Technical

The Determination of Specific Free Fatty Acids in Peanut Oil by Gas Chromatography

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ABSTRACT

Peanut oil is dissolved in 2,2,4-trimethylpentane containing n-heptadecanoic acid as an internal standard. The free acids are extracted into N,N-dimethylformamide as their sodium salts and treated with methyl iodide. The resulting methyl esters are analyzed by gas chromatography. A typical result for oleic acid is about 200 ppm with a relative standard deviation of 6%. An average recovery of 95% is obtained from a sample spiked with an additional 200 ppm of oleic acid. Evidence is presented that the procedure does not give high values as a result of sample hydrolysis.

INTRODUCTION

The determination of free fatty acids in vegetable oils is usually carried out by titration with alkali (1). Colorimetric (2,3) and coulometric (4) procedures have also been described. Several workers have used gas chromatography for the determination of free acids in fats of animal origin. Their procedures have involved a preliminary separation of free acids from fatty acid triglycerides by thin layer chromatography, (5) ion exchange (6), or extractive techniques (7,8). The free acid fraction has then been methylated, and the amount of each acid determined by gas chromatography. The use of gas chromatography for the measurement of free fatty acids in vegetable oils has not been reported, however.

This work arose from a need for quality control of peanut oil which was under consideration as an ingredient for formulation in an injectable. There was specific interest in determining the amount of free oleic acid present because of its reported irritant properties (9,10). A rapid and simple procedure was required which avoided the use of diazomethane because of its hazardous properties. Special attention was given to the problems of hydrolysis and transesterification of the fatty acid triglycerides during the sample preparation.

We describe here a convenient method of extracting the free fatty acids, as their sodium salts, from peanut oil into N,N-dimethylformamide. Salt formation is accomplished by the addition of one equivalent of sodium phenoxide, as determined by a phenolphthalein indicator. The methyl esters are then formed by reaction with methyl iodide using the method of Greeley (11). By combining the use of an indicator, a relatively weak base, and essentially anhydrous conditions, significant hydrolysis is avoided. The sample preparation removes most of the triglycerides, leaving a dilute solution of fatty acid methyl esters, including an internal standard, which may be concentrated as desired for greater sensitivity.

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EXPERIMENTAL PROCEDURES

Apparatus and Reagents

All mixing operations were conducted by placing the sample in a Vortex-Genie Mixer (Scientific Products) operated at full speed for about 15 sec. Centrifugation was carried out using an International Equipment Co. Model HN-S centrifuge operated for 5 min at 2000 (920 x g at the tip).

All solvents were reagent grade or better and were stored over "Linde" Type 3A Molecular Sieve (Matheson, Coleman, and Bell) to reduce water content. Oleic and heptadecanoic acids were purchased from Applied Science Laboratories with a stated purity of 99+%.

Sodium phenoxide, 0.1 N, in methanol was prepared fresh daily using sodium methoxide (Baker) and a 10% excess of reagent phenol. Solutions of n-heptadecanoic (internal standard) and oleic acids in 2,2,4-trimethylpentane were prepared by accurate weighing and dilution so that the final concentration of each solution was about 0.05 mg/ml. A 0.01 N solution of phenolphthalein in methanol was prepared as an indicator.

Sample Preparation

About 250 mg of peanut oil was accurately weighed into a 15-ml centrifuge tube. To this were added 1 ml internal standard solution, 2 ml 2,2,4-trimethylpentane, 1 ml N,N-dimethylformamide, and 4 drops indicator solution. The contents of the tube were mixed and then titrated to a faint pink or purple end point using a 50 mcl syringe containing the 0.1 N sodium phenoxide solution. The sample was mixed after each addition of base to ensure complete extraction. After centrifugation, the upper (trimethylpentane) layer was removed and discarded. To the remaining lower layer was added 3 ml of trimethylpentane and mixing to wash out any residual triglycerides. After centrifugation, the upper layer was removed and discarded as before. The sample was transferred to a fume hood, and 50 mcl methyl iodide was added. Methyl iodide has been cited as a carcinogen (12) and proper precautions should be observed during this and subsequent steps. After mixing the reactants, the sample was allowed to stand for 5 min. To the reaction mixture were added 1 ml trimethylpentane, mixing, then 2 ml water followed by further mixing. After centrifugation, the lower (water)

TABLE I

Free Fatty Acids Measured in Samples of Peanut Oil (ppm)

Lot	Number of determinations	Palmitic	Oleic	Linoleic	Total
A	6	50	220	130	390
B	1	30	190	110	330
C	1	30	120	90	240
D	1	20	170	100	290
E	1	70	310	190	570
F	1	---	100	60	160

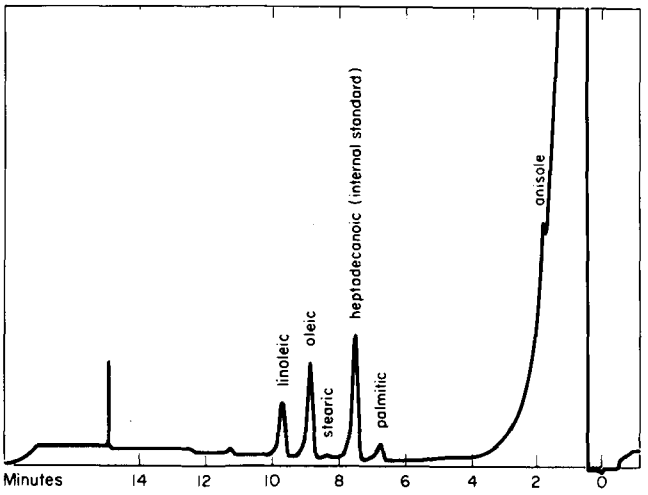

FIG. 1. Chromatogram of methyl esters of free fatty acids from peanut oil (Lot A).

layer was removed and discarded. An additional 2 ml water was added and mixed to wash out the remaining dimethylformamide. After centrifugation, the upper (trimethylpentane and esters) layer was transferred to a 2-ml vial and was ready for injection into the gas chromatograph. A standard was prepared by adding 1 ml each or oleic acid solution, internal standard solution, trimethylpentane, and dimethylformamide to a 15-ml centrifuge tube. After addition of 4 drops of indicator solution, the standard was taken through the procedure described for the sample.

Gas Chromatography

A Perkin-Elmer Model 910 gas chromatograph equipped with flame ionization detection was use. A stainless-steel column, 6 ft x 1/8 in. OD, was packed with 10% SP-2340 on 100/120 mesh Supelcoport (Supelco). The injector and detector temperatures were 200 C and 250 C respectively. A temperature program was used for the column as follows: 160 C for 2 min, 8 C/min to 240 C, hold at 240 C for 3 min. Nitrogen was the carrier gas, with a flow rate of about 20 ml/min. The amount of sample injected was 3 mcl.

Calculations

An Autolab System IV Digital Integrator (Spectra-Physics) was used to measure peak areas. Oleic acid, which was the principal free acid present, was determined directly by comparison to the standard. Palmitic and linoleic acids were quantitated using the response factor obtained for oleic acid. Other acids, such as stearic, which were present in only relatively small amounts, were not measured.

RESULTS AND DISCUSSION

This method has been used to analyze six different samples of U.S.P. grade peanut oil (Table I). The relative amounts of the different free acids found are roughly equivalent to those reported for saponified peanut oil (13). Six determinations on Lot A gave the average values shown, with a relative standard deviation of 6%. A typical chromatogram obtained with this lot is shown in Figure 1. Excess sodium phenoxide reacts with methyl iodide to form anisole, which elutes on the tail of the solvent peak, at about 2 min. A small peak for anisole indicates that the titration has been done correctly, with only a small amount of excess base added.

A series of seven recovery experiments was conducted by spiking Lot A with about 200 ppm additional oleic acid. Each spiked sample was compared with an unspiked sample analyzed on the same day. The difference between the two was divided by the amount of oleic acid added and expressed as percent. Results ranges from 85% to 100%, with an average of 95%. Titration of Lot A by the U.S.P. procedure (1) gave about 0.0023 meq/g, or about 650 ppm total free acids calculated as oleic. The total by gas chromatography was significantly lower since several minor components, as well as the short chain acids, were not measured.

Special attention was given to the problems of hydrolysis and transesterification of the fatty acid triglycerides during the sample preparation. Because of the low level of total free fatty acids involved (less than 0.004 meq/g to meet U.S.P. requirements) (1) these side reactions had to be minimized to avoid obtaining high values. This required a careful choice of the methods used for isolation and derivatization. For example, a virtually complete separation of triglycerides and free acids would have been necessary prior to derivatization with acidic reagents, such as boron trifluoride:methanol, which promote transesterification.

In the initial development of this procedure, phenyltrimethylammonium hydroxide, the base used by Greeley (11), was used as the titrant. The results were about the same as those obtained with sodium phenoxide when the sample was titrated just to the phenolphthalein end point. However, a 100% excess of the quaternary ammonium hydroxide resulted in a dramatic increase in "free acids" measured, indicating that excess hydroxide produced rapid hydrolysis of the fatty acid triglycerides. In contrast, the results obtained with sodium phenoxide were much less sensitive to the amount of base added, as shown in Table II. Even when the sample solution was saturated with water prior to analysis, hydrolysis from excess sodium phenoxide was not significant.

The procedure described has been found useful in determining free oleic acid in peanut oil, and in estimating the amounts of palmitic and linoleic acids present. The techniques utilized should have other applications in determining small amounts of specific free acids in the presence of derivatives of those acids which are easily hydrolyzed or transesterified.

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TABLE II

The Effect of Type and Amount of Titrant Used on Assay Results for Oleic and Linoleic Acids (ppm)

	1 Equivalent		2 Equivalents	
	Oleic	Linoleic	Oleic	Linoleic
Phenyltrimethylammonium hydroxide	210	130	330	320
Sodium phenoxide	200	120	210	140
Sodium phenoxide (sample equilibrated with water)	210	130	220	140

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