Triglyceride Analysis by Combined Argentation/Nonaqueous Reversed Phase High Performance Liquid Chromatography

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Full analysis of triglycerides of natural fats and oils has been investigated by the combination of argentation high performance liquid chromatography (HPLC) with nonaqueous reversed phase (NARP) HPLC. An infrared detector was used in argentation HPLC, because it indicated molar responsibility to all triglycerides. After peak trapping with argentation HPLC, each triglyceride fraction was analyzed with NARP chromatography using the glyceride-selective post-column reactor detector. The results of the analyses of triglycerides of palm oil and cocoa butter by the proposed method agreed well with those reported earlier.

In a previous paper (1), a new post-column reactor detector (PCRD) for the analysis of triglycerides with high sensitivity, high selectivity and molar responsibility was developed. By using this glyceride-selective PCRD (GS-PCRD), triglycerides of natural fats and oils were sufficiently separated and quantitatively analyzed with nonaqueous reversed phase (NARP) chromatography with octadecyl chemically bonded silica (ODS) column. Better separations were obtained by using a gradient solvent system; however, complete separation of all triglyceride species could not be accomplished even with a gradient elution. By NARP chromatography, it seemed very difficult to separate positional isomers such as 1,3-disaturated-2-unsaturated (SUS) and 1-unsaturated-2,3-disaturated (SSU) triglycerides.

On the other hand, Smith et al. (2) described the separation of triglyceride mixtures including the positional isomers, SUS and SSU, by argentation HPLC (Ag HPLC) with a refractive index (RI) detector. It is expected that semi-preparative Ag HPLC combined with a subsequent NARP chromatographic analysis of the trapped peak will enable full separation of all triglyceride species except positional isomers of trisaturated (SSS) triglycerides. For Ag HPLC an infrared detector is considered to be the most suitable, for it is expected to have molar responsibility to all triglyceride species. Furthermore, high sensitivity and molar responsibility of the GS-PCRD makes it possible to analyze a small amount of the trapped triglycerides by NARP chromatography. Therefore, the combination of Ag HPLC with NARP chromatography was investigated for the full analysis of triglycerides.

This paper deals with the combination of Ag HPLC using an IR detector with NARP chromatography using the GS-PCRD for the full analysis of triglyceride species; it also deals with the analyses of triglycerides of palm oil and cocoa butter.

EXPERIMENTAL PROCEDURES

Apparatus. The liquid chromatograph with the GS-PCRD is similar to that described in a previous paper (1). The liquid chromatograph for Ag HPLC consisted of a reciprocating piston pump (Model 638-50, Hitachi, Tokyo, Japan), an IR detector (HPIR-100, Japan Spectroscopic Co., Tokyo, Japan), an injector (Model 7125, Rheodyne, Berkeley, California), and a circulator (Model D8-G, Haake Inc., Karlsruhe, West Germany) for column temperature control. Chromatograms, peak areas and

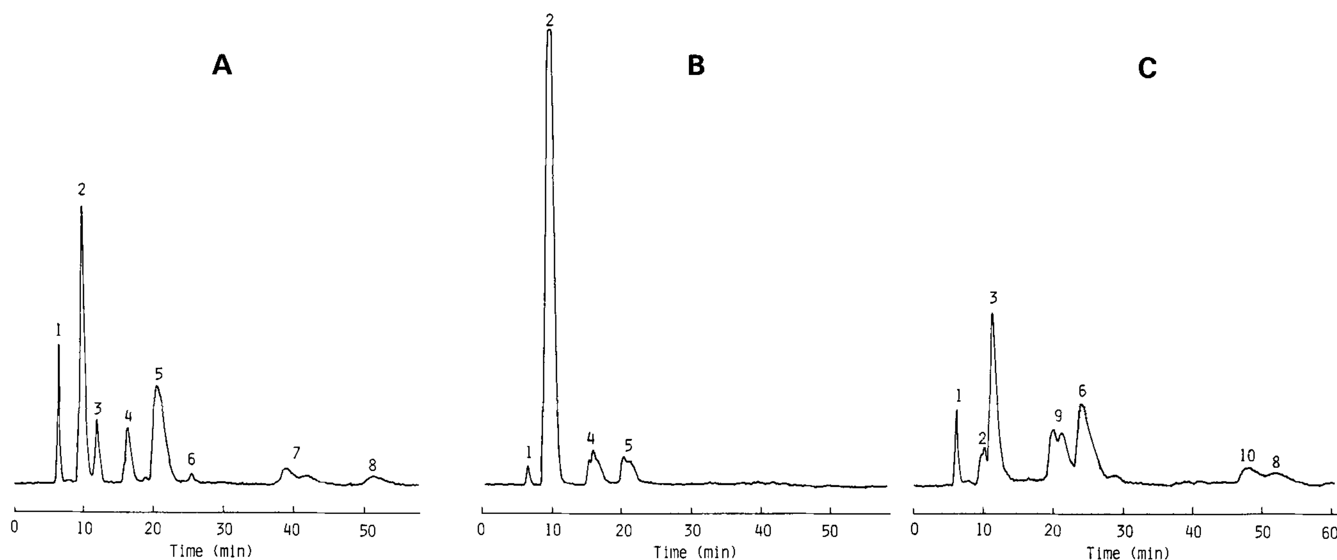

FIG. 1. Analysis of triglycerides of palm oil (A, injection amount 4 mg); cocoa butter (B, injection amount 3 mg), and lard (C, injection amount 4 mg) with argentation HPLC using an IR detector. 1, SSS; 2, SUS; 3, SSU; 4, SLS; 5, SUU; 6, USU; 7, SLU + SUL; 8, UUU; 9, SUU + SSL; 10, USL.

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TABLE 1

Relative Molar Response of Triolein to Tripalmitin with Infrared and Refractive Index Detectors

	Molar response	
Detector	Tripalmitin	Triolein
Infrared	1.00	1.02
Refractive index	1.00	0.68

retention times were obtained by using a data processor (CHROMATOPAC C-R3A, Shimadzu Co., Kyoto, Japan).

Reagents. Hitachi gel 3057 (Hitachi) was used as a stationary phase, which was ODS packings of average diameter 3 μm. For Ag HPLC, 10% silver nitrate impregnated silica (Develosil 60-3, average diameter 3 μm, Nomura Chemical Co., Seto, Japan) was prepared according to Smith et al. (2). Authentic triglycerides were purchased from Sigma Chemical Co. (St. Louis, Missouri) and P-L Biochemicals (Milwaukee, Wisconsin). Acetonitrile and ethanol of HPLC analysis grade were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). Other reagents were of analytical reagent grade.

Procedure for triglyceride analysis. For Ag HPLC, two stainless columns (4.6 mm i.d., 150 mm long) packed with 10% silver nitrate-impregnated Develosil 60-3 were connected together and kept at 25 C. (The column could be used for over one mo.) Benzene was used as a mobile phase at a flow rate of 1.5 ml/min. The column effluent was monitored at 1745 cm^{-1}, and each peak was trapped for the subsequent analysis with NARP chromatography. A sample, containing a few mg of triglycerides dissolved in 20–50 μl benzene, was injected into HPLC.

TABLE 2

Analysis of Triglycerides of Palm Oil by Argentation HPLC

Type of triglyceride	Composition (mol %)	Coefficient of variation (%)
SSS	8.5	2.5
SUS	34.8	2.3
SSU	7.0	4.6
SLS	8.6	5.5
SUU	27.1	1.9
USU	1.4	17.7
SLU	8.1	5.2
UUU	3.6	22.9

For NARP chromatography, two stainless columns (4.6 mm i.d., 150 mm long) packed with Hitachi gel 3057 were connected together and kept at 35 C. Ethanol-acetonitrile (60/40) was used as a mobile phase at a flow rate of 0.8 ml/min. The effluent was monitored with the GS-PCRD. A sample, containing 0.2–200 μg triglycerides

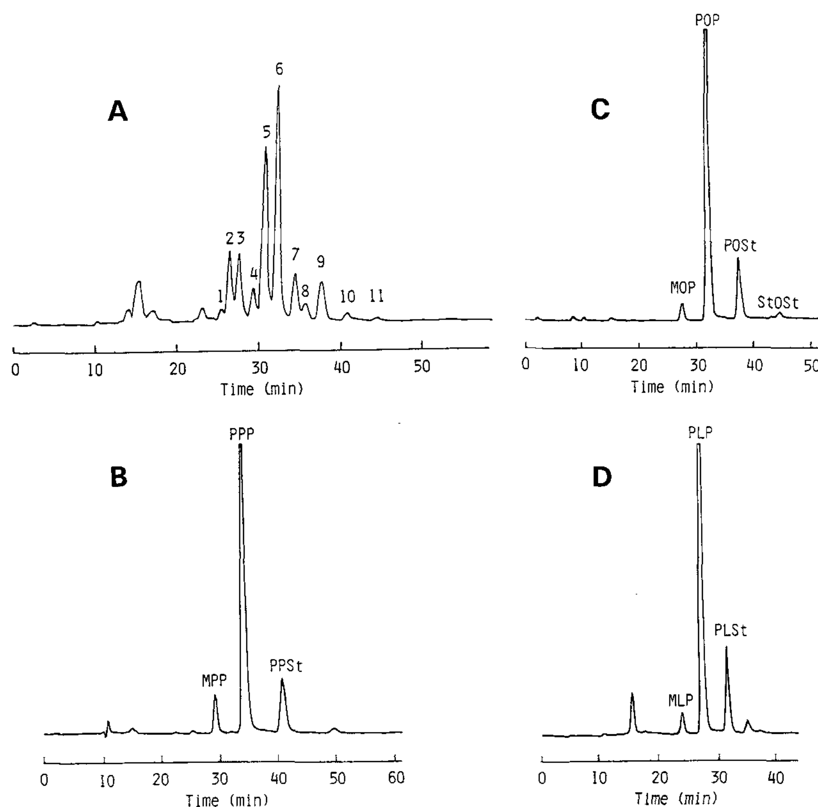

FIG. 2. Typical chromatograms of triglycerides of palm oil with NARP chromatography using the GS-PCRD after peak trapping with argentation HPLC. A, original triglycerides of palm oil (injection amount 40 μg): 1, LOO; 2, PLO; 3, PLP; 4, OOO; 5, POO; 6, POP; 7, PPP; 8, SOO; 9, POS; 10, SPP; 11, SOS. B, SSS fraction (injection amount 50 μg); C, SUS fraction (injection amount 50 μg); D, SLS fraction (injection amount 50 μg).

dissolved in 5-40 μl of acetone or less than 5 μl of tetrahydrofuran, was injected into HPLC.

RESULTS AND DISCUSSION

Separation of triglycerides with argentation HPLC. Smith et al. reported the separation of SSS, SUS, SSU, 2-diunsaturated-1,3-disaturated (SLS), 2,3-diunsaturated-1-saturated (SUU) and triunsaturated (UUU) triglycerides with Ag HPLC using benzene as a mobile phase (2). The resolution between SSU and SUS was poor at room temperature; however, a decrease in column temperature rapidly increased the resolution, which led them to set the column temperature at 6.8 C. In the present study, the separation of triglycerides with Ag HPLC was carried out under the conditions described in the experimental section according to Smith et al., but the column temperature was set at 25 C, taking into account resolution, peak shape and column pressure. A typical chromatogram of triglycerides of palm oil is shown in Figure 1A, where assignments of a few peaks are indicated in addition to those reported by Smith et al. (2). These assignments were done by the analyses of the fatty acid moieties of triglycerides after peak trapping. Typical chromatograms of cocoa butter and lard also are shown in Figure 1B and 1C.

Responsibility and reproducibility of an infrared detector. The responsibility of the IR and RI detectors to tripalmitin and triolein is shown in Table 1. The responsibility of the IR detector was measured at 1745 cm^{-1}, because the highest sensitivity was obtained at 1745 cm^{-1}. In the case of the IR detector, the molar response of triolein is 1.02, while in the case of the RI detector it is 0.68. These results clearly indicate that the IR detector has molar responsibility to all triglyceride species. Furthermore, the stability of the IR detector was superior to that of the RI detector. These facts led us to use the IR detector for Ag HPLC.

Table 2 shows the results of the analyses of palm oil and the reproducibilities of the analyses. Main components of palm oil were SUS and SUU, at 34.8% and 27.1%, respectively. The reproducibilities decreased with decreases in the triglyceride content and increases in unsaturation of triglyceride, but they were within 6% except for USU and UUU.

Combination of Ag HPLC with NARP HPLC for the full triglyceride analysis. After peak trapping with Ag HPLC, each triglyceride fraction was analyzed with

TABLE 3

Compositions of Triglycerides of Palm Oil

		Composition (mol %)			
			Literature (3,4)		
Unsaturation	Triglyceride	Proposed method	Malaysia	Sumatra	Congo
0	PMP + MPP	0.7	0.7	0.9	0.5
	PPP	6.5	7.2	6.1	4.3
	PPSt + PStP	1.3	1.7	1.2	1.5
	PStSt	—	0.1	—	—
1	MOP	1.2	1.4	1.3	1.2
	POP	27.6	23.7	25.9	24.1
	POSt	5.5	3.1	3.1	7.0
	StOSt	0.7	—	—	0.5
	MPO	0.8	0.2	—	—
	PPO	5.3	6.9	6.0	3.6
	PStO + StPO	0.8	1.1	0.8	0.5
2	MOO	0.4	0.7	—	0.6
	POO	23.1	21.5	18.9	18.9
	StOO	3.0	1.4	2.6	2.8
	OPO	1.0	1.6	1.2	1.0
	OStO	—	0.2	—	—
	PPL	0.8	1.0	1.7	0.4
	PStL + StPL	—	0.2	—	—
	MLP	0.3	—	—	0.5
	PLP	7.0	6.3	6.8	7.8
	PLSt	1.5	0.8	1.9	2.3
3	OOO	3.4	5.1	3.2	2.7
	MOL + MLO	—	0.3	—	0.5
	POL + PLO + OPL	7.3	9.6	7.4	8.8
	StOL + StLO	0.8	0.6	0.5	1.2
1-3	Others	0.3	0.8	2.7	2.3
4		—	3.8	6.9	7.0

TABLE 4

Compositions of Triglycerides of Cocoa Butter

		Composition (mol %)	
Unsaturation	Triglyceride	Proposed method	Literature (3)
0	PPP	0.2	0.1
	PPSt + PStP	0.4	0.5
	PStSt + StPSt	0.5	1.1
	StStSt	0.3	0.4
	Others	0.3	0.1
1	MOP	—	0.9
	MOSt + POP	15.6	13.3
	POSt	38.1	34.8
	StOSt	26.1	25.2
	StOA	1.6	—
	PMO	—	0.2
	StMO + PPO	—	0.9
	StPO + PStO	—	1.7
	StStO	—	1.1
	Others	1.9	0.2
2	MOO	0.3	—
	POO	2.9	3.7
	StOO	3.7	4.9
	PLP	1.6	1.2
	PLSt	3.4	3.1
	StLSt	2.2	2.0
	Others	0.8	1.1
3		—	2.6
4		—	0.9

NARP chromatography using the GS-PCRD. No isomerization of triglycerides occurred during the analyses; this was confirmed by the analysis of a mixture of standard triglycerides. Figure 2A shows the chromatogram of original triglycerides of palm oil with NARP chromatography, while Figures 2B-2D show the chromatograms of the trapped triglyceride fractions of palm oil. These analyses could be effected by only one peak trapping.

Tables 3 and 4 show the results of full triglyceride analysis of palm oil and cocoa butter, respectively, by the proposed method. These results agree well with those reported by Jurriens et al. (3,4).

The proposed method is applicable to the analysis of triglycerides containing up to triunsaturation; however, the gradient elution method in Ag HPLC suggested by Aitzetmüller (5) will make it possible to analyze triglycerides with unsaturation higher than triunsaturation, which is now under investigation. On the other hand, it seems very difficult to analyze positional isomers of SSS, SSU, etc. by means of chromatography. However, it will become feasible by pancreatic lipase hydrolysis (3,6-8) of SSS and SSU.

ACKNOWLEDGMENT

Hideko Ono and Kunio Iida provided technical assistance.

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[Received June 20, 1986]