TABLE II
Molecular Distillation of Synthetic Triglycerides at 40 Microns Pressure

Fraction	Temp C	Wt in g
1	118–170	80.1
2	235	27.0
3	238	24.9
4	240	23.6
5	260	188.3
6	275	487.6
7	280–300	50.3
Residue	.....	96.0

the synthetic triglycerides. The observed differences are within the generally accepted experimental error of the method and tend to support the conclusions from the chemical analysis that only slight selectivity occurred during the esterification reaction.

Preparation of Triglycerides

Into a reaction flask equipped with mechanical stirrer, thermometer and gas-bubbling tube were placed 1100 g (3.45 moles) molecularly distilled menhaden oil fatty acids (N.E. 319; I.V. 326; 1.66% conjugated diene and 0.03% conjugated triene; Gardner No. 6–7) and 108 g (1.11 moles) 95% glycerol. The fatty acid-glycerol mixture was vigorously stirred under an atmosphere of purified (99.99%) nitrogen and heated to 225C. The catalyst, 2.2 g (0.2% based on weight of fatty acids) zinc dust, was added in four equal portions at 15-min intervals. The reaction was allowed to proceed for 15 min after the final addition of zinc dust.

The hot reaction mixture was immediately poured onto one kg cracked ice to quench the reaction. This mixture was then transferred to a separatory funnel, and the crude product was extracted with petroleum ether (bp 30–60C). The petroleum ether extract was washed with equal volumes of water, 5% hydrochloric acid, 5% sodium bicarbonate, saturated sodium chloride solution, and water—in that order. Deoxygenated water was used throughout the washing procedure.

The petroleum ether extract was dried over anhydrous sodium sulfate, filtered, and the solvent was then removed under vacuum on a water bath. The crude product (1111 g) obtained was light yellow (Gardner No. 4).

Analysis of the crude product by TLC (Fig. 1) indicated approx 75% triglycerides. The remaining 25% consisted of unreacted fatty acids, mono- and diglycerides, and a small amt of polymeric material.

Molecular distillation was used to purify the crude reaction product. The results of the molecular distillation of 996 g crude product show in Table II. All fractions were analyzed by TLC (Fig. 1) and the principal fraction (Fraction 6) was found to be approximately 95% synthetic triglycerides (S.E. 330; I.V. 308; 3.55% conjugated diene, 2.13% conjugated triene, and no tetraene; Gardner No. 2).

REFERENCES

1. Norris, F. A., Oil & Soap *12*, 257 (1940).
2. Malkin, T., and T. H. Bevan, "Progress in the Chemistry of Fats and Other Lipids," Vol. 4, ed R. T. Holman, W. O. Lundberg and T. Malkin, Pergamon Press, New York, 1957, p. 64–77.
3. Feuge, R. O., E. A. Kraemer and A. E. Bailey, Oil & Soap *22*, 202 (1945).
4. Feuge, R. O., and A. E. Bailey, *Ibid. 23*, 259 (1946).
5. Clarkson, C. E., and T. Malkin, J. Chem. Soc., 666 (1934).
6. Daubert, B. F., and A. R. Baldwin, J. Am. Chem. Soc. *66*, 997 (1944).
7. Eckey, E. W., Ind. Eng. Chem. *40*, *1183 (1948)*.
8. Eckey, E. W., and C. C. Clark, (The Procter and Gamble Co.), U.S. Pat. 2,065,520 (1936).
9. Lundberg, W. O., and J. R. Chipault, Official Digest, Federation of Paint and Varnish Production Clubs, January, 11 (1949).
10. Chipault, J. R., M. J. Hendrickson and W. O. Lundberg, *Ibid.* January, 53 (1950).
11. Malins, D. C., and H. K. Mangold, JAOCS *37*, 576 (1960).
12. Verkade, P. E., J. Van Der Lee and W. Meerburg, Rec. Trav. Chim. *51*, 850 (1932).
13. Quinlin, P., and H. S. Weiser, Jr., JAOCS *35*, 325 (1958).
14. *Schlenk, H., and J. L. Gellerman, Anal. Chem. 32, 1412 (1960).*
15. Gauglitz, E. J., Jr., and L. W. Lehman, JAOCS *40*, 197 (1963).

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Spectrophotometric Determination of Small Amounts of 1-Monoglycerides in Fats

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Abstract

A method is described for the determination of small amounts of 1-monoglycerides in fats. The method consists of a periodic acid oxidation, the resulting glycol aldehyde fatty acid ester then being converted to the 2,4-dinitrophenylhydrazone derivative, which is determined spectrophotometrically. The colored solutions are stable and follow Beer's law in the range 0.0–1.0% 1-monoglycerides with a standard deviation of ±0.024%. Glycerol does not interfere.

Introduction

PERIODIC ACID oxidation has been widely employed for the quantitative determination of 1-monoglycerides (1–5, 9, 11, 12). Amounts of less than 1–3% 1-monoglycerides, however, cannot be accurately assessed by these methods. In 1959 Jensen et al. (6,7,8) published papers on the estimation of 0.1–1.0% 1-monoglycerides in milk. According to their method the formaldehyde produced by periodic acid oxidation is reacted with chromotropic acid and determined colorimetrically. Glycerol and carbohydrates interfere and have to be removed prior to the oxidation.

The present method assesses the fatty acid ester of glycol aldehyde produced by periodic acid oxidation after conversion to its 2,4-dinitrophenylhydrazone. Since the oxidation is quantitative, the 1-monoglyceride content of the sample is proportional to the increase in absorption of the dinitrophenylhydrazones obtained from the oxidised fat as compared with the original value. Glycerol does not interfere.

The oxidation step is based on the AOCS Method (1) for 1-monoglyceride determination, except that carbonyl-free benzene is used as a solvent instead of chloroform and the aqueous extraction before the oxidation is omitted. Excess periodic and iodic acids are removed by adding an aqueous potassium iodide solution and then slightly overtitrating with 0.1 *N* sodium thiosulfate. The aqueous phase is separated and re-extracted with benzene.

The method for assessing the carbonyl compounds is based on that of Lohman (10).

Experimental Procedures and Data

Reagents and Materials. Carbonyl-free benzene was prepared by refluxing 4 liters benzene for 3–4 hours

TABLE I

Absorbances of Tallow—Monoglyceride Mixtures

	Absorbance				
Blank	0.068	0.059	0.055	0.053	0.051
Unoxidized sample		0.094	0.108	0.081	0.084
Oxidized sample	0.119	0.158	0.158	0.137	0.155
Sample + 0.0931% 1-monostearate[a]	0.168 0.171	0.214	0.194	0.190	
Sample + 0.186% 1-monostearate		0.279	0.252		
Sample + 0.279% 1-monostearate				0.301	
Sample + 0.372% 1-monostearate		0.365	0.382		
Sample + 0.466% 1-monostearate				0.409	
Sample + 0.559% 1-monostearate					0.438
Sample + 0.745% 1-monostearate					0.553
Sample + 0.931% 1-monostearate					0.648

[a] Added as 0.1, 0.2, 0.3% etc. Myverol.

with 20 g of 2:4-dinitrophenylhydrazine and 4 g trichloroacetic acid. The benzene is then distilled through a fractionating column (18 in. high) packed with Fenske Helices and insulated with asbestos rope. Those parts of the apparatus which are exposed to the light and which contain hot benzene vapours are wrapped in brown paper and the distillate is collected in a dark bottle, the first and last portions of the distillate being rejected. This benzene was used throughout the procedure described below. Carbonyl-free ethanol was prepared in a manner similar to carbonyl-free benzene; *n*-hexane was Merck reagent for UV spectroscopy.

Periodic acid solution was prepared by dissolving 5.4 g of the acid in 100 ml distilled water and then adding 1900 ml glacial acetic acid. After mixing thoroughly, the solution was stored in a dark glass-stoppered bottle. Trichloracetic acid (TCA) solution was prepared by dissolving 4.3 g TCA in 100 ml carbonyl-free benzene. 2:4-Dinitrophenylhydrazine (DNPH) solution was prepared by dissolving 0.05 g DNPH (recrystallized from carbonyl-free alcohol) in 100 ml carbonyl-free benzene.

Monoglyceride was Myverol 18–05 (Distillation Products) containing 93.1% 1-monoglyceride expressed as monostearate (or 2.6 mmole/g) with a fatty acid composition (by gas chromatography) of 70.6% stearic, 27.6% palmitic, 1.8% lauric acid and an average mol wt (calculated from gas chromatography data) of 349.

Instrument. Unicam SP.700 recording spectrophotometer, range 31,000–27,000 cm^{-1} with 1 cm silica cells.

Procedure. Two sample solutions are needed for the carbonyl determination: 1) a solution containing an aliquot of the fat in which any monoglycerides have been oxidized to the corresponding glycol aldehyde fatty esters; 2) a solution of another aliquot of the same fat in which the monoglycerides have not been oxidized.

Periodic Acid Oxidation. First, 25 ml sample solution containing 1.00 ± 0.01 g fat in benzene and 25 ml periodic acid solution are pipetted into a 250 ml separatory funnel and mixed well. After standing for 30 min, 10 ml 15% potassium iodide solution are added to the mixture. The iodine liberated by the iodic acid and excess periodic acid is then slightly over-titrated with 0.1 *N* sodium thiosulphate solution (usually ca. 26 ml). The two phases are separated and the aqueous phase washed once more with 15 ml benzene. The organic layers are collected in a 50 ml volumetric flask and diluted to volume with ethanol.

TABLE II

Comparison of Added and Experimentally Found Monoglyceride Contents

1-Monoglyceride			
Δ a	C added %	C found %	Δ C %
0.049	0.093	0.081	−0.012
0.052	0.093	0.087	−0.006
0.053	0.093	0.089	−0.004
0.056	0.093	0.094	+0.001
0.036	0.093	0.057	−0.036
0.094	0.186	0.166	−0.020
0.121	0.186	0.217	+0.031
0.164	0.279	0.298	+0.019
0.207	0.372	0.379	+0.007
0.224	0.372	0.411	+0.039
0.272	0.466	0.502	+0.036
0.283	0.559	0.523	−0.036
0.398	0.745	0.740	−0.005
0.493	0.931	0.919	−0.012

Simultaneously 25 ml of benzene are subjected to the above treatment, yielding 50 ml of a "blank solvent." Part of this is used for the solution of 0.5 ± 0.005 g unoxidized fat in a final volume of 25 ml.

The flasks should be stored in the dark until required for the colour reaction. A deep red colour soon develops upon exposure to light, but this does not seem to affect the final results to any appreciable extent.

Carbonyl Determination. Aliquots of 5 ml of both the above solutions are pipetted into test tubes, 3 ml TCA and 5 ml DNPH solutions are added and the test tubes are placed in a 60C water bath for 30 min. After cooling, contents of the test tubes are transferred into separatory funnels containing 20 ml 1.0% $NaHCO_3$ and 75 ml ethanol. The test tubes are washed several times with small volumes of hexane, totalling 20 ml. The separatory funnel is shaken vigorously and after separation of the two phases the hexane layer is collected in a 100 ml volumetric flask. The aqueous layer is washed twice with 20 ml hexane. The combined hexane extracts are diluted to volume with ethanol. The absorbances of both solutions are determined at 28,600 cm^{-1}, using hexane as a reference.

For calibration purposes known amt of 1-monoglyceride were added to tallow samples and the increases in absorbance determined. Table I contains the experimental data.

By applying the method of least squares to the data in Table I it was found that the increase in absorbance, Δ a, was a linear function of the added 1-monoglyceride concentration:

$$\Delta a = 0.006 + 0.53\ c$$

where c = % 1-monoglyceride expressed as 1-monostearate in sample.

Table II compares the per cent added 1-monoglyceride and the amounts calculated from Δ a values with the above equation. The error is not proportional to the 1-monoglyceride content. The standard deviation is ±0.024% 1-monoglyceride expressed as 1-monostearate whereas the maximum error is 0.039%.

When determining the 1-monoglyceride content (expressed as per cent 1-monostearate) of a fat:

$$\Delta a = A_o - A_u$$

Where A_o = absorbance of DNPH from oxidized fat
A_u = absorbance of DNPH from untreated fat, and

$$C\%\ \text{1-monostearate} = \frac{\Delta a - 0.006}{0.53}$$

Experiments conducted in the presence of added glycerol proved that glycerol does not interfere. Addition of as much as 0.6% glycerol gave no increase in absorbance.

REFERENCES

1. Method Cd 11-57, Alpha-Monoglycerides, "Official and Tentative Methods of AOCS," Chicago, 1960.
2. Becker, E., and L. Krull, Fette, Seifen, Anstrichmittel *60*, 449-52 (1598).
3. Handschumaker, E., and L. Linteris, JAOCS *24*, 143-5 (1947).
4. Hartman, L., Analyst *81*, 67-8 (1956).
5. Hartman, L., Fette, Seifen, Anstrichmittel *62*, 271-4 (1950).
6. Jensen, R. G., and M. E. Morgan, J. Dairy Sci. *42*, 232-9 (1959).
7. Jensen, R. G., and M. E. Morgan, *Ibid. 42*, 1235-6 (1959).
8. Jensen, R. G., G. W. Gander and A. M. Duthie, *Ibid. 42*, 1913-6 (1959).
9. Kruty, M., J. B. Segur and C. S. Miner Jr., JAOCS *31*, 466-9 (1954).
10. Lohman, F. H., Anal. Chem. *30*, 972-4 (1958).
11. Pohle, W. D., V. C. Mehlenbacher and J. H. Cook, Oil & Soap *22*, 115-9 (1945).
12. Pohle, W. D., and V. C. Mehlenbacher, JAOCS *27*, 54-7 (1950).

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Efficient Production of Biosynthetically Labeled Fatty Acids[1]

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Abstract

By short-term exposure of a photosynthesizing oilseed plant at seed-setting stage to high levels of $C^{14}O_2$, radioactivity is efficiently incorporated into glycerides yielding randomly labeled fatty acids of high specific activity.

Introduction

BIOSYNTHETIC METHODS for labeling fatty acids in seeds of higher plants have involved their culture from seedling stage to maturity in the presence of a radioactive isotope (3). This procedure assures randomness of labeling; however, there are two disadvantages: a) fatty acids have very low specific activities, necessarily limited by the lethal levels of radiation, and b) providing a plant with a continuous supply of an isotope during the growth period increases production costs of the desired fatty acids by labeling the undesired leaf and stem portions of the plant.

An efficient procedure has been developed to produce high specific activity, randomly labeled fatty acids by short-term exposure of a nearly mature plant to levels of radioactivity that would be lethal in the continuous culture method.

Experimental

Production of Labeled Fatty Acids. Several species of oilseed plants were employed for biosynthetic labeling at various times, including flax, soybean and safflower. The use of a perilla plant *(Perilla frutescens)* is described here because its fatty acid composition is favorable for the isolation of labeled linolenate (6). The plant was grown in a pot, buried in the ground with the top level with the surface, for five months to the seed-setting stage under otherwise ordinary field conditions. It was then exposed to radioactive carbon dioxide of high specific activity in a closed system (Fig. 1) as described by Burris et al. (2), but with modifications. The pressure in the system was reduced by 2 cm to prevent leaks to the outside; the pressure increased only slightly during the exposure of the plant.

$C^{14}O_2$ (0.5 mc in 0.34 cc gas) was generated into the glass exposure vessel (volume was 36 liters) by the reaction of 3 mg Ba $C^{14}O_3$, specific activity of 32.8 mc/mmole, with 3 ml 30% perchloric acid. Photosynthetic uptake of CO_2 was stimulated by irradiating with twenty-six 20-w fluorescent tubes cylindrically arranged about the vessel. To counteract the heating effects of the lights, tap water was sprayed on the top and sides of the vessel. Absorption of $C^{14}O_2$ by the plant was monitored by a continuous-flow ion chamber in a recycling pumping system. A 2-hr period of illumination was required for absorption of 90% of radioactivity; illumination was continued for an additional 2 hr. Figure 2 is the ion current recording showing the generation and loss of $C^{14}O_2$. During a 12-hr dark period the plant, maintained in the exposure chamber with the pumping system off, respired carbon dioxide. As shown in the lower curve of Fig. 2, the radioactivity respired amounted to 30% of the original dosage and required an additional 3.5-hr of illumination for reabsorption. Before returning the plant to natural light conditions, the chamber was flushed with air and the plant was enclosed in a battery jar with a loose fitting lid to ensure recovery of any leaves which might drop off.

After allowing 12 days for maturing, the seeds (1.06 g) were harvested, and the lipids were extracted by crushing them in a micro-Waring Blendor with 20 ml diethyl ether. The ether solution was decanted, filtered, dried over anhydrous Na_2SO_4, and filtered again. Two additional 20-ml ether extractions were made with the volumes added together. Ether was removed *in vacuo* on a rotary pump. The wt of lipids recovered was 371 mg, corresponding to a 35.1% yield; the meal weighed 615 mg.

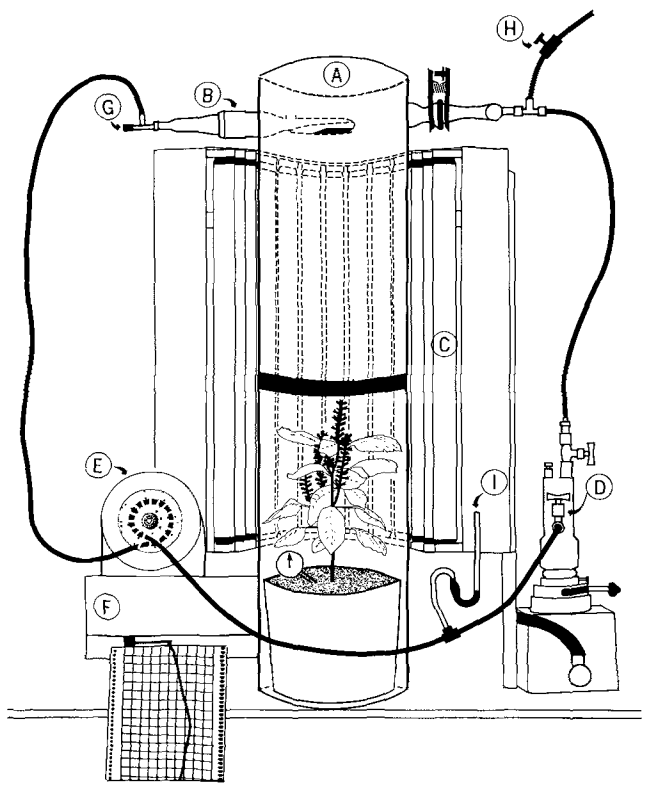

FIG. 1. Gas exposure system: A, sealed exposure vessel; B, $C^{14}O_2$ generator; C, fluorescent tubes: D, continuous-flow ion chamber; E, recycling pump; F, recorder; G, rubber injection septum; H, vacuum line; I, open-end manometer.

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