Analysis of Neutral Lipids and Glycerolysis Products from Olive Oil by Liquid Chromatography

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A simple method was developed to separate model triglycerides, 1,3-diglycerides, 1,2-diglycerides, monoglycerides and free fatty acids and products generated in enzymatic glycerolysis of olive oil by high-performance liquid chromatography with a laser light-scattering detector. The separation was carried out with a silica column at 30°C. A gradient elution with mobile phase A (hexane/chloroform/formic acid) and mobile phase B (hexane/acetone/chloroform) can separate the compounds into distinct peaks in less than 20 min.

KEY WORDS: Glycerolysis, HPLC, lipids analysis.

Separation of free fatty acids (FFA), monoglycerides (MAG), diglycerides (DAG) and triglycerides (TAG) is important in the food industry (1). Quantitative determinations of these compounds are also important in enzymatic glycerolysis of triglycerides, where they are the reaction products and demand a quick and easy method for analysis (2,3). Most of the analysis methods for this purpose were developed based on gas chromatography (4,5) or thin-layer chromatography (6,7). Although some studies showed that high-performance liquid chromatography (HPLC) is also capable of separating these neutral lipid classes and FFA, none of these methods achieved a total separation of all compounds in a single run (7,8). Trathnigg and Mittelbach (8) carried out the separation by coupling a cyano-modified silica column with two gel permeation chromatography columns and using a mobile phase containing 0.6% ethanol in chloroform. Although connecting three columns in series, they still could not separate 1,3-DAG and 1,2-DAG, and the separation between FFA and MAG was also poor. Greenspan and Schroeder (9) successfully separated TAG, DAG and oleic acid with an Ultrasil silica column and a mobile phase containing formic acid, isooctane and tetrahydrofuran. However, they did not mention the separation of MAG, 1,2-DAG and 1,3-DAG.

In this paper we report a new HPLC method for separating neutral lipid classes and FFA by means of the laser light-scattering detector. This method was also tested for analyzing products from enzymatic glycerolysis of olive oil.

EXPERIMENTAL PROCEDURES

Lipase (E.C. 3.1.1.3) from *Pseudomonas fluorescence* (type AK) was obtained from Amano International Enzyme Co. (Troy, VA). Olive oil and lipid standards (triolein, 1,3-diolein, 1,2-dioleoyl-*rac*-glycerol, 1-monooleoyl-*rac*-glycerol, and oleic acid) were obtained from Sigma Chemical Co. (St. Louis, MO). Formic acid, 2-methyl-2-propanol, hexane, chloroform and acetone were obtained from Aldrich Chemical Co. (Milwaukee, WI). The Econosil silica column (250 mm × 4.6 mm ID, 5 μ particle size) was purchased from Alltech Associates Inc. (Arlington Heights, IL). The HPLC system consisted of two Waters 501 pumps, a Waters WISP 712 autosampler (Milford, MA), and a Varex ELSD II laser light-scattering detector. Program control, data acquisition and analysis were carried out with Baseline 810 software. Nebulization temperature for the detector was 80.6°C, and exhaust temperature was between 49.3 to 50°C. Column temperature was controlled at 30°C by a column heater. Mobile phase A contained hexane/chloroform/formic acid (60:40:0.2 by volume) and mobile phase B contained hexane/acetone/chloroform (25:35:65 by volume). The mobile phase ratios are shown in Table 1. All samples were dissolved in chloroform prior to injection.

Enzymatic glycerolysis of olive oil was carried out in 10 mL of 2-methyl-2-propanol containing 25% olive oil, 10 μL of water, 0.5 g of glycerol, and 200 mg of lipase AK. The solution was mixed in a 50-mL flask by shaking at 300 rpm at 35°C for 1 hr. One mL of reaction mixture was withdrawn and 2 mL of chloroform was added to terminate the reaction. The solution was then filtered through a 0.45-μm membrane filter to remove the lipase before HPLC analysis.

TABLE 1

Mobile Phase Composition for HPLC Analysis of Neutral Lipids

Time (min)	Flow (mL/min)	Mobile phase A (%)	Mobile phase B (%)	Curve type
0.0	1.5	90	10	—
1.0	1.5	99	1	6
5.0	1.5	99	1	6
6.0	1.5	2	98	6
19.0	1.5	2	98	6
20.5	1.5	90	10	6
30.0	1.5	90	10	6

TABLE 2

Retention Time for Lipid Standards and Glycerolysis Products from Olive Oil

Lipid classes[a]	Retention time of standards (min)	Retention time of products (min)
TAG	2.10	2.11
FFA	4.15	4.32
1,3-DAG	6.45	6.42
1,2-DAG	11.89	11.21
MAG	14.81	14.39 and 14.76

[a]TAG, triglyceride; FFA, free fatty acid; DAG, diglyceride; MAG, monoglyceride.

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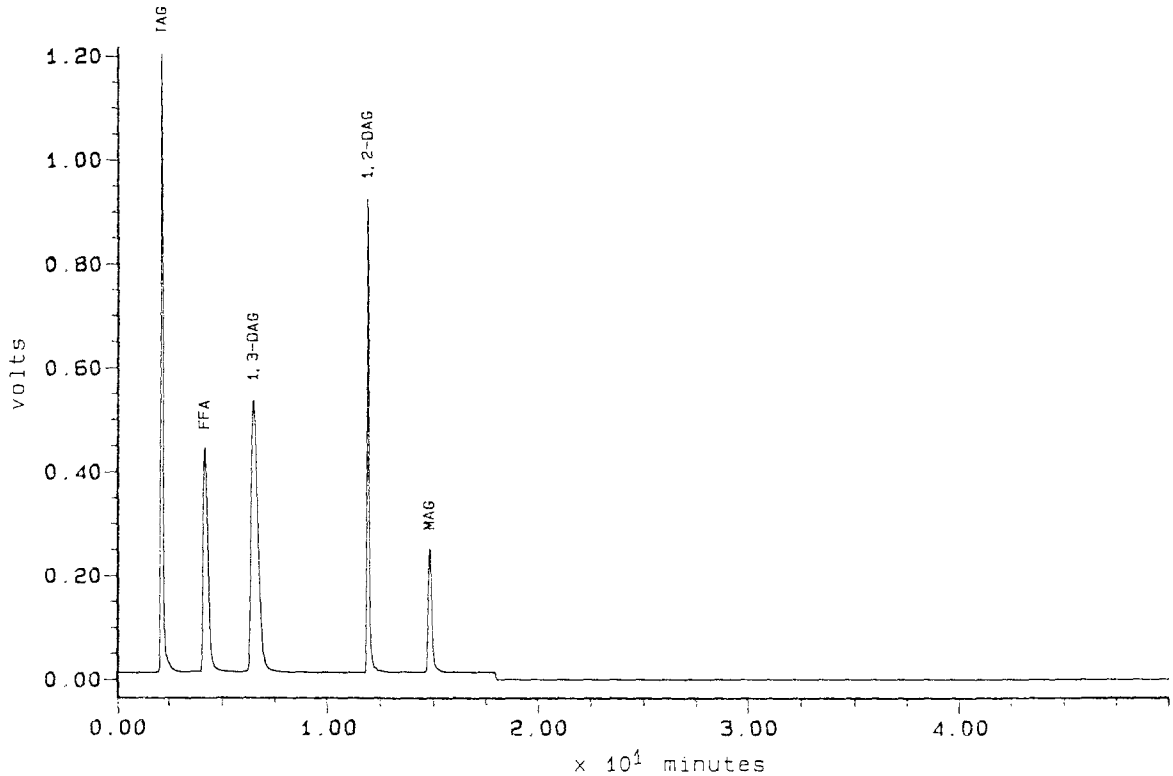

FIG. 1. HPLC chromatogram of lipid standards obtained with the mobile phases in Table 1 and an Econosil column (250 mm × 4.6 mm ID, 5 μ) at 30°C. Key to symbols as in Table 2.

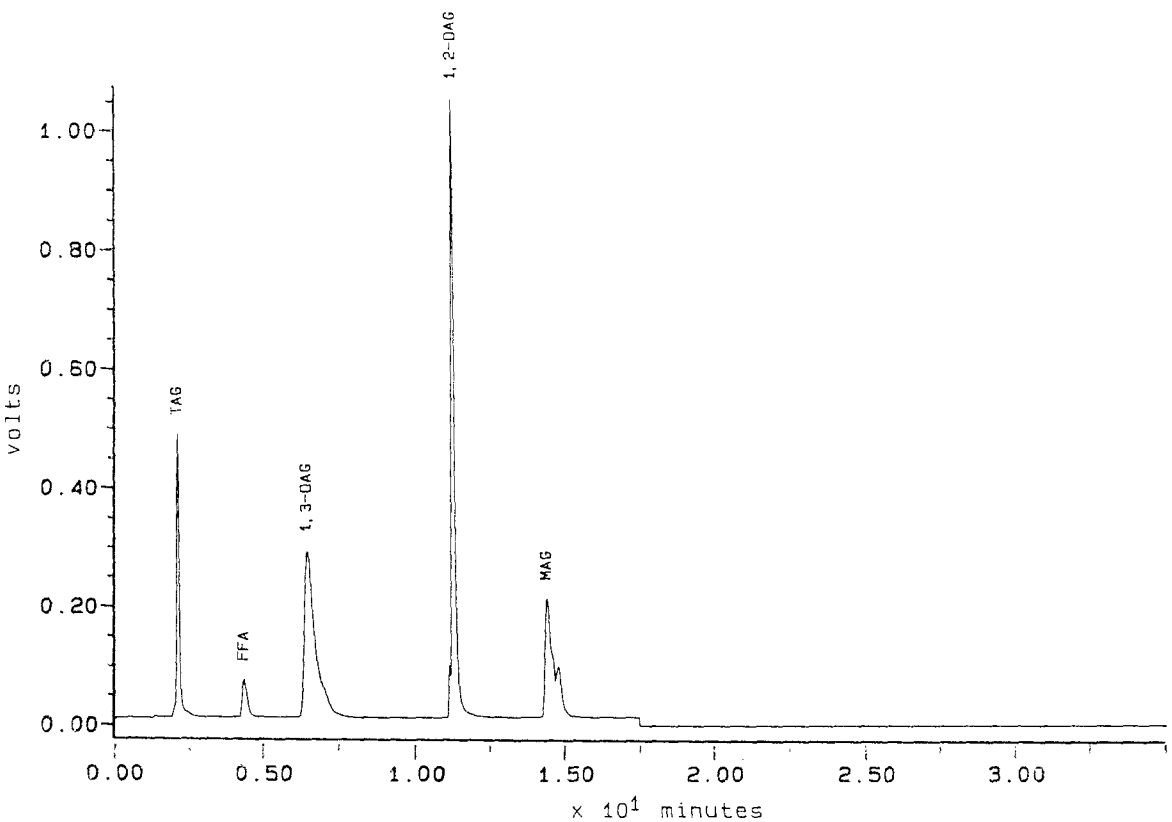

FIG. 2. HPLC chromatogram of products from enzymatic glycerolysis of olive oil obtained with the mobile phases in Table 1 and an Econosil column (250 mm × 4.6 mm ID, 5 μ) at 30°C. Key to symbols as in Table 2.

RESULTS AND DISCUSSION

Figures 1 and 2 report the HPLC chromatograms for lipid standards and enzymatic glycerolysis products of olive oil, respectively. Complete separation of all compounds was observed within 16 min, with an additional 14 min for equilibration of the column. The MAG peak in Figure 2 appeared to include two separate peaks, which may be 1-MAG and 2-MAG. Linearity of detector response has been checked by constructing calibration curves for each standard. The concentration range of standard used in the calibration was 10.6–53.0 mg/mL, 2.8–14 mg/mL, 3.0–15 mg/mL, 4.0–21 mg/mL and 7.4–37 mg/mL for TAG, 1,3-DAG, 1,2-DAG, MAG and FFA, respectively. In all cases, a linear relationship was confirmed with regression coefficients between 0.992 to 0.999. The retention time of neutral lipids and FFA are included in Table 2 for both standards and glycerolysis products, and excellent agreement between both samples was found. This new HPLC method is expected to provide easy quantitative determination of enzymatic glycerolysis products of triglycerides.

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