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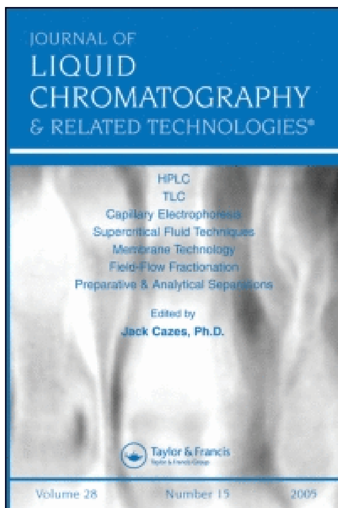

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

ANALYSIS OF VEGETABLE OIL TRANSESTERIFICATION PRODUCTS BY GEL PERMEATION CHROMATOGRAPHY

D. Darnoko[a]; Munir Cheryan[a]; E. G. Perkins[a]
[a] Department of Food Science and Human Nutrition, Agricultural Bioprocess Laboratory, University of Illinois, Urbana, IL, U.S.A.

Online publication date: 13 September 2000

To cite this Article Darnoko, D. , Cheryan, Munir and Perkins, E. G.(2000) 'ANALYSIS OF VEGETABLE OIL TRANSESTERIFICATION PRODUCTS BY GEL PERMEATION CHROMATOGRAPHY', Journal of Liquid Chromatography & Related Technologies, 23: 15, 2327 — 2335
To link to this Article: DOI: 10.1081/JLC-100100491
URL: http://dx.doi.org/10.1081/JLC-100100491

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J. LIQ. CHROM. & REL. TECHNOL., 23(15), 2327–2335 (2000)

ANALYSIS OF VEGETABLE OIL TRANSESTERIFICATION PRODUCTS BY GEL PERMEATION CHROMATOGRAPHY

D. Darnoko, Munir Cheryan,* E.G. Perkins

University of Illinois
Department of Food Science and Human Nutrition
Agricultural Bioprocess Laboratory
1302 W. Pennsylvania Ave.
Urbana, IL 61801, USA

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ABSTRACT

A method for simultaneous analysis of transesterification reaction products — triglycerides, diglycerides, monoglycerides, methyl esters, and glycerol — was developed using gel permeation chromatography. Two Phenogel columns are coupled in series to a refractive index detector, and tetrahydrofuran is used as the mobile phase. Sample preparation involves only dilution and neutralization. The column was calibrated with standard solutions of the compounds. Reproducibility of the method was good: analysis of palm oil transesterification products at different levels of conversion showed a relative standard deviation of 0.27% - 3.87%.

INTRODUCTION

Several analytical methods have been developed for the determination of mono-, di-, triglycerides, methyl esters, and glycerol such as thin layer chromatography with flame ionization detection,[1,2] gas chromatography,[2-5] and liquid chromatography using a density detector.[6] These methods require careful sample preparation since the glycerol must be separated from the methyl esters by phase separation. In addition, gas chromatography methods require derivatiza-

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tion of the samples, which can be laborious and require long analysis times. Single peaks of tri-, di-, and monoglycerides are not produced by gas chromatography. High performance size exclusion chromatography (HPSEC) of fatty acids, mono-, di-, and triglyceride mixture has been reported[7,8] but glycerol was not detected in these analyses. Filippis et al.[9] developed a method of controlling methyl ester content based on viscosity, but the tri-, di-, and monoglycerides could not be detected.

This work reports on the simultaneous analysis of all transesterification products — tri-, di-, monoglycerides, methyl esters, and glycerol — in a vegetable oil methanolysis mixture using HPSEC without prior separation of the glycerol from the methyl ester and with only minimal sample pretreatment.

EXPERIMENTAL

Materials

Reference standards, such as, tripalmitin, triolein, diolein, monoolein, methyl oleate, methyl palmitate, and glycerol of >99% purity were purchased from Nu-Chek-Prep, Inc. (Elysian, MN) and Sigma Chemical Co., St. Louis, MO. Palm oil methyl esters were prepared by transesterification of RBD (refined-bleached-deodorized) palm oil obtained from Archier-Daniels-Midland, Decatur, IL, with methanolic KOH (1% KOH and 1:6 oil-to-solvent molar ratio) at 60°C for one hour, as described by Darnoko.[10]

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Instrumentation

The HPLC system consisted of a Waters HPLC pump with Waters 600E System Controller, a Waters model 401 differential refractive index detector, a Waters 717 Plus Autosampler, and Waters Millennium software (Millipore Co., Milford, MA). The columns were two 300 x 7.6 mm Phenogel columns of 5 µm and 50Å pore size (Phenomenex, Torrance, CA) connected in series and protected by a 50 x 7.6 mm guard column of the same packing material. The mobile phase was HPLC grade tetrahydrofuran at a flow rate of 1 mL/min at room temperature and the sample injection size was 10 µL.

Sample Preparation

Samples of reaction mixture (about 300 mg) were taken from the transesterification reactor using a Pasteur pipette and placed in a vial. The sample was neutralized by adding 5 mL HPLC grade tetrahydrofuran and one drop of 0.6N hydrochloric acid. The dilution and neutralization stopped the reaction imme-

diately. The samples were then kept at –20°C until analysis. Before injection, samples were filtered through a 0.2 μm PTFE syringe filter.

RESULTS AND DISCUSSION

Separation of Components

This method was developed specifically for the analysis of methyl esters derived from palm oil, which is composed of mainly oleic acid and palmitic acid. Thus, the standards were selected to represent the fatty acid composition of palm oil. Figure 1 shows the chromatogram of reference standard solutions of tripalmitin, diolein, monoolein, methyl oleate, methyl palmitate, and glycerol. Complete peak separation was obtained with these compounds. Different classes of triglycerides, e.g., tripalmitin and triolein, did not differ in elution time, but different methyl esters gave slightly different elution times when injected separately. When present together in the same sample, methyl palmitate and methyl oleate produced one asymmetric peak. Table 1 shows the retention time of the standard compounds.

The major advantage of using gel permeation chromatography is its short analysis time since there is no interaction between samples and the polymeric gel material in the column. Separation of individual compounds is based solely on their hydrodynamic volume or effective molecular size in solution. The difference in molecular weight of different glycerides produced in the transesterification reaction is about 250. Even though their steric hindrance is also different, it still resulted in only one peak for each lipid class. Having different classes of triglycerides, diglycerides, or monoglycerides, each in its own peak, makes quantitation of these compounds simple and accurate. With gas chromatography, on the other hand, a difference of 2 carbon atoms of triglycerides resulted in completely different peaks which makes calculation more difficult.[2-5]

Quantitative Analysis

Calibration curves were made with reference compounds tripalmitin, diolein, monoolein, methyl oleate, and glycerol. Standard solutions of at least 5 concentration levels were injected three times each into the HPLC and the corresponding peak areas were plotted against concentration (Figure 2). Statistical analysis (Table 2) showed that all standard solutions have good linearity within the concentration range examined in this work, as shown by the high correlation coefficients.

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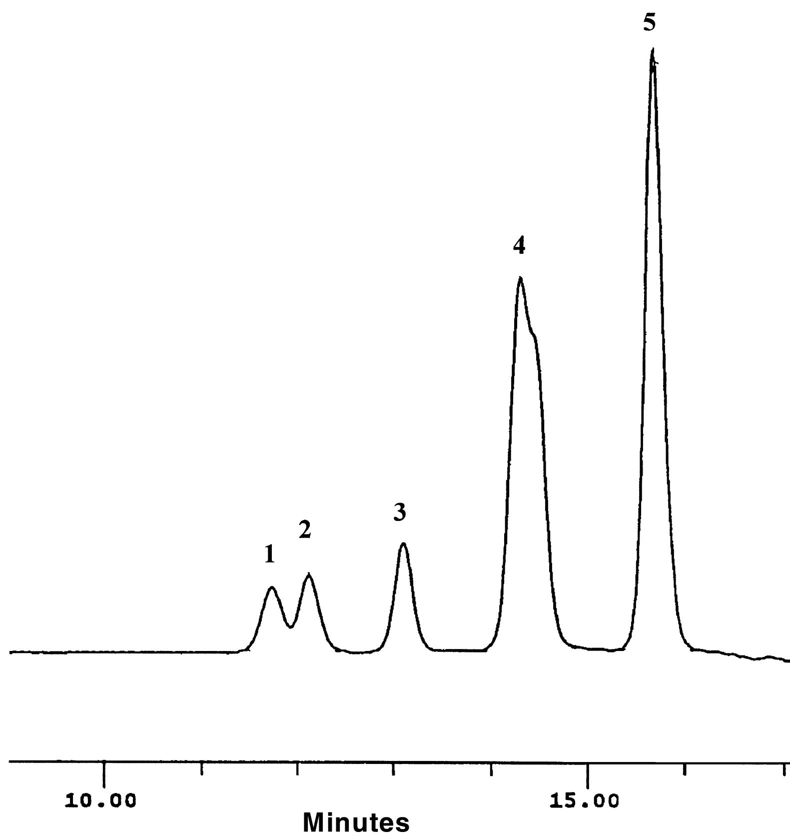

Figure 1. Gel permeation chromatogram of standard compounds. 1 = tripalmitin, 2 = diolein, 3 = monoolein, 4 = methyl oleate + methyl palmitate, 5 = glycerol.

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Table 1

Retention Time of Standard Compounds

Compound	Retention Time (min)
Tripalmitin/triolein	11.73
Diolein	12.13
Monoolein	13.12
Methyl oleate	14.35
Methyl palmitate	14.48
Glycerol	15.70

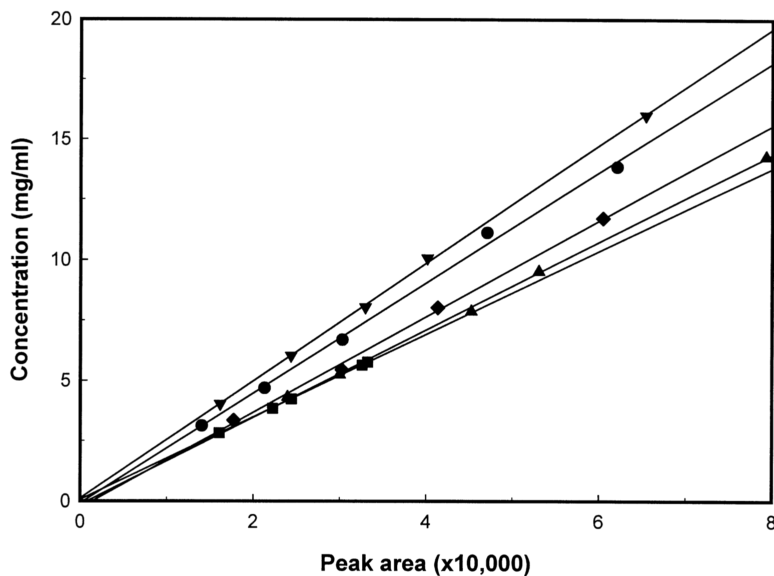

Figure 2. Calibration curves of tripalmitin (1), diolein (2), monoolein (3), methyl oleate (4) and glycerol (5).

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Table 2

Calibration Function of Standard Solutions*

Component	a	b	Standard error	R^2
Tripalmitin	-0.0877	2.2842	0.0474	0.9940
Diolein	0.0577	1.7158	0.0099	0.9995
Monoolein	-0.1544	1.8152	0.0155	0.9991
Methyl oleate	0.1095	2.4378	0.0169	0.9994
Glycerol	-0.2831	1.9822	0.0308	0.9969

* C (mg/ml) = a + b*Peak Area.

Table 3

Analysis of Standard Mixtures*

Sample	Triglycerides	Diglycerides	Monoglycerides	Methyl Esters	Glycerol
Mixture 1					
Injection 1	2.5651	2.5541	2.5095	12.0377	7.7516
Injection 2	2.7230	2.6397	2.5934	12.3787	7.9920
Injection 3	2.7291	2.6312	2.6438	12.4348	7.9905
Mean	2.6724	2.6083	2.5823	12.2837	7.9114
Standard deviation	0.0930	0.0472	0.0679	0.2149	0.1384
Relative S.D. (%)	3.4785	1.8078	2.6276	1.7497	1.7489
Actual	2.7025	2.5250	2.6021	12.7551	8.0250
Recovery (%)	98.8802	103.2990	99.2391	96.3042	98.5844
Mixture 2					
Injection 1	1.6007	1.5001	1.7925	14.9143	11.5542
Injection 2	1.6725	1.5114	1.7989	15.1291	11.6872
Injection 3	1.6944	1.5301	1.8669	15.2088	11.8618
Mean	1.6559	1.5138	1.8194	15.0840	11.7011
Standard deviation	0.0490	0.0152	0.0413	0.1523	0.1543
Relative S.D. (%)	2.9581	1.0017	2.2675	1.0098	1.3186
Actual	1.5950	1.5250	1.9050	15.0750	11.7250
Recovery (%)	103.8182	99.2658	95.5066	100.0597	99.7962
Mixture 3					
Injection 1	0.7156	1.0845	1.4838	20.2784	3.2537
Injection 2	0.6782	1.0249	1.4418	20.2896	3.1996
Injection 3	0.6622	1.0026	1.4839	20.1712	3.2233
Mean	0.6853	1.0373	1.4698	20.2464	3.2255
Standard deviation	0.0274	0.0423	0.0243	0.0654	0.0271
Relative S.D. (%)	4.0033	4.0789	1.6506	0.3231	0.8409
Actual	0.6750	1.0002	1.5000	21.1250	3.3500
Recovery (%)	101.5259	103.7093	97.9867	95.8409	96.2836

* Concentrations are in mg/mL.

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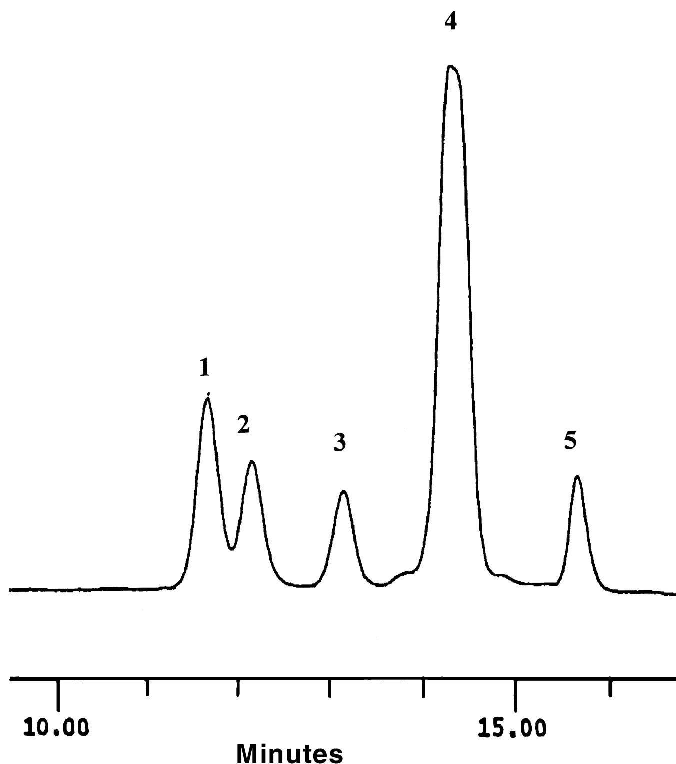

Figure 3. Gel permeation chromatogram of palm methyl esters. 1 = triglycerides, 2 = diglycerides, 3 = monoglycerides, 4 = methyl esters, 5 = glycerol.

To study the recovery of each component, mixtures of known amounts of standard components were prepared and injected three times into the HPLC. The intercept and slope in Table 2 were used to quantitate each component. In general, as shown in Table 3, the difference between the measured and actual values was less than 5%, which indicates good accuracy and precision.

The reproducibility of the method was studied by analyzing samples of palm oil transesterification products produced under 3 different reaction conditions as described by Darnoko.[10] Each sample was injected 5 times. Figure 3 shows a typical chromatogram of crude palm methyl esters mixture and the statistical data are shown in Table 4. The relative standard deviation of the analysis was 0.268-3.87% which indicates good reproducibility.

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Table 4

Composition of Crude Palm Methyl Esters (mg/mL)

Sample	Triglycerides	Diglycerides	Monoglycerides	Methyl Esters	Glycerol
Sample 1					
Injection 1	9.0822	4.6319	3.2053	37.2187	3.1742
Injection 2	9.1300	4.5356	3.1612	35.9562	3.2067
Injection 3	9.0534	4.6463	3.1632	36.1114	3.2204
Injection 4	9.0493	4.5787	3.1408	36.2697	3.2015
Injection 5	9.0911	4.5035	3.1472	35.9705	3.1567
Mean	9.0812	4.5792	3.1635	36.3053	3.1919
Standard deviation	0.0326	0.0610	0.0252	0.5261	0.0258
Relative S.D. (%)	0.3595	1.3329	0.7951	1.4491	0.8097
Sample 2					
Injection 1	2.2575	1.3089	0.7221	43.2093	5.1248
Injection 2	2.2686	1.3755	0.7682	46.6025	5.2035
Injection 3	2.2113	1.3202	0.7404	43.1706	5.2341
Injection 4	2.2801	1.3329	0.7856	46.4514	5.2703
Injection 5	2.2054	1.2468	0.7878	45.9914	5.3474
Mean	2.2446	1.3169	0.7608	45.0850	5.2360
Standard deviation	0.0341	0.0466	0.0287	1.7446	0.0822
Relative S.D. (%)	1.5187	3.5372	3.7829	3.8696	1.5694
Sample 3					
Injection 1	0.6012	0.3240	0.2409	50.8231	6.2741
Injection 2	0.5907	0.3261	0.2258	50.6914	6.2224
Injection 3	0.5909	0.3092	0.2251	50.5469	6.2192
Injection 4	0.5710	0.3039	0.2296	50.6878	6.1861
Injection 5	0.5772	0.3237	0.2207	50.4769	6.2360
Mean	0.5862	0.3174	0.2284	50.6452	6.2276
Standard deviation	0.0120	0.0101	0.0076	0.1356	0.0318
Relative S.D. (%)	2.0502	3.1737	3.3462	0.2678	0.5112

CONCLUSIONS

A simple and reproducible method has been developed for simultaneous analysis of tri-, di-, and monoglycerides, methyl esters, and glycerol from transesterification reaction mixtures. Different classes of triglycerides, diglycerides, or monoglycerides produce only a single peak which makes calculation easier.

Sample preparation is simple and easy: no prior separation of glycerol is needed. The method can be suitably automated for routine analysis.

ACKNOWLEDGMENTS

This study was partly supported by the Illinois Agricultural Experiment Station, University of Illinois, and the Indonesian Oil Palm Research Institute, which provided a leave of absence and a scholarship to D. Darnoko (Present address: Indonesian Oil Palm Research Institute, Jl. Brigjen Katamso 51, Medan 20001, Indonesia).

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Received January 29, 2000 Author's Revisions April 30, 2000
Accepted February 25, 2000 Manuscript 5238

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