

NOTE

Analysis of Glucosylceramides from Various Sources by Liquid Chromatography-Ion Trap Mass Spectrometry

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Abstract: Liquid chromatography-mass spectrometry is one of the most powerful methods for the identification and detection of chemical structures of lipids. In this study, we attempted to identify the chemical structures of glucosylceramides from maize, rice, mushroom (maitake) and sea cucumber by liquid chromatography-ion trap mass spectrometry. For structural analysis of glucosylceramides, $[M + H]^+$, $[M + H - 18]^+$ or $[M + H - 162]^+$ in the positive scan mode was used for MS/MS analysis to obtain product ion spectra. The typical signals which are characteristic for the sphingoid base moieties were observed while the isomers could not be distinguished. This method should be useful for the structural determination of diverse glucosylceramide molecular species.

Key words: liquid chromatography-ion trap mass spectrometry, glucosylceramide, chemical structures of lipids

1 INTRODUCTION

Dietary sphingolipids have gained attention for their potential to protect intestine from inflammation and cancers¹⁻⁴. One plausible mechanism for these effects is believed to be via the hydrolysis of complex sphingolipids to bioactive ceramides and sphingoid bases, because those breakdown products play important roles as intracellular mediators^{5,6}. Our previous findings indicated that sphingoid bases prepared from dietary sources can induce apoptosis in colon cancer cell lines⁷⁻⁹, and dietary sphingolipids from plant and yeast prevent the formation of aberrant crypt foci in 1, 2-dimethylhydrazine-treated mice^{10,11}. In addition, other physiological functions of sphingolipids, such as improving the barrier function of skin, lowering plasma lipids and preventing of melanin formation, have also been reported¹²⁻¹⁴.

Sphingolipids are ubiquitous in all eukaryotic organisms, and constitute a family of compounds that have a sphingoid base (long-chain base) with an amide-linked fatty acid and a polar head group. It is well known that there are diverse structures of sphingoid bases in nature^{15,16}. The most common sphingoid base of mammalian sphingolipids is sphingosine (*trans*-4-sphingene, d18:1^{4t}). Smaller amounts of

other sphingoid bases, such as sphinganine (dihydrosphingosine, d18:0) and phytosphingosine (4-hydroxysphinganine, t18:0) are frequently encountered. The structure of sphingoid bases in higher plants is more complicated than in mammals, because they can be desaturated at the C8-position by a Δ 8-sphingolipid desaturase, yielding *cis*- and *trans*- isomers of Δ 8-unsaturated sphingoid bases (*cis/trans*-8-sphingene, d18:1^{8c/t}; *trans*-4, *cis/trans*-8-sphingadiene, d18:2^{4t, 8c/t}; 4-hydroxy-*cis/trans*-8-sphingene, t18:1^{8c/t})¹⁷. In fungi, 9-methyl-*trans*-4, *trans*-8-sphingadiene (d19:2^{4t, 8t}) is a typical structure¹⁶. In addition, sphingolipids of marine invertebrates have unique triene types of sphingoid bases with a conjugated diene such as 2-amino-4, 8, 10-octatriene-1, 3-diol (d18:3^{4, 8, 10}) and 2-amino-9-methyl-4, 8, 10-octatriene-1, 3-diol (d19:3^{4, 8, 10})¹⁸. Thus, sphingolipids having various structures of sphingoid bases are ingested daily from foodstuff. Determination of their diverse structures including variation of sphingoid backbone must be important for understanding the functional and nutritional significance of dietary sphingolipids.

Liquid chromatography-mass spectrometry is one of the

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most powerful methods for identification and detection of chemical structures of lipids including sphingolipids^{19,20}. In this study, we attempted to identify the structures of glucosylceramide (GluCer, Fig. 1), one of the predominant sphingoglycolipids, from various sources by liquid chromatography-ion trap mass spectrometry.

2 EXPERIMENTAL

2.1 Materials

GluCer were prepared from maize, rice, mushroom (maitake) and sea cucumber by a silica gel column after lipid extraction and saponification as described previously^{9,21}. All other chemicals and solvent were of reagent grade.

2.2 LC-MS/MS analyses

A prominence HPLC system coupled to LCMS-IT-TOF equipped with an electrospray ionization interface (Shimadzu, Kyoto, Japan) was used. For structural analysis of GluCer, $[M+H]^+$, $[M+H-18]^+$ (loss of water) or $[M+H-162]^+$ (loss of glucose) in the positive scan mode was used for MS/MS analysis to obtain the product ions. A TSK gel ODS-100Z column (2.0 × 50 mm, 3 μm, Tosoh, Tokyo, Japan) was eluted with acetonitrile/water (95:5, v/v) at a flow rate of 0.2 mL/min. The MS was operated with the following conditions: probe voltage of 4.50 kV, CDL temperature of 200°C, block heater temperature of 200°C, nebulizer gas flow of 1.5 L/min, ion accumulation time of 100 msec, MS range of m/z 650 to 900, MS² range of m/z 200 to 300, and CID parameters were follows: energy, 60%; collision gas 60%.

The typical signals which are characteristic for the sphingoid base moieties were observed by auto MS/MS detection mode in this system. The structures and characteristic product ions of diverse sphingoid bases contained in GluCer are given in Fig. 2. Pairs of these structurally specific ions of sphingoid bases and their precursor ions were used for the identification of GluCer molecules.

3 RESULTS AND DISCUSSION

Figure 3 shows total ion and selected ion chromatograms of GluCer prepared from maize, and MS spectra of repre-

sentative peaks. In the positive full scan mode, $[M+Na]^+$, $[M+H]^+$ and $[M+H-H_2O]^+$ were the predominant signals in each peak (Fig. 3). The transition of precursor ions $[M+H]^+$ and $[M+H-H_2O]^+$ to the product ions of the sphingoid bases was used for the identification of GluCer molecular species (Table 1). Nine molecules were determined by pairs of specific product ions of sphingoid bases and their precursor ions. In the case of GluCer molecules consisting of 4-hydroxy-8-sphingene (t18:1), the loss of glucose $[M+H-162]^+$ was clearly detected and was used as precursor ion, because the product ions could not be detected from $[M+H]^+$. In addition, $[M+H-H_2O]^+$ ion was not detected in this system. Detection of GluCer consisting of d18:2 and t18:1 was separated into two peaks, *cis*- and *trans*- isomers of Δ8-unsaturated sphingoid bases. Predominantly hydroxy fatty acids containing 16 to 26 carbon atoms were detected in maize GluCer. The present components of maize GluCer were in accordance with previous reports^{10,22}.

The total ion chromatograms of GluCer from rice, maitake and sea cucumber are shown in Fig. 4. The patterns of chromatograms in the present HPLC condition were different among GluCer sources. Molecular structures of rice GluCer were similar to those of maize (Table 2). Molecular species of GluCer in rice consisted of hydroxy fatty acids with 18 to 26 carbon atoms including odd numbered fatty acid (C19:0h). Detection of molecules consisting of d18:2 and t18:1 in rice was separated into two peaks as similar as maize. In the case of GluCer with t18:1, the loss of glucose $[M+H-162]^+$ was used as precursor ion (peaks 6, 8 and 12 in Fig. 4). GluCer molecules consisting of d18:1 were also detected in this sample. It has been known that the major sphingoid backbone in fungal sphingolipids is a unique 9-methyl branched sphingoid base (9-methyl-4,8-sphingadienine, d19:2)^{16,23,24}. In this study, GluCer containing d19:2 acylated to hydroxy fatty acids with 14-24 carbon atoms were identified in maitake (Table 3). Odd numbered hydroxyl fatty acids (C15:0h and C17:0h) were also predominant. Sphingadienine (d18:2) acylated to C20:0h was found in GluCer from maitake. GluCer molecules consisting of d18:1 and non-hydroxy fatty acid were also detected. It has been reported that the sphingoid bases in marine invertebrates were quite different from those in mammals and plants^{9,18}. The product ions detected from sea cucumber GluCer were identified as d17:1, d18:1,

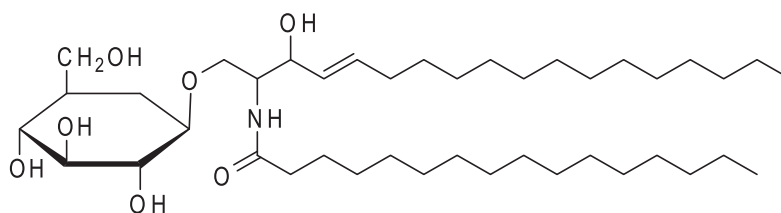


Fig. 1 Representative Structure of Glucosylceramide (D-Glucosyl-β-1,1'-N-palmitoylsphingosine).

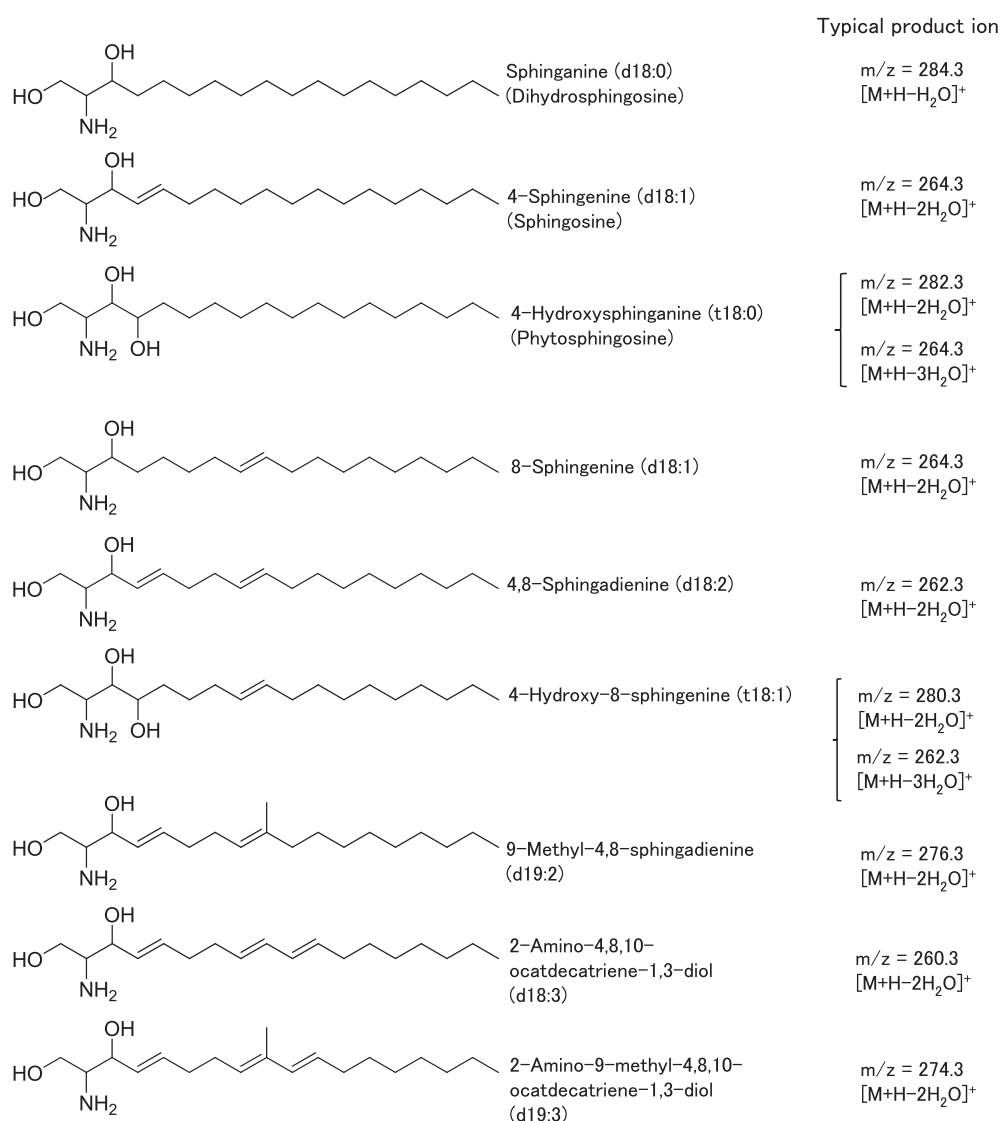


Fig. 2 Structures and Characteristic Product Ions of Sphingoid Bases. The name and shorthand designations were described by Karlsson¹⁵⁾. M = m/z of each sphingoid base.

Table 1 Detection and Identification of Glucosylceramide Molecular Species from Maize by HPLC-MS/MS Analysis.

Peak No. in Fig. 3	Precursor ion m/z			Product ion m/z	Species
	[M+H] ⁺	[M+H-18] ⁺	[M+H-162] ⁺		
1	714.6	696.6		262.3	d18:2-C16:0h
2	742.6	724.6		262.3	d18:2-C18:0h
3	816.7		654.6	280.3 262.3	t18:1-C22:0h
4	770.6	752.6		262.3	d18:2-C20:0h
5	844.7		682.6	280.3 262.3	t18:1-C24:0h
6	798.7	780.6		262.3	d18:2-C22:0h
7	826.7	808.7		262.3	d18:2-C24:0h
8	840.7	822.7		262.3	d18:2-C25:0h
9	854.7	836.7		262.3	d18:2-C26:0h

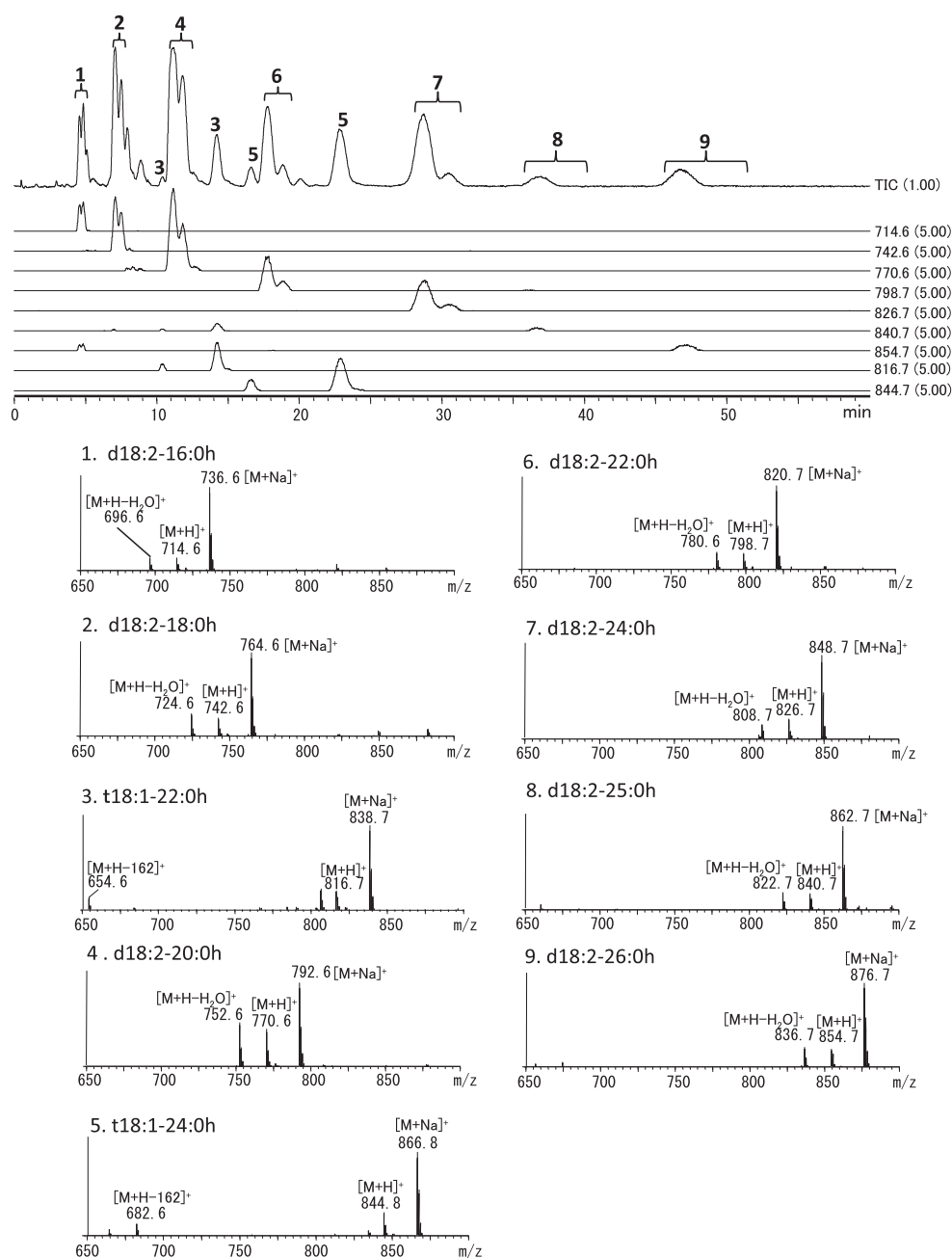


Fig. 3 Total Ion and Selected Ion Chromatograms of Maize Glucosylceramide and Mass Spectra of Peak Components.

d18:2, d18:3, d19:1, d19:2 and d19:3 in the present study (Table 4). This result was similar to the previous report⁹. The fatty acid moieties of sea cucumber GluCer used in this study were mainly non-hydroxy fatty acids.

We previously reported that daily intake of plant-origin GluCer in Japan was estimated to be 50 mg by their contents in foodstuff²⁵. Vesper *et al.* have summarized the amount of sphingolipids in food including dairy, meat and egg products, and estimated that sphingolipids consumption in United States was 300-400 mg/day²⁶. Yunoki *et al.* determined sphingolipid content in Japanese meals by us-

ing HPLC-ELSD, and showed that total amounts of sphingolipids in typical high- and low-calorie meal were 128-292 mg/day and 45-81 mg/day, respectively²⁷. Therefore, significant amounts of sphingolipids having various structures of sphingoid bases are ingested daily from foodstuff. Dietary sphingolipids consisted of sphingosine and sphinganine can be hydrolyzed to sphingoid bases, fatty acids and the polar head group by intestinal enzymes, and are then taken up by mucosal cells^{28, 29}. However, a large portion of sphingosine is metabolized to fatty acids after absorption and a small part is resynthesized to complex sphingolipids^{30, 31}.

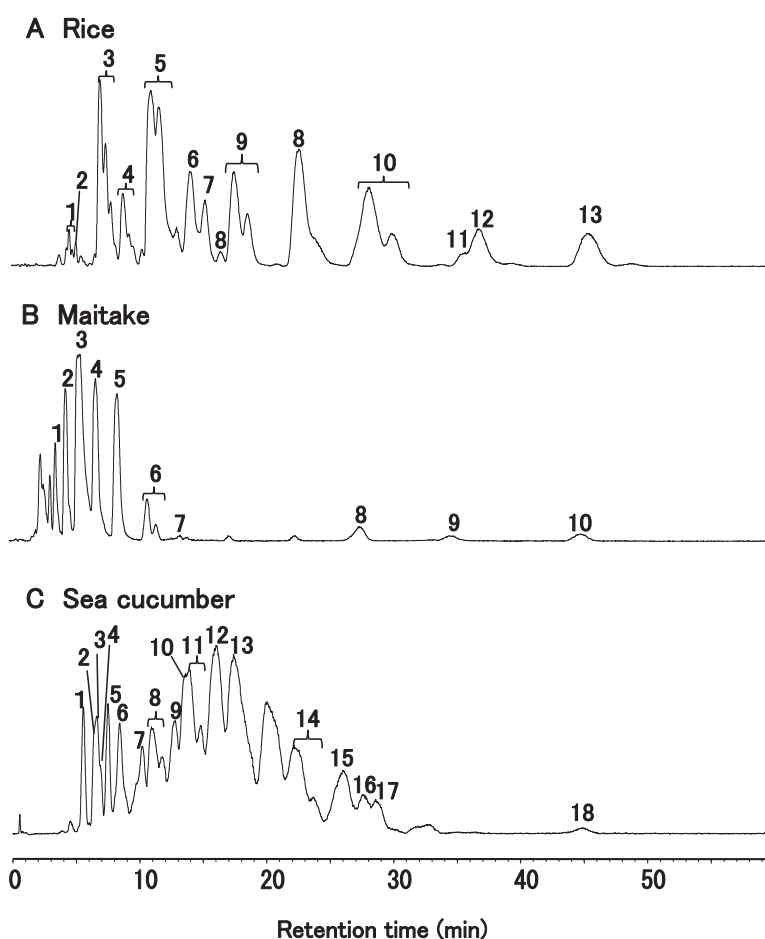


Fig. 4 Total Ion Chromatograms of Glucosylceramides from Rice (A), Maitake (B) and Sea Cucumber (C).

Table 2 Detection and Identification of Glucosylceramide Molecular Species from Rice by HPLC-MS/MS Analysis.

Peak No. in Fig. 4	Precursor ion m/z			Product ion m/z	Species
	[M+H] ⁺	[M+H-18] ⁺	[M+H-162] ⁺		
1	714.6	696.6		262.3	d18:2-C16:0h
2	740.6	722.6		262.3	d18:2-C18:1h
3	742.6	724.6		262.3	d18:2-C18:0h
4	756.6	738.6		262.3	d18:2-C19:0h
5	770.6	752.6		262.3	d18:2-C20:0h
6	816.7		654.6	280.3 262.3	t18:1-C22:0h
7	772.6	754.6		264.2	d18:1-C20:0h
8	844.7		682.6	280.3 262.3	t18:1-C24:0h
9	798.7	780.6		262.3	d18:2-C22:0h
10	826.7	808.7		262.3	d18:2-C24:0h
11	798.7	780.6		264.3	d18:1-C23:0
12	872.7		710.5	280.3 262.3	t18:1-C26:0h
13	812.7	794.7		264.3	d18:1-C24:0

Table 3 Detection and Identification of Glucosylceramide Molecular Species from Maitake by HPLC-MS/MS Analysis.

Peak No. in Fig. 4	Precursor ion m/z		Product ion m/z	Species
	[M+H] ⁺	[M+H-18] ⁺		
1	700.6	682.6	276.3	d19:2-C14:0h
2	714.6	696.6	276.3	d19:2-C15:0h
3	728.6	710.6	276.3	d19:2-C16:0h
4	742.6	724.6	276.3	d19:2-C17:0h
5	756.6	738.6	276.3	d19:2-C18:0h
6	770.6	752.5	262.3	d18:2-C20:0h
7	784.6	766.6	276.3	d19:2-C20:0h
8	784.6	766.6	264.3	d18:1-C22:0
9	840.7	822.7	276.3	d19:2-C24:0h
10	812.7	794.7	264.3	d18:1-C24:0

Table 4 Detection and Identification of Glucosylceramide Molecular Species from Sea Cucumber by HPLC-MS/MS Analysis.

Peak No. in Fig. 4	Precursor ion m/z		Product ion m/z	Species
	[M+H] ⁺	[M+H-18] ⁺		
1	728.6	710.6	276.3	d19:2-C16:0h
2	724.6	706.6	260.3	d18:3-C18:0
3	754.6	736.6	274.3	d19:3-C18:0h
4	700.6	682.6	264.3	d18:1-C18:0
5	738.6	720.6	274.3	d19:3-C19:0
6	726.6	708.5	262.3	d18:2-C18:0
7	808.7	790.7	274.3	d19:3-C23:0
8	784.6	766.6	250.3	d17:1-C23:0
9	822.7	804.7	274.3	d19:3-C24:0
10	822.7	804.7	260.3	d18:3-C25:0
11	798.7	780.6	250.3	d17:1-C24:0
12	836.7	818.7	274.3	d19:3-C25:0
13	812.7	794.7	250.3	d17:1-C25:0
14	826.7	808.7	264.3	d18:1-C25:0
15	800.7	782.6	250.3	d17:1-C23:0h
16	840.7	822.7	278.3	d19:1-C25:0
17	814.7	796.7	250.3	d17:1-C24:0h
18	812.7	794.5	264.3	d18:1-C24:0

We previously investigated the digestion and absorption of plant-derived sphingolipids and our finding indicated that the metabolic fate of plant-derived sphingoid bases, such as 4, 8-sphingadienine, within enterocytes differs from that of sphingosine²¹⁾. Sphingoid bases, except for sphingosine, appear to be transported out of cells across the apical membranes of enterocytes by P-glycoprotein after absorp-

tion and consequently the intestinal uptake is quite poor³²⁾, although the exact fate of dietary plant-derived sphingolipids is still not well understood. Thus, determination of sphingolipid structures including variation of sphingoid backbone from dietary sources is important for understanding their functional and nutritional significance.

In this study, we analyzed the chemical structures of

GluCer from maize, rice, maitake and sea cucumber by liquid chromatography-ion trap mass spectrometry. Various molecules of GluCer were able to be identified using this system. However, quantitative analysis of each molecule of glucosylceramide should be difficult because each standard molecule is required in LC-MS/MS system. It could be concluded from the present results that MS/MS analysis is a powerful method for identification of molecular structures of sphingolipids from various sources.

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