Analysis of Molecular Species of Glycolipids in Fruit Pastes of Red Bell Pepper (*Capsicum annuum* L.) by High-Performance Liquid Chromatography−Mass Spectrometry

Ryo Yamauchi,*,† Koichi Aizawa,‡ Takahiro Inakuma,‡ and Koji Kato†

Department of Bioprocessing, Faculty of Agriculture, Gifu University, 1-1 Yanagido, Gifu City, Gifu 501-1193, Japan, and Kagome Research Institute, Kagome Company Ltd., Nishinasuno, Tochigi 329-2762, Japan

Five major glycolipid classes (acylated steryl glucoside, steryl glucoside, monogalactosyldiacylglycerol, digalactosyldiacylglycerol, and glucocerebroside) from fruit pastes of red bell pepper were separated by silica gel column chromatography. The molecular species of each glycolipid were separated and characterized by reversed-phase high-performance liquid chromatography coupled with on-line mass spectrometry using atmospheric pressure chemical ionization. The molecular species of steryl glucoside were β-sitosteryl and campesteryl glucosides, and those of the acylated steryl glucoside were their fatty acid esters. The dilinolenoyl species was predominant in monogalactosyldiacylglycerol in addition to small amounts of another five molecular species, whereas digalactosyldiacylglycerol consisted of seven molecular species varying in their degree of unsaturation. The glucocerebroside class contained at least seven molecular species, which were characterized by proton nuclear magnetic resonance spectroscopy.

Keywords: *Red bell pepper; steryl glucoside; glyceroglycolipid; sphingoglycolipid; LC-MS*

INTRODUCTION

Ripe fruits of red bell pepper (*Capsicum annuum*) are used widely as vegetables and food additives, such as ground pepper (paprika) and oleoresin, which are good sources of carotenoid pigments. Capsanthin is the major carotenoid in red bell peppers and occurs acylated with fatty acids (*1*, *2*). This carotenoid, which does not possess provitamin A activity, has been shown to be effective as a free radical scavenger (*3*). Red bell peppers are also a good source of various lipids (*4*) and some micronutrients such as vitamins A, C, and E (*5*−*7*), but limited information is available on the content and composition of such nutrients in the fresh or processed products.

Edible plant glycolipids are thought to be nutrients in the human diet. Glycolipids in higher plants mainly consist of steryl glucosides, sphingoglycolipids, and glyceroglycolipids. These glycolipids are widely distributed, if not universal, in edible plants (*8*, *9*). The fruits of red bell pepper contain all three of the above-mentioned glycolipid classes (*4*, *10*). In previous studies (*11*, *12*), plant glycolipid classes have been directly separated and quantified by normal-phase high-performance liquid chromatography (HPLC). However, the molecular species of each glycolipid class were not fully characterized.

The objective of this study was to develop rapid and direct analyses of glycolipids from red bell pepper using HPLC coupled with on-line mass spectrometry (LC-MS). The glycolipid classes were first separated by silica gel column chromatography, and then the molecular species of each glycolipid were separated and characterized by reversed-phase LC-MS.

MATERIALS AND METHODS

Materials. The fruits of red bell pepper (*C. annuum* L. var. Capia, harvested in 1997) were supplied by a local distributor. The fruits were processed to pastes within the same day after harvest and stored at −30 °C until analyzed. Standard monogalactosyldiacylglycerol and digalactosyldiacylglycerol were isolated from spinach leaves (*13*).

Isolation of Glycolipid Classes. The lyophilized fruit pastes (100 g in dry weight) of red bell pepper were extracted with 600 mL of chloroform/methanol (2:1, v/v) three times, and the total lipids were obtained following the method of Folch et al. (*14*). The total lipids (3.26 g) were dissolved in 20 mL of chloroform and subjected to silica gel column chromatography (silica gel BW-820MH, 70−200 mesh; Fuji Silysia Chemical Ltd., Kasugai, Japan; 4.5 × 30 cm) with sequential elutions of chloroform (1 L), acetone (2 L), and methanol (1 L). Each solvent eluate was pooled, and the solvent was removed in vacuo to obtain neutral lipids (1.73 g) from the chloroform, glycolipids (0.79 g) from the acetone, and phospholipids (0.63 g) from the methanol, respectively. An aliquot of the glycolipid fraction was analyzed by silica gel thin-layer chromatography (silica gel 60 TLC, 0.25 mm thickness; Merck, Darmstadt, Germany) developed in chloroform/methanol (85:15, v/v). Compounds on the plate were visualized by charring after spraying with 50% H_2SO_4. Six major spots, **1**−**6**, were detected on the TLC plate (Figure 1). The bulk of the glycolipid fraction (0.79 g) was then separated into fractions **1**−**6** by silica gel column chromatography (2.5 × 30 cm). Fractions **1**−**6** were sequentially eluted by increasing the methanol concentration in mixtures of chloroform/methanol: a red pigment **1** (35 mg) was eluted with chloroform/methanol (99:1, v/v); fraction **2** (43 mg) with chloroform/methanol (98:2, v/v); fraction **3** (138 mg) with chloroform/methanol (95:5, v/v); fraction **4** (171 mg) with chloroform/methanol (90:10, v/v); fraction **5** (76 mg) with chloroform/methanol (85:15, v/v); and fraction **6** (141 mg) with chloroform/methanol (80:20, v/v). Fraction **5** was further separated into its molecular species by preparative HPLC.

* Author to whom correspondence should be addressed (fax +81-58-293-2930; e-mail yamautir@cc.gifu-u.ac.jp).

† Gifu University.

‡ Kagome Co. Ltd.

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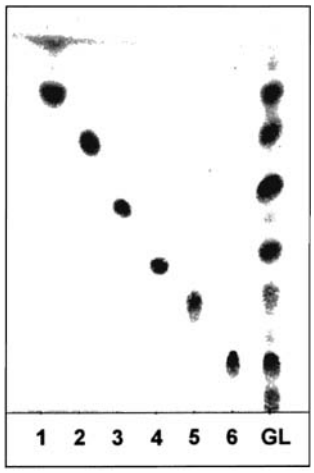

Figure 1. Thin-layer chromatogram of the glycolipid fractions from red bell pepper. The glycolipid fraction (GL) was further separated by silica gel column chromatography to obtain fractions **1**−**6**. The silica gel plate was developed with chloroform/methanol (85:15, v/v), and spots were visualized by charring after spraying with 50% H_2SO_4.

Reversed-phase HPLC was done with an Inertsil Prep-ODS column (1.0 × 25 cm; GL Sciences, Tokyo, Japan) developed with methanol at a flow rate of 5 mL/min. The eluate was monitored by its absorbance at 205 nm.

Analysis of Components in Glycolipids. The fatty acid composition was determined by gas−liquid chromatography (GLC) after transmethylation with 5% HCl in methanol (*15*). The sugar composition was determined by GLC as alditol acetates after hydrolysis (*15*). The sterol composition was determined by GLC as trimethylsilyl derivatives after saponification (*15*, *16*).

LC-MS. HPLC was carried out using a Shimadzu LC-10AV*vp* pump equipped with a Shimadzu SPD-10A*vp* UV−vis detector (Shimadzu Co., Kyoto, Japan). Sample lipids were separated isocratically on a Luna 3 μm C18(2) column (2.0 × 150 mm, Phenomenex, Torrance, CA) at 40 °C. The mobile phase was methanol/water (98:2, v/v) for the analyses of **1** and **3**−**6** or methanol/ethanol (3:2, v/v) for the analysis of **2**, respectively, with the flow rate maintained at 0.2 mL/min. Online UV detection at 205 nm was performed before MS detection. Atmospheric pressure chemical ionization (APCI)-MS was performed using a Shimadzu LCMS-QP8000α quadruple mass spectrometer. The MS parameters were optimized by direct infusion of polyethylene glycol standards into the source. An APCI probe voltage of 4.5 kV and a temperature of 400 °C were used. Nebulizing gas (nitrogen) was delivered at a flow rate of 2.5 L/min. The curved desolvation line (CDL) voltage was at −40 V with a temperature at 250 °C. The deflector voltage was maintained at +30 V for the analysis of capsanthin, at +70 V for the analysis of acylated steryl glucoside and steryl glucoside, or at +80 V for the analysis of monogalactosyldiacylglycerol, digalactosyldiacylglycerol, and cerebroside. Ionization was performed in the positive ion mode for all analyses, and mass spectra were acquired in the mass range *m*/*z* 200−1000 at a scan rate of 3 s.

Proton Nuclear Magnetic Resonance Spectrometry (^{1}H NMR). ^{1}H NMR spectra were recorded with a Varian Inova 400 FT-NMR spectrometer (Varian, Palo Alto, CA) with $CDCl_3/CD_3OD$ (2:1, v/v) as the solvent and tetramethylsilane as the internal standard. ^{1}H NMR was performed at 400 MHz, and the ^{1}H−^{1}H chemical shift-correlated (COSY) technique was employed to assign ^{1}H shifts and couplings.

RESULTS AND DISCUSSION

Isolation of Glycolipids. The glycolipid fraction in red bell pepper lipids contained six major spots, **1**−**6**, on the TLC (Figure 1). Pigments and glycolipids in spots **1**−**6** were completely separated from each other by silica gel column chromatography. From the elution order and the TLC behavior of the standard samples, fractions **1**−**6** were assumed to be capsanthin (**1**), acylated steryl glucoside (**2**), monogalactosyldiacylglycerol (**3**), steryl glucoside (**4**), glucocerebroside (ceramide monoglucoside, **5**), and digalactosyldiacylglycerol (**6**), respectively (Figure 2). Fractions **1**−**6** were further analyzed by reversed-phase LC-MS to separate and identify their molecular species.

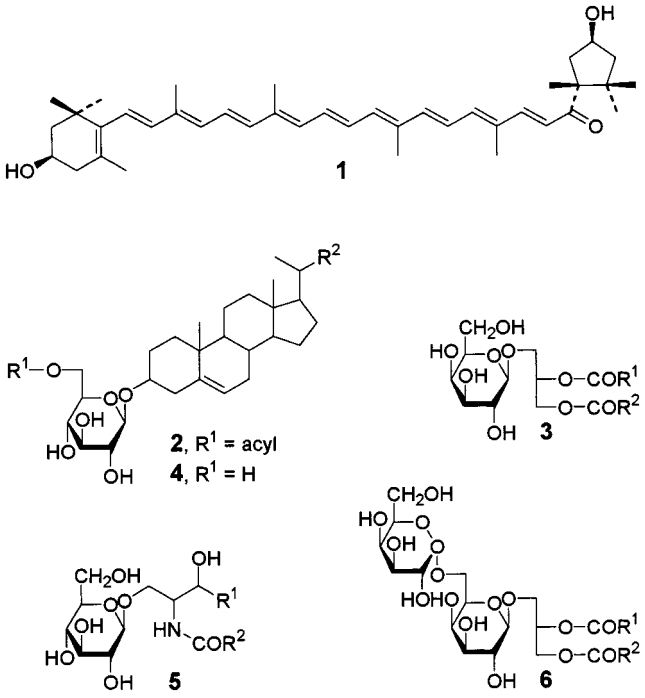

Figure 2. Representative structures of capsanthin (**1**), acylated steryl glucoside (**2**), monogalactosyldiacylglycerol (**3**), steryl glucoside (**4**), glucocerebroside (**5**), and digalactosyldiacylglycerol (**6**).

Capsanthin. Glycolipid fraction **1** included a red pigment. The HPLC chromatogram of **1** gave one major peak around 3.5 min, and its structure was identified as capsanthin by the APCI-MS: *m*/*z* (fragment, percentage relative intensity) 585 ($[M + H]^{\bullet +}$, 100), 567 ($[M - OH]^{\bullet +}$, 61), 479 (28), and 387 (37). Capsanthin is the major carotenoid in red bell peppers, accounting for 60% of the total carotenoids (*1*). Capsanthin is esterified with saturated fatty acids as ripening of the fruits progresses (*1*, *17*). In the fully ripe fruit, the percentages of the free carotenoids and the partially and totally esterified forms of these have been estimated to be 21.3, 35.6, and 43.1%, respectively (*17*). In the present study, diacylated capsanthins were eluted in the neutral lipid fraction obtained by silica gel column chromatography (data not shown).

Molecular Species of Acylated Steryl Glucoside and Steryl Glucoside. The components of acylated steryl glucoside (**2**) and steryl glucoside (**4**) were analyzed by GLC after hydrolysis. Fractions **2** and **4** contained campesterol and β-sitosterol as the sterol moieties, the percentages of which, respectively, were 24.3 and 75.7% for **2** and 27.7 and 72.3% for **4**. Glucose was the only sugar detected in both compounds (data not shown). Furthermore, **2** contained fatty acids; the fatty acid composition was determined to be palmitic acid (16:0, 49.9%), stearic acid (18:0, 8.7%), linoleic acid (18:2, 33.1%), and α-linolenic acid (18:3, 8.3%).

LC-MS analysis of acylated steryl glucoside (**2**) indicated at least seven molecular species, **2a**−**g** (Figure 3). The APCI-MS of each peak exhibited the Na^+ adduct

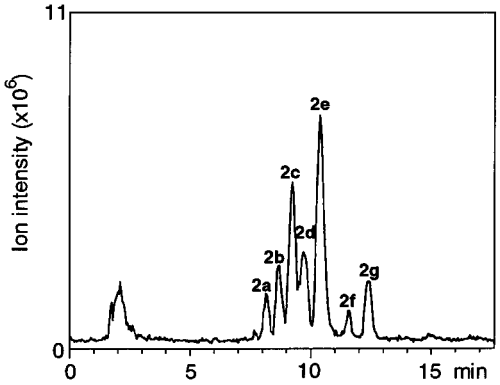

Figure 3. Total ion chromatogram of acylated steryl glucoside from red bell pepper by LC-MS. Glycolipid fraction **2** was separated on a Luna C18 column (2 × 150 mm) developed with methanol/ethanol (3:2, v/v) at 0.2 mL/min. The eluate was monitored by total ions of APCI-MS.

($[M + Na]^{\bullet+}$), fragment ions corresponding to campesterol, *m/z* 383 ($[C_{28}H_{47}]^{\bullet+}$), 397 ($[C_{28}H_{45}O]^{\bullet+}$), and 215 ($[C_{16}H_{23}]^{\bullet+}$), or β-sitosterol, *m/z* 397 ($[C_{29}H_{49}]^{\bullet+}$) 411 ($[C_{29}H_{47}O]^{\bullet+}$), and 215 ($[C_{16}H_{23}]^{\bullet+}$), and an ion from the fatty acyl moiety, $[R^2]^{\bullet+}$ (Table 1). Thus, compounds **2a**−**g** were identified as follows: **2a**, β-sitosteryl (6′-*O*-linolenoyl)glucoside; **2b**, campesteryl (6′-*O*-linoleoyl)-glucoside; **2c**, β-sitosteryl (6′-*O*-linoleoyl)glucoside; **2d**, campesteryl (6′-*O*-palmitoyl)glucoside; **2e**, β-sitosteryl (6′-*O*-palmitoyl)glucoside; **2f**, campesteryl (6′-*O*-stearoyl)-glucoside; and **2g**, β-sitosteryl (6′-*O*-stearoyl)glucoside. The peak area percentage of **2a**−**g** indicated in Table 1 corresponded to the percentage calculated from the data of sterol and fatty acid compositions described above.

The LC-MS analysis of steryl glucoside (**4**) showed two peaks, **4a** and **4b** (Figure 4A). They were identified as campesteryl glucoside (**4a**), APCI-MS *m/z* (fragment, percentage relative intensity) 585 ($[M + Na]^{\bullet+}$, 22), 397 ($[C_{28}H_{45}O]^{\bullet+}$, 74), 383 ($[C_{28}H_{47}]^{\bullet+}$, 100), and 215 ($[C_{16}H_{23}]^{\bullet+}$, 33), and β-sitosteryl glucoside (**4b**), APCI-MS *m/z* 599 ($[M + Na]^{\bullet+}$, 17), 411 ($[C_{29}H_{47}O]^{\bullet+}$, 100), 397 ($[C_{29}H_{49}]^{\bullet+}$, 97), and 215 ($[C_{16}H_{23}]^{\bullet+}$, 32). The peak area percentages of **4a** and **4b** on the total ion chromatogram in Figure 4A were calculated to be 26.1 and 73.9%, respectively, which corresponded to the sterol composition of fraction **4** determined by GLC after saponification.

Molecular Species of Monogalactosyldiacylglycerol and Digalactosyldiacylglycerol. The fatty acid compositions of monogalactosyldiacylglycerol (**3**) and digalactosyldiacylglycerol (**6**) were determined by GLC as follows: 16:0 (3.6%), 18:0 (1.6%), oleic acid (18:1, 1.5%), 18:2 (8.6%), and 18:3 (86.0%) for **3**; and 16:0 (13.9%), 18:0 (7.8%), 18:1 (1.5%), 18:2 (12.5%), 18:3 (64.3%) for **6**. Fractions **3** and **6** were analyzed by LC-MS (Figure 4B,C). One major peak (**3a**) and five minor peaks (**3b**−**f**) appeared on the total ion chromatogram of monogalactosyldiacylglycerol (Figure 4B), whereas seven peaks (**6a**−**g**) appeared in that of digalactosyldiacylglycerol (Figure 4C). The APCI-MS of each peak exhibited the Na^+ adduct ($[M + Na]^{\bullet+}$) and fragment ions corresponding to diacylglycerol ($[CH_2(OCOR^1)CH(OCOR^2)CH_2OH_2]^{\bullet+}$ and $[CH_2(OCOR^1)CH(OCOR^2)CH_2]^{\bullet+}$), monoacylglycerol ($[CH_2(OCOR^1)CH(OH)CH_2]^{\bullet+}$ and $[CH_2(OH)CH(OCOR^2)CH_2]^{\bullet+}$), and fatty acyl moieties ($[R^1]^{\bullet+}$ and $[R^2]^{\bullet+}$) (Tables 2 and 3). Thus, the molecular species of monogalactosyldiacylglycerol were found to be 18:3/18:3 (**3a**), 18:2/18:3 (**3b**), 16:0/18:3 (**3c**), 18:1/18:3 (**3d**), 16:0/18:2 (**3e**), and 18:0/18:3 (**3f**); and those of digalactosyldiacylglycerol were 18:3/18:3 (**6a**), 18:2/18:3 (**6b**), 16:0/18:3 (**6c**), 18:1/18:3 (**6d**), 16:0/18:2, (**6e**), 18:0/18:3 (**6f**), and 18:0/18:2 (**6g**). The molecular species compositions calculated from the total ion chromatograms indicated that the 18:3/18:3 species was predominant in monogalactosyldiacylglycerol, whereas the 18:3/18:3, 16:0/18:3, and 18:0/18:3 species were predominant in digalactosyldiacylglycerol (Tables 2 and 3).

The reversed-phase HPLC technique has been reported to allow rapid and reproducible separations of the main molecular species of the plant galactolipids monogalactosyldiacylglycerol and digalactosyldiacylglycerol (*13*, *18*). However, collection of the separated fractions and analysis of the component fatty acids by GLC was required to identify molecular species. The present on-line APCI-MS technique has enabled direct identification of the molecular species of these galactolipids without such processing, although it did not give information on the *sn* positioning of the two fatty acid moieties including two different fatty acids.

Molecular Species of Glucocerebroside. The glucocerebroside fraction (**5**) gave seven peaks (**5a**−**g**) in the total ion chromatogram of LC-MS (Figure 4D). Peaks **5a**−**g** were further separated by preparative reversed-phase HPLC, and their structures were identified by ^1H NMR (*19*−*21*). Peak assignments were confirmed by $^1H-^1H$ COSY analysis (data not shown). In each spectrum, the signals for methyl residues were found at δ 0.89, which corresponded to the terminal methyl groups of the sphingoid and the acyl moiety. The doublet at δ 4.27−4.28 corresponded to the anomeric proton, and the coupling constant (J = 7.7−8.1 Hz) indicated the presence of a β-D-glucopyranoside linkage. Thus, the following structures were identified, where the numbering systems used are the standard numbering systems for the sphingoid, fatty acid, and glucose moieties (*21*): (8*E*)-*N*-2′-hydroxypalmitoyl-1-*O*-β-D-glucopyranosyl-4-hydroxy-8-sphingenine (**5a**) [^1H NMR ($CDCl_3/CD_3OD$ = 2:1, v/v) δ 0.89 (m, 6H, H-18,16′), 1.27 (m, 40H, $-CH_2-$), 1.40 (m, 4H, H-5,4′), 1.60 (m, 1H, H-3′a), 1.78 (m, 1H, H-3′b), 1.99 (m, 2H, H-10), 2.06 (m, 2H, H-7), 3.23 (t, J = 8.4 Hz, 1H, H-2″), 3.31 (m, 1H, H-5″), 3.36 (m, 1H, H-4″), 3.40 (m, 1H, H-3″), 3.56 (m, 1H, H-1a), 3.71 (dd, J = 5.1, 12.1 Hz, 1H, H-6″a), 3.83 (m, 2H, H-1b,4), 3.88 (dd, J = 2.2, 12.1 Hz, 1H, H-6″b), 4.02 (m, 1H, H-2′), 4.06 (dd, J = 5.9, 10.6 Hz, 1H, H-3), 4.25 (m, 1H, H-2), 4.28 (d, J = 7.7 Hz, 1H, H-1″), 5.36 (m, 1H, H-8), and 5.41 (m, 1H, H-9)]; (4*E*,8*Z*)-*N*-2′-hydroxypalmitoyl-1-*O*-β-D-glucopyranosyl-4,8-sphingadienine (**5b**) [^1H NMR ($CDCl_3/CD_3OD$ = 2:1, v/v) δ 0.89 (m, 6H, H-18,16′), 1.26 (m, 36H, $-CH_2-$), 1.55 (m, 1H, H-3′a), 1.75 (m, 1H, H-3′b), 2.01 (m, 2H, H-10), 2.10 (m, 4H, H-6,7), 3.25 (t, J = 8.3 Hz, 1H, H-2″), 3.29 (m, 1H, H-5″), 3.39 (m, 1H, H-4″), 3.41 (m, 1H, H-3″), 3.73 (m, 2H, H-1a,6″a), 3.87 (m, 2H, H-1b,6″b), 4.03 (m, 2H, H-2,2′), 4.13 (t, J = 6.6 Hz, 1H, H-3), 4.27 (d, J = 7.9 Hz, 1H, H-1″), 5.36 (m, 2H, H-8,9), 5.48 (dd, J = 7.1, 15.3 Hz, 1H, H-4), 5.74 (dt, J = 6.6, 15.2 Hz, 1H, H-5), and 7.51 (d, J = 8.6 Hz, 1H, N*H*)]; (8*Z*)-*N*-2′-hydroxypalmitoyl-1-*O*-β-D-glucopyranosyl-8-sphingenine (**5c**) (*21*) [^1H NMR ($CDCl_3/CD_3OD$ = 2:1, v/v) δ 0.89 (m, 6H, H-18,16′), 1.27 (m, 40H, $-CH_2-$), 1.41 (m, 4H, H-4,4′), 1.61 (m, 1H, H-3′a), 1.78 (m, 1H, H-3′b), 2.02 (m, 4H, H-7,10), 3.24 (dd, J = 8.1, 8.8 Hz, 1H, H-2″), 3.29 (m, 1H, H-5″), 3.36 (m, 1H, H-3″), 3.40 (m, 1H, H-4″), 3.63 (m, 1H, H-3), 3.72 (m, 2H, H-1a,6″a), 3.87 (dd, J = 2.6, 12.1 Hz, 1H, H-6″b), 3.94 (m, 1H, H-2), 4.05 (dd, J = 3.7, 7.7 Hz, 1H, H-2′), 4.10 (dd, J = 5.1, 10.3 Hz, 1H, H-1b), 4.27 (d, J = 7.7 Hz, 1H, H-1″), and 5.35 (m, 2H,

Table 1. APCI-MS Data of the Acylated Steryl Glucosides Separated by Reversed-Phase HPLC

	peak[a]						
ion	**2a**	**2b**	**2c**	**2d**	**2e**	**2f**	**2g**
$[M + Na]^{\bullet+}$	859 (15)[b]	847 (24)	861 (20)	823 (19)	837 (21)	851 (8)	865 (8)
sterol moiety							
$[R^1C_{19}H_{26}O]^{\bullet+}$	411 (53)	397 (66)	411 (61)	397 (64)	411 (58)	397 (34)	411 (76)
$[R^1C_{19}H_{28}]^{\bullet+}$	397 (100)	383 (100)	397 (100)	383 (100)	397 (97)	383 (100)	397 (100)
$[C_{16}H_{23}]^{\bullet+}$	215 (20)	215 (19)	215 (20)	215 (22)	215 (13)	215 (11)	215 (16)
acyl moiety							
$[R^2]^{\bullet+}$	261 (19)	263 (32)	263 (23)	239 (84)	239 (100)	267 (79)	267 (80)
molecular species (fatty acid/sterol)[c]	18:3/sit	18:2/cam	18:2/sit	16:0/cam	16:0/sit	18:0/cam	18:0/sit
%[d]	7.5	11.2	22.4	13.1	31.4	5.1	9.2

[a] Peak numbers correspond to those indicated in Figure 3. [b] *m/z* (percentage relative intensity). [c] Abbreviations: 18:3, α-linolenic acid; 18:2, linoleic acid; 18:1, oleic acid; 18:0, stearic acid; 16:0, palmitic acid; sit, β-sitosterol; cam, campesterol. [d] Values indicate the peak percentage of total peak area measured by the total ion chromatogram described in Figure 3.

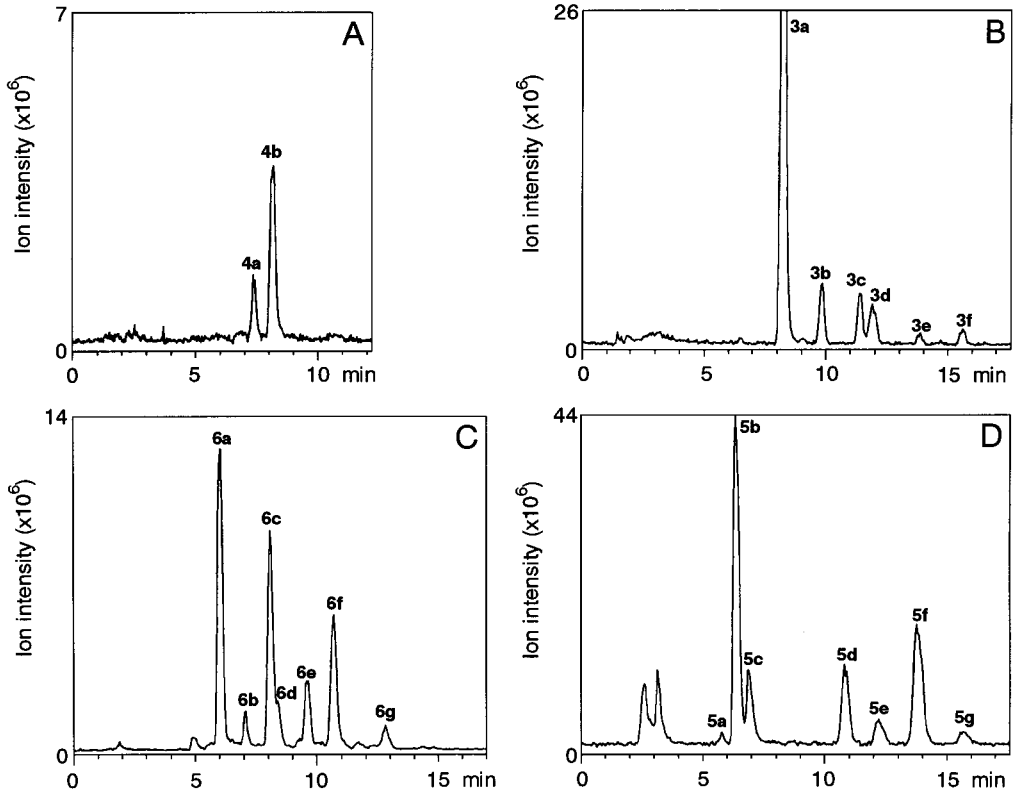

Figure 4. Total ion chromatograms of steryl glucoside (A), monogalactosyldiacylglycerol (B), digalactosyldiacylglycerol (C), and glucocerebroside (D) from red bell pepper by LC-MS. Glycolipid fractions **3**−**6** were separated on a Luna C18 column (2 × 150 mm) developed with methanol/water (98:2, v/v) at 0.2 mL/min. The eluate was monitored by total ions of APCI-MS.

H-8,9)]; (8*E*)-*N*-2′-hydroxydocosanoyl-1-*O*-β-D-glucopyranosyl-4-hydroxy-8-sphingenine (**5d**) [^{1}H NMR ($CDCl_3$/CD_3OD = 2:1, v/v) δ 0.89 (m, 6H, H-18,22′), 1.27 (m, 50H, −CH_2−), 1.40 (m, 4H, H-5,4′), 1.59 (m, 1H, H-3′a), 1.79 (m, 1H, H-3′b), 2.00 (m, 2H, H-10), 2.04 (m, 2H, H-7), 3.23 (dd, *J* = 8.1, 8.4 Hz, 1H, H-2″), 3.30 (m, 1H, H-5″), 3.36 (m, 1H, H-4″), 3.41 (m, 1H, H-3″), 3.52 (m, 1H, H-4), 3.57 (t, *J* = 6.2 Hz, 1H, H-3), 3.72 (dd, *J* = 5.1, 12.1 Hz, 1H, H-6″a), 3.82 (m, 1H, H-1a), 3.88 (dd, *J* = 2.2, 12.1 Hz, 1H, H-6″b), 4.03 (m, 1H, H-2′), 4.06 (dd, *J* = 5.9, 10.6 Hz, 1H, H-1b), 4.25 (m, 1H, H-2), 4.28 (d, *J* = 8.1 Hz, 1H, H-1″), 5.36 (m, 1H, H-8), and 5.41 (m, 1H, H-9)]; (8*E*)-*N*-2′-hydroxytricosanoyl-1-*O*-β-D-glucopyranosyl-4-hydroxy-8-sphingenine (**5e**) [^{1}H NMR ($CDCl_3$/CD_3OD = 2:1, v/v) δ 0.89 (m, 6H, H-18,23′), 1.27 (m, 52H, −CH_2−), 1.40 (m, 4H, H-5,4′), 1.59 (m, 1H, H-3′a), 1.78 (m, 1H, H-3′b), 1.97 (m, 2H, H-10), 2.07 (m, 2H, H-7), 3.23 (dd, *J* = 8.1, 8.5 Hz, 1H, H-2″), 3.31 (m, 1H, H-5″), 3.35 (m, 1H, H-4″), 3.41 (m, 1H, H-3″), 3.53 (m, 1H, H-4), 3.57 (t, *J* = 6.2 Hz, 1H, H-3), 3.71 (dd, *J* = 5.1, 12.1 Hz, 1H, H-6″a), 3.83 (m, 1H, H-1a), 3.88 (dd, *J* = 2.2, 12.1 Hz, 1H, H-6″b), 4.03 (dd, *J* = 3.7, 8.1 Hz, 1H, H-2′), 4.06 (dd, *J* = 5.9, 10.6 Hz, 1H, H-1b), 4.26 (m, 1H, H-2), 4.28 (d, *J* = 7.7 Hz, 1H, H-1″), 5.36 (m, 1H, H-8), and 5.41 (m, 1H, H-9)]; (8*E*)-*N*-2′-hydroxytetracosanoyl-1-*O*-β-D-glucopyranosyl-4-hydroxy-8-sphingenine (**5f**) [^{1}H NMR ($CDCl_3$/CD_3OD = 2:1, v/v) δ 0.89 (m, 6H, H-18,24′), 1.27 (m, 54H, −CH_2−), 1.4 (m, 4H, H-5,4′), 1.60 (m, 1H, H-3′a), 1.80 (m, 1H, H-3′b), 1.99 (m, 2H, H-10), 2.05 (m, 2H, H-7), 3.23 (dd, *J* = 7.7, 8.1 Hz, 1H, H-2″), 3.30 (m, 1H, H-5″), 3.36 (m, 1H, H-4″), 3.41 (m, 1H, H-3″), 3.54 (m, 1H, H-4), 3.57 (t, *J* = 6.2 Hz, 1H, H-3), 3.72 (dd, *J* = 5.1, 12.1 Hz, 1H, H-6″a), 3.83 (m, 1H, H-1a), 3.87 (dd, *J* = 2.2, 12.1 Hz, 1H, H-6″b), 4.03 (dd, *J* = 3.8, 8.1 Hz, 1H, H-2′), 4.06 (dd, *J* = 5.9, 10.6 Hz, 1H, H-1b), 4.25 (m, 1H, H-2), 4.28 (d, *J* = 7.7 Hz, 1H, H-1″), 5.36 (m, 1H, H-8), and 5.41 (m, 1H, H-9)]; (8*E*)-*N*-2′-hydroxypentacosanoyl-1-*O*-β-D-glucopyranosyl-4-hydroxy-8-sphingenine (**5g**) [^{1}H NMR ($CDCl_3$/CD_3OD = 2:1, v/v) δ 0.88 (m, 6H, H-18,25′), 1.26 (m, 56H, −CH_2−), 1.40 (m, 4H, H-5,4′), 1.59 (m, 1H, H-3′a), 1.76 (m, 1H, H-3′b), 2.01 (m, 2H, H-10), 2.07 (m,

Table 2. APCI-MS Data of the Molecular Species of Monogalactosyldiacylglycerol Separated by Reversed-Phase HPLC

ion	peak[a] 3a	3b	3c	3d	3e	3f
$[M + Na]^{\bullet+}$	797 (9)[b]	799 (9)	775 (4)	801 (13)	777 (6)	803 (4)
diacylglycerol moiety						
$[CH_2(OCOR^1)CH(OCOR^2)CH_2OH_2]^{\bullet+}$	613 (100)	615 (80)	591 (2)	617 (80)	593 (5)	619 (6)
$[CH_2(OCOR^1)CH(OCOR^2)CH_2]^{\bullet+}$	595 (28)	597 (33)	573 (20)	599 (65)	575 (100)	601 (24)
monoacylglycerol moiety						
$[CH_2(OCOR^1)CH(OH)CH_2]^{\bullet+}$ and $[CH_2(OH)CH(OCOR^2)CH_2]^{\bullet+}$	335 (66)	337 (39), 335 (43)	313 (60), 335 (43)	339 (55), 335 (38)	313 (36), 337 (18)	341 (56), 335 (43)
acyl moiety						
$[R^1]^{+\bullet}$ and $[R^2]^{+\bullet}$	261 (79)	263 (38), 261 (100)	239 (33), 261 (100)	265 (32), 261 (100)	239 (76), 263 (76)	267 (37), 261 (100)
molecular species (fatty acid/fatty acid)[c]	18:3/18:3	18:2/18:3	16:0/18:3	18:1/18:3	16:0/18:2	18:0/18:3
%[d]	72.1	9.3	7.9	5.3	2.4	3.0

[a] Peak numbers correspond to those indicated in Figure 4B. [b] *m/z* (percentage relative intensity). [c] The abbreviations used are the same as described in Table 1, and the *sn* positions of fatty acids are not determined. [d] Values indicate the peak percentage of total peak area measured by the total ion chromatogram described in Figure 4B.

Table 3. APCI-MS Data of the Molecular Species of Digalactosyldiacylglycerol Separated by Reversed-Phase HPLC

ion	peak[a] 6a	6b	6c	6d	6e	6f	6g
$[M + Na]^{\bullet+}$	959 (6)[b]	961 (1)	937 (3)	963 (9)	939 (4)	965 (1)	967 (3)
diacylglycerol moiety							
$[CH_2(OCOR^1)CH(OCOR^2)CH_2OH_2]^{\bullet+}$	613 (100)	615 (91)	591 (8)	617 (91)	593 (1)	619 (7)	621 (1)
$[CH_2(OCOR^1)CH(OCOR^2)CH_2]^{\bullet+}$	595 (29)	597 (28)	573 (34)	599 (75)	575 (100)	601 (15)	603 (81)
monoacylglycerol moiety							
$[CH_2(OCOR^1)CH(OH)CH_2]^{+\bullet}$ and $[CH_2(OH)CH(OCOR^2)CH_2]^{\bullet+}$	335 (79)	337 (50), 335 (37)	313 (70), 335 (48)	339 (100), 335 (73)	313 (45), 337 (32)	341 (58), 335 (47)	341 (45), 337 (27)
acyl moiety							
$[R^1]^{\bullet+}$ and $[R^2]^{\bullet+}$	261 (97)	263 (45), 261 (100)	239 (38), 261 (100)	265 (95), 261 (98)	239 (61), 263 (79)	267 (35), 261 (100)	267 (100), 263 (73)
molecular species (fatty acid/fatty acid)[c]	18:3/18:3	18:2/18:3	16:0/18:3	18:1/18:3	16:0/18:2	18:0/18:3	18:0/18:2
%[d]	37.4	5.5	24.0	3.4	9.1	17.1	3.5

[a] Peak numbers correspond to those indicated in Figure 4C. [b] *m/z* (percentage relative intensity). [c] The abbreviations used are the same as described in Table 1, and the *sn* positions of fatty acids are not determined. [d] Values indicate the peak percentage of total peak area measured by the total ion chromatogram described in Figure 4C.

Table 4. APCI-MS Data of the Molecular Species of Glucocerebroside Separated by Reversed-Phase HPLC

ion	peak[a] 5a	5b	5c	5d	5e	5f	5g
$[M + H]^{\bullet+}$	732 (5)[b]	714 (4)	716 (10)	816 (9)	830 (8)	844 (21)	858 (7)
$[M - OH]^{\bullet+}$	714 (5)	696 (98)	698 (6)	798 (9)	812 (5)	826 (5)	840 (3)
ceramide moiety							
$[M - C_6H_9O_5]^{+\bullet}$	570 (100)	nd[c]	554 (100)	654 (100)	668 (100)	682 (100)	696 (100)
$[M - C_6H_{11}O_6]^{+\bullet}$	552 (90)	534 (77)	536 (97)	636 (88)	650 (73)	664 (85)	678 (48)
$[M - C_6H_{13}O_7]^{+\bullet}$	534 (21)	516 (97)	518 (18)	618 (25)	632 (12)	646 (24)	660 (8)
sphingoid moiety							
$[R^1CH(OH)CH(NH_2)CH_2OH_2]^{\bullet+}$	316 (13)	nd	300 (24)	316 (18)	316 (8)	316 (11)	316 (6)
$[R^1CH(OH)CH(NH_2)CH_2]^{\bullet+}$	298 (34)	280 (76)	282 (73)	298 (24)	298 (20)	298 (17)	298 (13)
$[R^1CH{=}CH(NH_2)CH_2]^{\bullet+}$	280 (48)	262 (100)	264 (20)	280 (55)	280 (22)	280 (32)	280 (22)
$[\text{sphingoid} - (H_2O)_2]^{\bullet+}$	262 (39)	nd	nd	262 (17)	262 (13)	262 (10)	262 (18)
α-hydroxy fatty acid moiety							
$[R^2CH(OH)CONH_3]^{\bullet+}$	272 (15)	272 (27)	272 (25)	356 (12)	370 (5)	384 (11)	398 (4)
molecular species (sphingoid/fatty acid)[d]	t18:1/16h:0	d18:2/16h:0	d18:1/16h:0	t18:1/22h:0	t18:1/23h:0	t18:1/24h:0	t18:1/25h:0
%[e]	3.2	47.4	11.7	11.5	5.0	18.0	3.2

[a] Peak numbers correspond to those indicated in Figure 4D. [b] *m/z* (percentage relative intensity). [c] nd, not detected. [d] Abbreviations: t18:1, 4-hydroxy-8-sphingenine; d18:2, 4,8-sphingadienine; d18:1, 8-sphingenine; 16−25h:0, 2-hydroxy fatty acids having carbon chain length 16−25. [e] Values indicate the peak percentage of total peak area measured by the total ion chromatogram described in Figure 4D.

2H, H-7), 3.23 (dd, J = 8.1, 8.4 Hz, 1H, H-2″), 3.30 (m, 1H, H-5″), 3.36 (m, 1H, H-4″), 3.41 (m, 1H, H-3″), 3.54 (m, 1H, H-4), 3.56 (dd, J = 5.1, 6.2 Hz, 1H, H-3), 3.71 (dd, J = 5.1, 12.1 Hz, 1H, H-6″a), 3.83 (m, 1H, H-1a), 3.87 (dd, J = 2.6, 12.1 Hz, 1H, H-6″b), 4.03 (dd, J = 3.7, 8.1 Hz, 1H, H-2′), 4.06 (dd, J = 5.9, 10.6 Hz, 1H, H-1b), 4.24 (m, 1H, H-2), 4.28 (d, J = 7.7 Hz, 1H, H-1″), 5.36 (m, 1H, H-8), and 5.41 (m, 1H, H-9)].

Table 4 shows the APCI-MS data of compounds **5a**−**g**. Each compound exhibited the expected quasi-molecular ion ($[M + H]^{\bullet+}$) and fragment ions corresponding to ceramide ($[M - C_6H_9O_5]^{\bullet+}$, $[M - C_6H_{11}O_6]^{\bullet+}$, and $[M - C_6H_{13}O_7]^{\bullet+}$), sphingoid ($[R^1CH(OH)CH(NH_2)CH_2OH_2]^{\bullet+}$, $[R^1CH(OH)CH(NH_2)CH_2]^{\bullet+}$, $[R^1CH{=}CH(NH_2)CH_2]^{\bullet+}$, and $[\text{sphingoid} - (H_2O)_2]^{\bullet+}$), and α-hydroxy fatty acyl moiety ($[R^2CH(OH)CONH_3]^{\bullet+}$). The mass spectra of **5a** and **5d**−**g** showed the same fragment ions at *m/z* 316, 298, 280, and 262, indicating the presence of 4-hydroxy-8-sphingenine as the sphingoid moiety. On the other hand, the main peak **5b** showed a different fragmentation pattern, which was characterized by intense fragment ions at *m/z* 696 ($[M - OH]^{\bullet+}$), 516 (ceramide moiety, $[M - C_6H_{13}O_7]^{\bullet+}$), and 262 ($[C_{15}H_{27}CH{=}CH(NH_2)CH_2]^{\bullet+}$) due to the presence of 4,8-sphin-

gadienine in the molecule. Whitaker (*10*) reported that reversed-phase HPLC of cerebrosides isolated from bell pepper fruits gave one major peak in addition to four minor peaks. He deduced that the structure of the major peak was 1-*O*-*β*-glucosyl-*N*-(2′-hydroxypalmitoyl)-4-*trans*-8-*cis*-sphingadienine, which corresponds to **5b** in our chromatogram, and aside from assignment of sphingoid cis/trans double bonds, he correctly deduced the structures of **5c**−**f**. Our analytical methods using APCI-MS have the advantage of direct identification of almost all of the molecular species of cerebroside in the red bell pepper without the need for hydrolysis and derivatization. However, the ^{1}H NMR analysis was essential for the identification of sphingoid cis/trans double bounds.

The ripening fruits of red bell pepper are an excellent source of natural colors and vital micronutrients such as carotenoids and vitamins C and E, which have been confirmed by many epidemiological studies to reduce the risk of cancer and cardiovascular disease (*22*, *23*). Capsanthin and its esters, the main pigments in paprika, have been reported to have antioxidant activity (*23*). In addition, edible plant glycolipids are believed to play a role in human diet as nutrients, but little is known about their processing and absorption in the digestive tract of mammals (*24*, *25*). Because molecular species containing α-linolenic acid (18:3) were the major species in mono- and digalactosyldiacylglycerols, these galactolipids would be an important source for this *n*−3 essential fatty acid. The average daily intake of glycolipids in humans has been reported to be 140 mg of acylated steryl glucoside, 65 mg of steryl glucoside, 50 mg of cerebroside, 90 mg of monogalactosyldiacylglycerol, and 220 mg of digalactosyldiacylglycerol (*12*). Fruits of red bell pepper appear to be a rich source of such glycolipids in addition to the sources of carotenoid pigments and some other micronutrients. The combination of HPLC and APCI-MS described in this study is convenient and reliable for the separation and identification of the molecular species of plant glycolipids without any chemical modifications.

LITERATURE CITED

(1) Gregory, G. K.; Chen, T.-S.; Philip, T. Quantitative analysis of carotenoids and carotenoid esters in fruits by HPLC: Red bell peppers. *J. Food Sci.* **1987**, *52*, 1071−1073.

(2) Biacs, P. A.; Daood, H. G.; Pavisa, A.; Hajdu, F. Studies on the carotenoid pigments of paprika (*Capsicum annuum* L. var Sz-20). *J. Agric. Food Chem.* **1989**, *37*, 350−353.

(3) Matsufuji, H.; Nakamura, H.; Chino, M.; Takeda, M. Antioxidant activity of capsanthin and the fatty acid esters in paprika (*Capsicum annuum*). *J. Agric. Food Chem.* **1998**, *46*, 3468−3472.

(4) Kinsella, J. E. Composition of the lipids of cucumber and peppers. *J. Food Sci.* **1971**, *36*, 865−866.

(5) Howard, L. R.; Smith, R. T.; Wagner, A. B.; Villalon, B.; Burns, E. E. Provitamin A and ascorbic acid content of fresh pepper cultivars (*Capsicum annuum*) and processed jalapeños. *J. Food Sci.* **1994**, *59*, 362−365.

(6) Howard, L. R.; Talcott, S. T.; Brenes, C. H.; Villalon, B. Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum* species) as influenced by maturity. *J. Agric. Food Chem.* **2000**, *48*, 1713−1720.

(7) Márkus, F.; Daood, H. G.; Kapitány, J.; Biacs, P. A. Change in the carotenoid and antioxidant content of spice red pepper (paprika) as a function of ripening and some technological factors. *J. Agric. Food Chem.* **1999**, *47*, 100−107.

(8) Allen, C. F.; Good, P.; Davis, H. F.; Chisum, P.; Fowler, S. D. Methodology for the separation of plant lipids and application to spinach leaf and chloroplast lamellae. *J. Am. Oil Chem. Soc.* **1966**, *43*, 223−231.

(9) Mudd, J. B.; Garcia, R. E. Biosynthesis of glycolipids. In *Recent Advances in the Chemistry and Biochemistry of Plant Lipids*; Galliard, T., Mercer, E. I., Eds.; Academic Press: New York, 1975; Chapter 6, pp 161−201.

(10) Whitaker, B. D. Cerebrosides in mature-green and red-ripe bell pepper and tomato fruits. *Phytochemistry* **1996**, *42*, 627−632.

(11) Picchioni, G. A.; Watada, A. E.; Whitaker, B. D. Quantitative high-performance liquid chromatography analysis of plant phospholipids and glycolipids using light-scattering detection. *Lipids* **1996**, *31*, 217−221.

(12) Sugawara, T.; Miyazawa, T. Separation and determination of glycolipids from edible plant sources by high-performance liquid chromatography and evaporative light-scattering detection. *Lipids* **1999**, *34*, 1231−1237.

(13) Yamauchi, R.; Kojima, M.; Isogai, M.; Kato, K.; Ueno, Y. Separation and purification of molecular species of galactolipids by high performance liquid chromatography. *Agric. Biol. Chem.* **1982**, *46*, 2847−2849.

(14) Folch, J.; Lees, M.; Sloane-Stanley, G. H. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497−509.

(15) Christie, W. W. In *Lipid Analysis. Isolation, Separation and Structural Analysis of Lipids*, 2nd ed.; Pergamon Press: Oxford, U.K., 1982.

(16) Bortolomeazzi, R.; De Zan, M.; Pizzale, L.; Conte, L. S. Mass spectrometry characterization of the 5α-, 7α-, and 7*β*-hydroxy derivatives of *β*-sitosterol, campesterol, stigmasterol, and brassicasterol. *J. Agric. Food Chem.* **1999**, *47*, 3069−3074.

(17) Mínguez-Mosquera, M. I.; Hornero-Méndez, D. Changes in carotenoid esterification during the fruit ripening of *Capsicum annuum* cv. Bola. *J. Agric. Food Chem.* **1994**, *42*, 640−644.

(18) Demandre, C.; Tremolieres, A.; Justin, A.-M.; Mazliak, P. Analysis of molecular species of plant polar lipids by high-performance and gas liquid chromatography. *Phytochemistry* **1985**, *24*, 481−485.

(19) Kawai, G.; Ikeda, Y. Chemistry and functional moiety of fruiting-inducing cerebrosides in *Schizophyllum commune*. *Biochim. Biophys. Acta* **1983**, *754*, 243−248.

(20) Kawai, G.; Ikeda, Y. Structure of biological active and inactive cerebrosides prepared from *Schizophyllum commune*. *J. Lipid Res.* **1985**, *26*, 338−343.

(21) Kawai, G.; Ohnishi, M.; Fujino, Y.; Ikeda, Y. Stimulatory effect of certain plant sphingolipids on fruiting of *Schizophyllum commune*. *J. Biol. Chem.* **1986**, *261*, 779−784.

(22) Charleux, J.-L. *β*-carotene, vitamin C, and vitamin E: The protective micronutrients. *Nutr. Rev.* **1996**, *54*, S109−S114.

(23) Van den Berg, H.; Faulks, R.; Granado, H. F.; Hirschberg, J.; Olmedilla, B.; Sandmann, G.; Southon, S.; Stahl, W. The potential for the improvement of carotenoid levels in foods and the likely systemic effects. *J. Sci. Food Agric.* **2000**, *80*, 880−912.

(24) Andersson, L.; Bratt, C.; Arnoldsson, K. C.; Herslöf, B.; Olsson, N. U.; Sternby, B.; Nilsson, Å. Hydrolysis of galactolipids by human pancreatic lipolytic enzymes and duodenal contents. *J. Lipid Res.* **1995**, *36*, 1392−1400.

(25) Ohlsson, L.; Blom, M.; Bohlinder, K.; Carlsson, A.; Nilsson, Å. Orally fed digalactosyldiacylglycerol is degraded during absorption in intact and lymphatic duct cannulated rats. *J. Nutr.* **1998**, *128*, 239−245.

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