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Composition analysis of normal plant triacylglycerols and hydroperoxidized *rac*-1-stearoyl-2-oleoyl-3-linoleoyl-*sn*-glycerols by liquid chromatography–atmospheric pressure chemical ionization mass spectrometry

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Abstract

Liquid chromatography–atmospheric-pressure chemical ionization mass spectrometry (LC–APCI-MS) of some plant triglycerols gave mass spectra with the peaks of $[M+H]^+$ and $[M-R_1(R_3)\ COOH+H]^+$ ions, in which $[M+H]^+$ and $R_1(R_3)COOH$ represent, respectively, the protonated molecular ion and fatty acid at the *sn*-1-(or -3-)position of the triacylglycerol. It was possible to discriminate fatty acids between *sn*-1-(or -3-) and -2-positions. The ratio of the molecular extinction coefficient (ε) of triacylglycerol at 220 nm to that of cholesterol depended on the total number of double bonds in the triacylglycerol. Thus, triacylglycerols in naturally occurring oils could be determined by using LC–APCI-MS with spectrophotometry. LC–APCI-MS of hydroperoxidized triacylglycerols gave characteristic fragment ions $[M-H_2O_2+H]^+$, $[M-H_2O+H]^+$ and $[M-R_1(R_3)\ COOH-H_2O_2+H]^+$.

Keywords: Oils; Triacylglycerols; Glycerols

1. Introduction

Stereospecific analysis of triacylglycerols has been performed for many years [1]. However, the most recent method [2] is still complicated and time consuming because techniques such as HPLC, TLC and GC must be combined skillfully. From the viewpoint of the nutritive value of the triacylglycerols, it is sufficient to discriminate between fatty acids at the *sn*-1-(or -3-) and -2-positions of the triacylglycerol. Liquid chromatography–atmospheric-pressure chemical ionization mass spectrometry (LC–APCI-MS) was found to be useful for this purpose. Further, it may be possible to apply this technique to the analysis of hydroperoxydized triacylglycerols.

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2. Experimental

2.1. Reagents

Acetonitrile, *n*-hexane and 2-propanol (HPLC grade) were purchased from Nacalai Tesque (Kyoto, Japan). Racemic (*rac*)-1,3-dioleoyl-2-stearoyl-*sn*-glycerol, *rac*-1-palmitoyl-2-oleoyl-3-stearoyl-*sn*-glycerol, *rac*-1-stearoyl-2-oleoyl-3-linoleoyl-*sn*-glycerol, *rac*-1,3-dilinoleoyl-2-stearoyl-*sn*-glycerol and tripalmitin were purchased from Larodan Fine Chemicals (Malmo, Sweden). The edible plant oils perilla oil (Nihonyushi), corn oil (Azinomoto) and olive oil (Azinomoto) were used.

2.2. Preparation of hydroperoxidized triacylglycerols

Hydroperoxidization of the unsaturated acyl-containing tricylglycerols was performed by the following method. Each triacylglycerol (100–200 μg) was suspended in 1 ml of 0.2% sodium deoxycholate, then, the solution was irradiated with a tungusten lamp (40 W) at a distance of 5 cm for 20 h at 15°C and extracted with chloroform.

2.3. LC–APCI-MS

A Hitachi (Tokyo, Japan) M-2000 double-focusing mass spectrometer equipped with a Hitachi L-6200 HPLC instrument and a Hitachi APCI interface system was used. HPLC was performed using a reversed-phase Cosmosil $_5C_{18}$-packed column with 5-μm particles (250 mm × 4.6 mm I.D.) (Nacalai Tesque). The column temperature in the column was maintained at 40°C. The eluate from the LC column was conducted to the mass spectrometer via a photometric cell and the APCI interface system. Spectrophotometric chromatograms at a wavelength of 220 nm were processed with a 7000-B-type chromatogram processor (System Instrument, Tokyo, Japan). The drift voltage of the APCI interface system was 100 V and the temperatures of the vaporizer and desolvator were 250°C and 385°C, respectively. The multiplier voltage of the mass spectrometer was 2000 V.

3. Results

3.1. LC–mass spectra of authentic triacylglycerols

The LC–mass spectra of authentic triplamitin, *rac*-1,3-dioleoyl-2-stearoyl-*sn*-glycerol and *rac*-1-palmitoyl-2-oleoyl-3-stearoyl-*sn*-glycerol were determined (Fig. 1). In the spectra, the peaks of [molecule (M) + H]$^+$ and [M − $R_1(R_3)$ COOH + H]$^+$ ions were observed (either one is the base peak). [M + H]$^+$ and $R_1(R_3)$COOH represent, respectively, the protonated molecular ion and fatty acid at the *sn*-1-(or -3-)position in the molecule. The mass spectra of triacylglycerols such as *rac*-1-stearoyl-2-oleoyl-3-linoleoyl-*sn*-glycerol and *rac*-1,3-dilinoleoyl-2-stearoyl-*sn*-glycerol were observed to be discriminated. Therefore, it is possible to discriminate between fatty acyl groups at *sn*-1-(or -3-) and -2-positions of triacylglycerols.

3.2. LC–MS of plant oils

The edible plant oils perilla oil, corn oil and olive oil (50–200 μg) were applied to the LC–mass spectrometer system equipped with a spectrophotometer. HPLC was monitored by measuring the spectrophotometric absorption at 220 nm. Several peaks were observed for these oils (Fig. 2). The eluate from the LC column was conducted to the mass spectrometer with the APCI interface system or to the LC–MS system after fractionation of these peaks. Examples of the mass spectra are shown in Fig. 3. These indicated that peaks 2 and 3 in Fig. 2C were 1,3-dioleoyl-2-linolenoyl-*sn*-glycerol and 1-(or -3-)palmitoyl(or linoleyl)-2-linoleyl-*sn*-glycerol, respectively. The molecular species of the acyl groups and their stereospecific numbers in triacylglycerols are shown in Table 1.

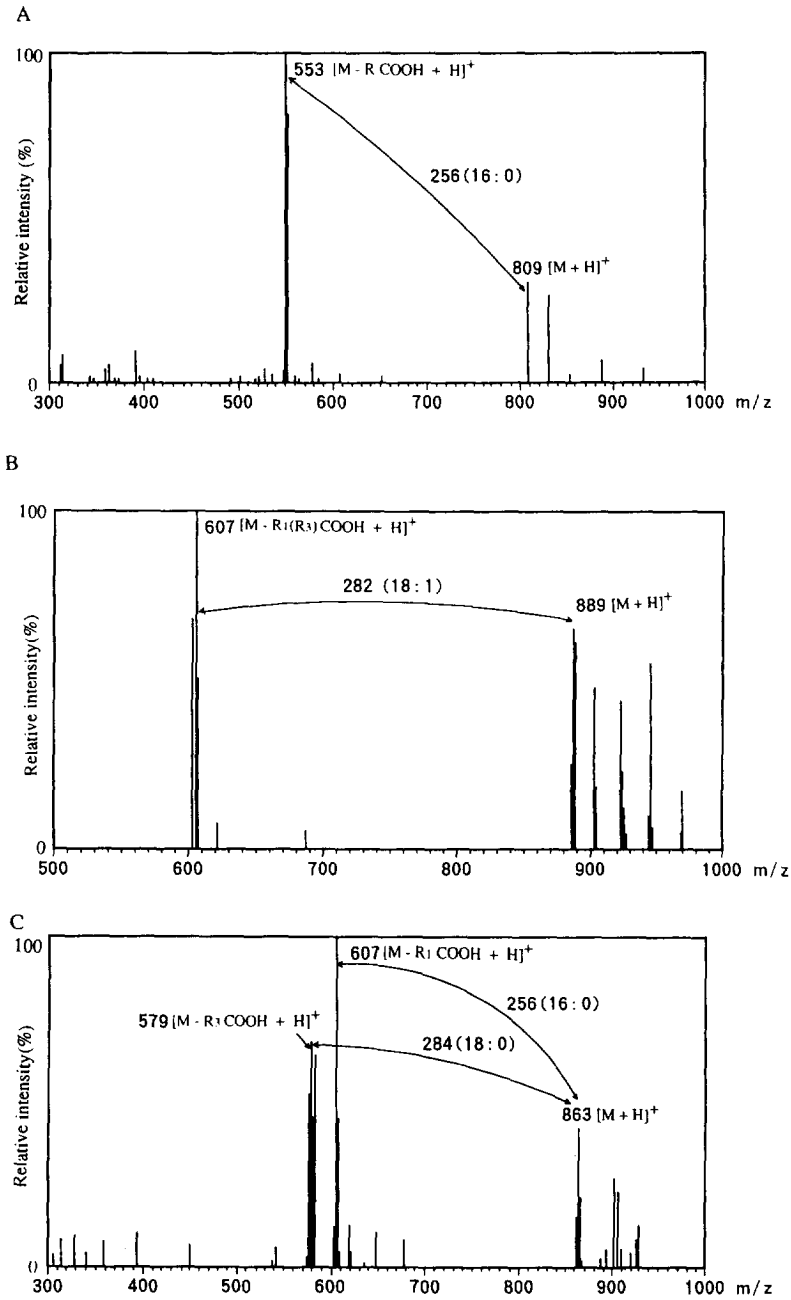

Fig. 1. APCI-mass spectra of (A) tripalmitin, (B) *rac*-1,3-dioleoyl-2-stearoyl-*sn*-glycerol and (C) *rac*-1-palmitoyl-2-oleoyl-3-stearoyl-*sn*-glycerol. Mobile phase, *n*-hexane–acetonitrile–2-propanol (10:80:10, v/v/v); amount of each sample applied, 5 μg.

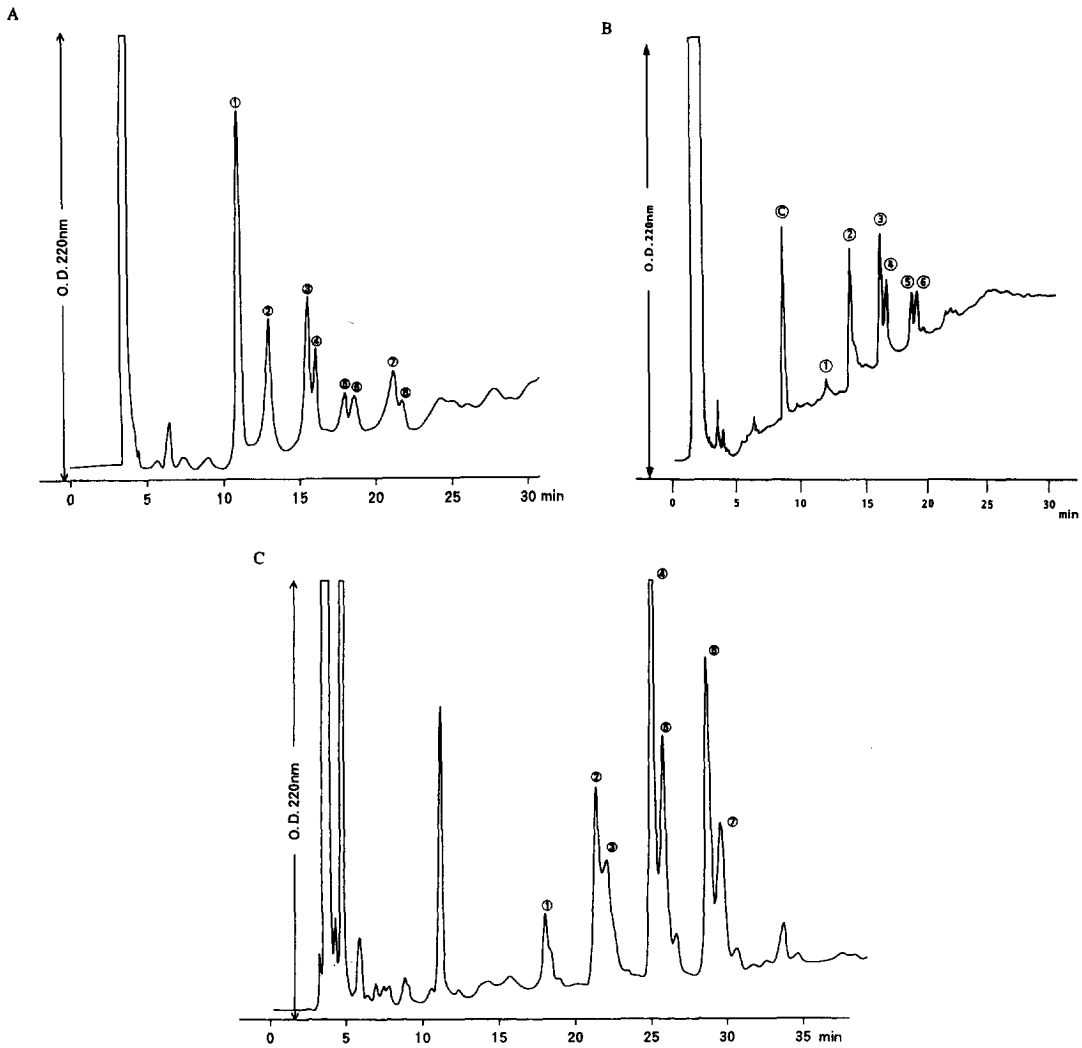

Fig. 2. HPLC profiles of (A) perilla oil, (B) corn oil and (C) olive oil with spectrophotometric detection at 220 nm. The starting mobile phase was acetonitrile–2-propanol (60:40, v/v) and *n*-hexane was added, with acetonitrile decreasing, in a linear gradient at 0.33%/min, the final mobile phase being *n*-hexane–acetonitrile–2-propanol (20:40:40, v/v/v). Amounts of 50–200 μg of each oil (A, C) and 200 μg of corn oil (B), to which 40 μg of cholesterol (peak C) were added as an internal standard, were applied.

3.3. Determination of triacylglycerols

The ratio of the molecular extinction coefficient (ε) of each triacylglycerol (TG) at 220 nm to that of cholesterol was found to depend on the total number of double bonds in each triacylglycerol (Table 2). Thus, triacylglycerols could be determined by measuring the absorbance at 220 nm (optical density, OD_{220}) of each triacylglycerol using cholesterol (Cho) as an internal standard. The amount of each triacylglycerol was calculated as follows:

$$\text{nmol of TG} = \text{nmol of Cho} \cdot \frac{\text{peak area of TG}}{\text{peak area of Cho}} \cdot \frac{1}{a}$$

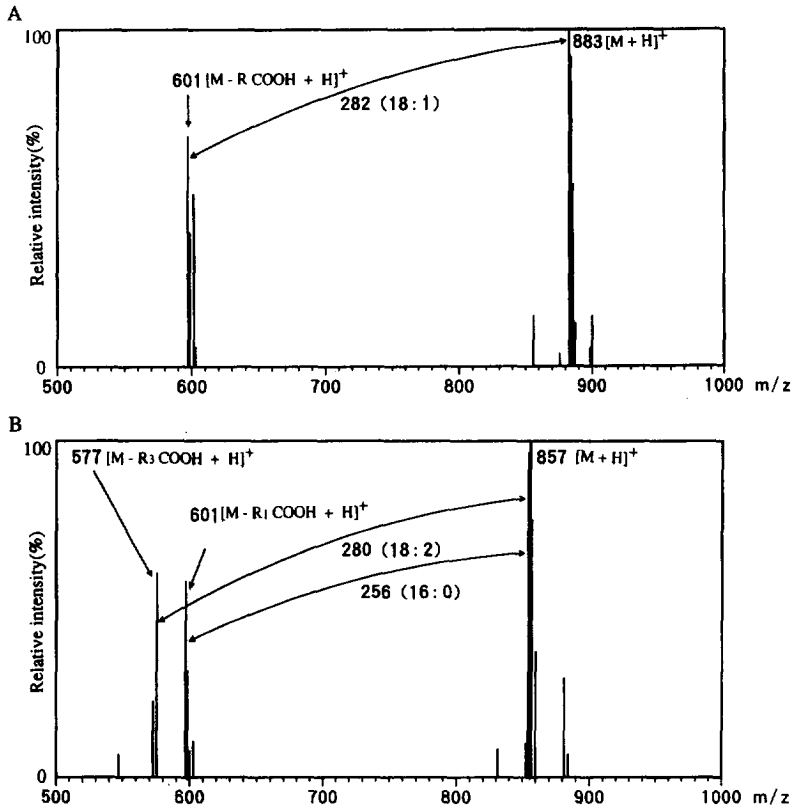

Fig. 3. Mass spectra of (A) peak 2 and (B) peak 3 in Fig. 2C.

Table 1
Molecular species of acyl groups and their stereospecific numbers in triacylglycerols

Peak No.	Perilla oil (Fig. 2A)		Corn oil (Fig. 2B)		Olive oil (Fig. 2C)	
	sn-1 or -3	-2	*sn*-1 or -3	-2	*sn*-1 or -3	-2
1	18:2 18:3	18:3	18:2 18:2	18:3	18:1 18:3	18:2
2	18:1 18:3	18:3	18:2 18:2	18:2	18:1 18:1	18:3
3	18:1 18:2	18:3	18:1 18:2	18:2	16:0 18:2	18:2
4	16:0 18:2	18:3	16:0 18:2	18:2	18:1 18:1	18:2
5	18:1 18:2	18:2	18:1 18:2	18:1	16:0 18:2	18:1
6	16:0 18:3	18:1	16:0 18:1	18:2	18:1 18:1	18:1
7	18:0 18:1	18:3			16:0 18:1	18:1
8	16:0 18:3	18:0				

Table 2
Dependence of ratio of ε (TG/Cho) to the total double bond number (total carbon number of acyl group = 52 or 54)

Total double bond No.	Ratio of ε	Total double bond No.	Ratio of ε
1	0.48	4	1.06
2	0.66	7	1.61
3	0.78	8	1.68

Table 3
Contents of triacylglycerols in corn oil

Peak No. In Fig. 2B	Triacylglycerols (mol-%)	Peak No. in Fig. 2B	Triacylglycerols (mol-%)
1	3.6	4	21.8
2	24.2	5	10.1
3	25.6	6	14.6

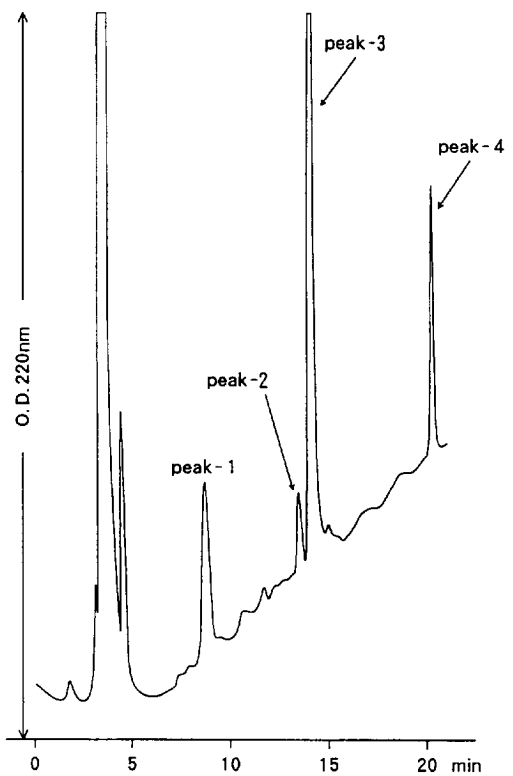

Fig. 4. HPLC profile of hydroperoxidized *rac*-1-stearoyl-2-oleoyl-3-linoleoyl-*sn*-glycerol with spectrophotometric detection at 220 nm. The mobile phase for HPLC was the same as that in Fig. 2 except that *n*-hexane was added at 1%/min.

where a is the ratio of the molecular extinction coefficient (ε) of each triacylglycerol at 220 nm to that of cholesterol.

The amount of triacylglycerols in corn oil (Fig. 2B) calculated according to the above equation is shown in Table 3.

3.4. LC–mass spectra of hydroperoxidized triacylglycerols

Triacylglycerols were hydroperoxydized as described under Experimental. Fig. 4 shows the HPLC spectrophotometric profiles. Peak 4 could be identified as an intact (non-hydroperoxidized) triacylglycerol from the mass spectrum and the elution time. Peaks 1, 2 and 3 were observed only after the hydroperoxidization reaction. Peak 1 is still unknown. Peaks 2 and 3 could be identified as hydroperoxidized triacylglycerols, because the mass spectra (Fig. 5) showed the main fragment ions $[M - H_2O_2 + H]^+$, $[M - H_2O + H]^+$ and $[M - R_1(R_3)\ COOH - H_2O_2 + H]^+$. These are similar to those ($[M - H_2O_2 + H]^+$ and $[M - H_2O + H]^+$ ions) of 7-methoxy-1,4-benzoxazin-2-one-3-methyl ester (MB) derivatives of hydroperoxy fatty acids [3]. In principle, application to the analysis of naturally occurring hydroperoxytriacylglycerols may be possible.

4. Discussion

The identification and determination of the molecular species of triacylglycerols by HPLC coupled to MS have been studied for years [4–9], but the methods developed are still not satisfactory enough to discriminate between fatty acyl groups at *sn*-1-(or -3-) and -2-positions in triacylglycerols. Kallio et al. [10] reported a new method for the analysis of triacylglycerols using selected ion-monitoring MS. They demonstrated the discrimination between fatty acids at the *sn*-1-(or -3-) and -2-positions in some authentic triacylglycerols. However, it is unclear whether their method can be employed for unknown triacylglycerols in general.

The present method is very simple and not time consuming. In addition, it requires only

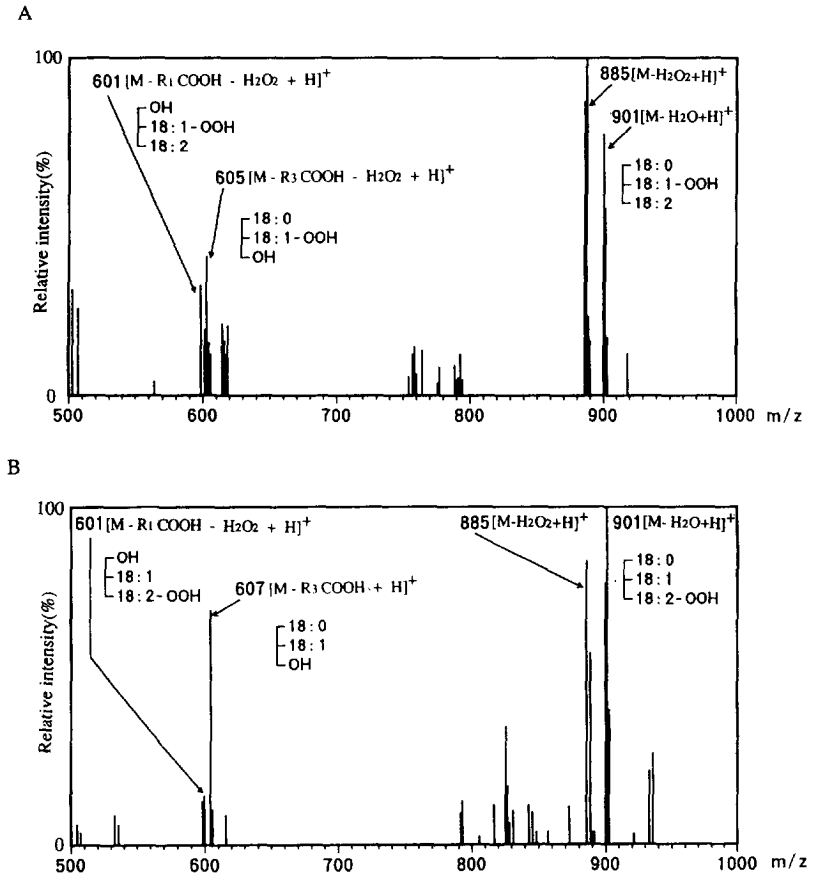

Fig. 5. Mass spectra of (A) peak 2 and (B) peak 3 in Fig. 4. The proposed triacylglycerol structures are shown.

nanomole levels of triacylglycerols without derivatization.

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