts, it has seemed desirable to establish a means of measuring the favored reactions and establish a material balance for the distribution of energy absorbed in the radiation process. Accordingly, a scheme has been devised to measure the yield of all the possible radiation products formed in a single substance and, if possible, account for their distribution. For reasons described in detail below, ethyl palmitate was chosen as the test substance. The results are presented in this manuscript.

EXPERIMENTAL

Practical grade ethyl palmitate was obtained from Eastman Kodak Co., Inc., (Rochester, NY) and purified by spinning band distillation to remove trace amounts of esters of other fatty acids and other high boiling impurities. The purity was checked by gas chromatography (GC) under the same conditions used for subsequent analysis. Complete removal of compounds having boiling points close to that of ethyl palmitate was not achieved, but the impurities remaining were found not to interfere in the analysis (see Figs. 1 and 2). Volatile impurities were removed from ethyl palmitate