Quantification of Sphingolipids in Soybeans

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ABSTRACT: Soybean is believed to be a rich source of sphingolipids, a class of polar lipids with desirable biological activities. Analytical methods for sphingolipids vary, and quantitative data for sphingolipids in foods, including soybeans, are scarce. The objectives of this study were to establish a method for quantification of sphingolipids in soybeans and to determine whether genotype, stage of maturity, and growing location affect sphingolipid content in soybeans. Separation of neutral lipids and interfering polar lipids from sphingolipids by saponification, transesterification, and solvent partition was studied. Solvent partition accompanied by TLC purification was determined to be the most accurate sample preparation method for HPLC quantification of cerebroside. There were significant differences in cerebroside concentration among genotypes, with a range of 142 to 492 nmol/g seed (dry wt basis). The differences in cerebroside concentration between immature and mature seeds of one genotype and between two seed production locations of one genotype were considerable but not statistically significant.

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KEY WORDS: Cerebroside, HPLC, quantification, soybean, sphingolipids.

Sphingolipids are found primarily in the plasma membrane of all eukaryotes, some prokaryotes, and in all foods, with soybeans considered a rich source (1). Sphingolipids include free sphingoid bases, ceramides, sphingophospholipids, and glycosphingolipids. Sphingoid bases, usually 18-carbon amino alcohols, are N-acetylated to a long-chain FA to form ceramides. Polar head groups, such as sugar residues and phosphorylcholine, attach to the 1-ol position of ceramide to form more complex sphingolipids.

Soybean contains two classes of sphingolipids, ceramide (Cer) and cerebroside. Cerebroside is the predominating class in soybeans (2), and it is the simplest glycosphingolipid because it contains only one sugar residue. The only type of cerebroside found in soybean is glucosylceramides (GlcCer), which contain a glucose molecule (2).

Until recently, sphingolipids were recognized only as structural lipids. It has been discovered that their metabolites (i.e., ceramides and sphingosine) are involved in intracellular signaling, cell growth, differentiation, and apoptosis (3). Dietary sphingolipids have been shown to protect mice from skin and colon cancer (3) and decrease plasma cholesterol by 30% in rats (4).

Dietary sphingolipids have important positive health implications, but information on their total content in foodstuffs, including soybeans, is sparse and may not be accurate. Certain data have been obtained from incomplete, single studies in which the main sphingolipid class may not have been measured and/or the effects of processing/preparation or other aspects influencing sphingolipid content were not considered (2). Almost all quantification studies have used chemical hydrolysis or derivatization, both of which require many steps and may produce artifacts, causing over- or underestimation of sphingolipid concentration. The possible degradation or hydrolysis during these quantification studies often has not been reported. Because data for food are scarce, values from characterization or qualitative studies, which were not designed for accurate sphingolipid quantification, are often cited. The objectives of this study were to establish a method for quantification of sphingolipids in soybeans without significant alteration of their chemical structure, and to examine the effect of soybean genotype, stage of maturity, and growing location on the concentration of the major sphingolipids in the seed.

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EXPERIMENTAL PROCEDURES

Solvents and standards. All chemicals, except sodium methylate and petroleum ether, were obtained from Fisher Scientific (Fairlawn, NJ). Sodium methylate (5.4 M) was obtained from Sigma Chemical Co. (St. Louis, MO), and petroleum ether (b.p. 20–40°C) from J.T.Baker (Phillipsburg, NJ). Soybean GlcCer standard was obtained from Avanti Polar Lipids, Inc. (Alabaster, AL). All other standards, including Cer, were obtained from Matreya, Inc. (State College, PA).

Seed selection. For the determination of the effect of genotype on sphingolipid content, mature seeds from 10 soybean genotypes with different FA compositions and protein contents were obtained from the Iowa State University soybean breeding program. The genotypes were planted in adjacent plots at the Agricultural Engineering and Agronomy Research Center near Ames, Iowa, in spring 2002. Seeds from individual plants were harvested from the plots when the plants were mature. A five-seed bulk sample from each plant was analyzed for FA composition by GC. The seeds from plants of each genotype with similar FA compositions were bulked together to obtain the sample used for analysis. IA1008, one of the genotypes grown at Ames, was grown at the Iowa State University–University of Puerto Rico nursery near Isabela, Puerto Rico, in 2002. Mature seeds of IA1008 from Ames and Isabela were used to determine the effect of production location on sphingolipid content. To evaluate effect of seed maturity on sphingolipid content, seeds of Pioneer 3981 (Pioneer Hi-Bred International, Johnston, IA) were collected while

they were immature (August 28, 2002) and when they were fully mature (September 24, 2002). This genotype was grown by X.B. Yang of Iowa State University near Ames, Iowa.

Moisture, protein, oil, and FA compositional analyses. Mature seeds were dried according to AOCS Official Method Ca2c-25 (5) to determine moisture content. Immature beans were dried in a vacuum oven at 55°C until a constant weight was reached, usually for 4.5 h. Protein and oil contents of the mature seeds were determined using a Grainspec analyzer (Foss Electric North America, Eden Prairie, MN). The near-IR analyzer was calibrated according to the methods described by Hardy *et al.* (6). For FA composition analysis, five mature seeds of each genotype were crushed together at 40,000 psi using a hydraulic press (Pasadena Hydraulics, Inc., El Monte, CA). Hexane (1 mL) was added to the crushed beans for oil extraction. The hexane/oil mixture (200 mL) was transferred into GC vials along with 500 μL of a 1 N sodium methoxide solution to produce FAME. After 2 h of reaction, FAME were analyzed by a 5890 Series II (Hewlett-Packard, Avondale, PA) gas chromatograph equipped with a FID and Supelco-2330 capillary column (15 m length, 0.25 mm i.d., 0.2 μm film thickness, with 80% biscyanopropyl and 20% cyanopropyl phenyl siloxane coating material; Supelco, Bellefonte, PA). The oven temperature was 220°C, inlet and detector temperatures were both 250°C, and the split ratio was 1:100. Protein and oil analyses were performed in duplicate. Moisture and FA analyses were replicated four times.

Lipid extraction. Mature soybeans were ground using a Wiley mill (Thomas Scientific, Swedesboro, NJ) equipped with a 20-mesh delivering tube. The immature seeds, blanched in boiling water for 5 min immediately after harvest, were ground with a mortar and pestle. Ten grams of ground sample (as-is basis) was sequentially extracted with 50 mL of each of the following solvents for 4 h each with stirring: hexane, chloroform/methanol (2:1, vol/vol), water-saturated butanol two times. For the second water-saturated butanol extraction, the soybean cake was sonicated while in solvent for 45 s (ultrasonic liquid processor, Model XL2020; Misonix, Farmingdale, NY; sonicator setting: continuous mode at setting 3). The 4-h extraction was determined by a yield vs. extraction time optimization process. The second butanol extraction was necessary because it resulted in additional lipid recovery. For greater efficiency, the first hexane extraction may be omitted. The four extracts were pooled, and the solvent was removed by a rotary evaporator at 60–70°C. The crude lipid was purified using the wash method of Folch *et al.* (7) to remove the polar contaminant, and the lipid was measured gravimetrically.

Analytical techniques investigated for sphingolipid isolation from the total lipid extract.

(i) Saponification to remove phospholipids and neutral lipids from sphingolipids. Saponification is the most commonly used method to remove glycerol lipids. To test whether it has any degradative effect on sphingolipids, saponification was performed on sphingolipid standards. A 2-mg sample of Cer standard was treated with 1.6 mL of 1 M KOH for 6 h at 40°C with stirring, and a 3 mg sample was treated with 2.4 mL in the same manner. After 6 h, the samples were neutralized with 1 M acetic acid, then washed using the method of Folch *et al.* to remove salt or residual acid or base. The extract was then quantified with HPLC and an ELSD using the conditions outlined under in the HPLC quantification section of this paper.

(ii) Transesterification to remove phospholipids and neutral lipids from sphingolipids. Oil sample (2 g), and Cer and GlcCer standards (1 mg) were treated separately with 0.2 mL of 5.4 M sodium methylate and 3.2 mL of methanol for either 20 or 45 min at ambient temperature to convert glycerol fatty esters to methyl esters. After the reaction, samples were washed using the method of Folch *et al.* (for standards) or purified by solvent partition extraction (for the oil sample) as described in the following section, and quantified with HPLC/ELSD.

(iii) Solid-phase extraction (SPE) of sphingolipids from other lipids. To determine the efficacy of sphingolipid separation from saponified and transesterified samples, model systems were used. Silica cartridges (5000 mg) (Alltech Associates, Inc., Deerfield, IL) were used to extract 6 mg Cer from 2 g oleic acid and 6 mg Cer from 2 g soybean methyl esters. The cartridge was eluted with 75 mL hexane/diethyl ether (95:5 vol/vol) to remove the FA, FAME, and other unsaponifiable neutral lipids followed by 50 mL of each of acetone, methanol, and methanol/water (95:5, vol/vol) to collect the alkali-stable sphingolipids. The combination of the last three fractions is referred to as the polar lipid.

(iv) Separation of sphingolipids from neutral lipids by solvent partition. A modification of the petroleum ether/87% ethanol partition extraction procedure (8) was made to separate polar and neutral lipids. Before extraction, both solvents were saturated with each other by thoroughly mixing equal amounts of the two solvents, and the two equilibrated and separated layers were used. Lipid was first dissolved into 25 mL of petroleum ether, to which 8.2 mL of 87% ethanol was added, and the funnel was shaken thoroughly. The equilibrated lower ethanol phase was transferred to a second funnel containing 25 mL petroleum ether, and the funnel was shaken thoroughly so that the neutral lipids extracted by ethanol would be redistributed into the petroleum ether phase. The equilibrated lower ethanol phase was transferred to a flask to complete one cycle of extraction. To begin another cycle, 8.2 mL of 87% ethanol was added to the first funnel to extract the polar lipids and the ethanol layer was transferred to the second funnel containing petroleum ether. Eight cycles were performed to complete one extraction. Eight cycles of ethanol extraction were reported as necessary to extract the polar lipids completely (with about 100% recovery) (8). The ethanol extract may contain a considerable amount of neutral lipids, and another set of multiple-cycle extraction was performed when necessary. Although this procedure seems tedious, each extraction can be done in about 20 min. This solvent partition method was the only technique adopted into this study's methodology for sphingolipid isolation and quantification.

TLC purification of lipid. After solvent partition of sphingolipids from neutral lipids, preparative silica chromatography plates (500-μm Absorbosil Plus 1; Alltech) were used to

separate GlcCer from other polar lipids. The lipid extract from solvent partition was streaked onto the 20 × 20 cm plate that also was co-chromatographed with GlcCer standard. The plate was developed with chloroform/methanol/ether/hexane/acetic acid (100:20:20:10:1.5, by vol). Only the GlcCer standard on the plate was sprayed with 2′,7′-dichlorofluorescein (Sigma) in methanol (0.1%) and visualized under UV light. The GlcCer silica band was identified, scraped off, and extracted five times with approximately 30 mL of methanol/water (95:5, vol/vol) to ensure complete extraction. The extracts were pooled, and the solvent was evaporated using a rotary evaporator. The extracted lipid was redissolved into chloroform/methanol (2:1, vol/vol) for HPLC analysis.

HPLC quantification of sphingolipids. A Beckman Coulter (Fullerton, CA) HPLC system equipped with auto sampler, solvent delivery system, silica column (Solvent Miser Silica with 5 μm particle size, 250 mm length, 2.1 mm i.d.; Alltech), and ELSD (Model 2000; Alltech) was used. Two mobile phases and a gradient program were created: "A" was hexane/THF (99:1, vol/vol), and "B" was isopropanol/methanol (50:50, vol/vol). The gradient elution program is presented in Table 1. A second gradient program, with slight modification of the first (i.e., it became polar faster at the beginning of the program than the first gradient), was created for the second replicate of samples and quantification because a new column was used, and separation of lipids using the gradient program for the first replicate could not be achieved. The mobile phase flow rate was 0.3 mL/min. Nitrogen at a 2.5 L/min flow rate was used to evaporate the solvent in the heated (68°C) chamber of the ELSD. A standard calibration curve for each replicate of analysis was made with soybean GlcCer standard (purity greater than 98%) using the foregoing HPLC/ELSD conditions. The concentration range used for establishing the standard curve was 0.25–5 mg/mL. The two curves are presented as follows (*X* represents mg/mL of standard and *Y* represents peak area):

$$\text{for first replicate analysis: } Y = 13{,}900{,}000X^{1.4200} \quad R^2 = 0.9980 \quad [1]$$

$$\text{for second replicate analysis: } Y = 20{,}000{,}000X^{1.5438} \quad R^2 = 0.9975 \quad [2]$$

The nonlinear response of the detector is due to the nature of light-scattering mechanism (9). A standard solution used for the calibration curves was run several times on the same day the samples were analyzed to detect any changes in the detector's original response during HPLC analysis. Reproducibility was good for each replicate analysis, and the coefficient of variation, on average, was 2.9%.

Statistical data analysis. GlcCer was extracted from each genotype in two replications conducted 2 mon apart. For each replication, all genotypes were prepared together for GlcCer isolation. After GlcCer extracts from all genotypes were prepared, they were analyzed by HPLC/ELSD. Data were analyzed by ANOVA using SAS software (10). Tukey–Kramer's mean comparison ($P = 0.05$) was used to determine differences between genotypes.

TABLE 1
Gradient Program of Mobile Phase Used for HPLC Quantification of Cerebroside[a]

Time (min)	Solvent A (%)	Solvent B (%)
0	95	5
5	90	10
10	90	10
22	0	100
24	0	100
34	100	0
36	100	0
51	100	0

[a]Solvent A: hexane/THF = 99:1, vol/vol; solvent B: methanol/isopropanol = 50:50, vol/vol.

RESULTS AND DISCUSSION

Effects of sample preparation methods on sphingolipid quantification.

(i) Saponification. An alkaline saponification treatment was initially considered essential if Cer was to be quantified because separation of Cer and esterified steryl glucoside (ESG) standards was unsuccessful under several HPLC conditions. ESG is a glycolipid found in soybeans, and its concentration in soybeans is typically 38 mg/100 g seed (11). Such an alkaline treatment would remove ESG in the sample by hydrolysis of the ester linkage between the FA and sugar molecule. Treating Cer standards alone with methanolic KOH caused degradation or hydrolysis, and the recovery of Cer was 76.5 ± 0.7%. This result was likely since nearly complete sphingolipid saponification occurs after 10 h in 1 M methanolic KOH under reflux (12). Samples were not refluxed so as to avoid saponification of the sphingolipids; however, a certain degree of hydrolysis of the Cer standards may have occurred. The saponification reaction conditions selected in this study were chosen based on the conditions that will be used for the treatment of the total lipid extract. Thorough investigation of Cer hydrolysis under various conditions will reveal more about the chemistry and stability of this compound; however, this was not the original intention of this research. The effect of saponification on GlcCer was not tested because we assumed that the same amide bond hydrolysis will occur in GlcCer as in Cer.

SPE was performed to examine the efficacy of isolating sphingolipids from the FFA after acidification of the saponified sample. A model system was used to represent a saponified and acidified sample, which consisted of 6 mg Cer standard and 2 g of pure oleic acid. Lipid extraction of 10 g of seed typically yielded 2 g of crude lipid in this study. The amount of Cer recovered with the polar solvents (acetone, methanol, and methanol/water) was about 20% as quantified by HPLC. A significant amount of oleic acid was recovered in the polar fractions. Hexane/diethyl ether (95:5, vol/vol) was chosen as the initial eluant because this solvent combination was recommended by Christie (12) to elute FFA from silica columns. It was also suggested that the amount of diethyl ether in hexane could be increased if elution of FFA was incomplete with 5% diethyl ether in hexane. Increasing diethyl

ether in hexane would risk more Cer loss during elution of the FA in this study. Therefore, the sphingolipid degradation/hydrolysis and the difficulty in separating large amounts of FFA from minor quantities of sphingolipids by SPE make saponification an inappropriate method of sample preparation.

In most reported studies involving isolation of both Cer and GlcCer from plant material, including one using soybean leaves, saponification was conducted after silica column chromatography (13–18). These qualitative studies utilized larger columns because they extracted total lipids from much greater amounts of starting material (11.8 kg to 110 g). Using a large column will involve a large amount of solvent, and saponification still has to be used after the major portion of the neutral lipids are removed so as to remove the residual neutral lipids and the interfering polar lipids. We chose to use 5000-mg silica columns and expected that other polar lipids, such as ESG and phospholipids, would interfere with the separation of sphingolipids from neutral lipids because only about 50 mg of polar lipids can be retained by the cartridge (Alltech representative, personal communication), and our samples likely contained much more than 50 mg of polar lipids. For this reason, the samples were saponified before using SPE to concentrate sphingolipids.

(ii) Transesterification. The effects of transesterification on sphingolipid quantification were investigated because this technique is considered a milder and a more rapid derivitization treatment than saponification (12). There was 75 ± 3.0% of the Cer standard recovered when Cer was treated for 20 min. Only 43 ± 20.5% of the GlcCer standard was recovered after 45 min of treatment. The treatment thought not to cause sphingolipid degradation was 0.00108 mol sodium methylate per 50 mg lipid treated for 10 min at 50°C (12). Our conditions included 0.001 mol sodium methylate per 2 g of lipid at ambient temperature for up to 45 min; these conditions were not more severe than those suggested (12). GlcCer was treated for 45 min because it was later found, after treatment of Cer standards, that this amount of time was necessary for complete transesterification of a lipid sample containing significant amounts of phospholipids.

SPE also was used to isolate Cer from a model system representing a transesterified sample. Cer (6 mg) was added to 2 g of soybean methyl esters to form the model, and the conditions for SPE were as previously described. Surprisingly, recovery of Cer was greater than 100%, which suggested possible production of an artifact during the transesterification of neutral oil. To validate this assumption, 2 g of purified soybean oil obtained by passage through a silica cartridge column (eluting with hexane) was transesterified alone under the same conditions as the Cer standards. HPLC analysis of the soybean oil sample verified that an artifact with a retention time similar to that of the Cer standard was produced in a significant amount, accounting for the greater than 100% recovery in the model system (Fig. 1). Several HPLC mobile phases and gradients were used for separating this unknown from Cer without success. It was later confirmed that this unknown was MAG (monoacylglycerol), which may have been produced from transesterification. Although they were present in very low concentration, they were enough to interfere with Cer quantification. Therefore, Cer and GlcCer quantification by transesterification was not considered.

(iii) Solvent partition separation. This extraction procedure was performed with both Cer and GlcCer standards alone. It was expected that recovery of Cer might be low because it is a relatively neutral lipid; its recovery was proved to be only 43 ± 0%. Recovery of GlcCer, a more polar lipid, was high (91.5 ± 2.1%). Recovery of GlcCer in a model sample (GlcCer standard in 2 g of purified oil) was 93.0 ± 0.04%. This extraction procedure was chosen as a final procedure for GlcCer quantification.

Overall, saponification caused some sphingolipid degradation. This treatment together with SPE did not allow separation of polar lipids from neutral lipids. Transesterification also may cause some degradation and/or produce artifacts that would interfere with sphingolipid quantification. Solvent partition extraction resulted in good recovery and quantification of GlcCer, but severe loss of Cer. Therefore, only GlcCer was quantified in this study. Ohnishi and Yasuhiko (17) reported the GlcCer content in soybeans to be almost three times more than Cer. Quantification of only the predominant GlcCer is temporarily satisfactory; in the future, Cer will also be quantified.

Final procedure for GlcCer isolation and quantification. After recovery of total lipids and the Folch wash, two petroleum ether/ethanol extractions were performed to remove contaminating neutral lipids. Silica plates were used to isolate GlcCer from other polar lipids, and GlcCer was quantified using HPLC.

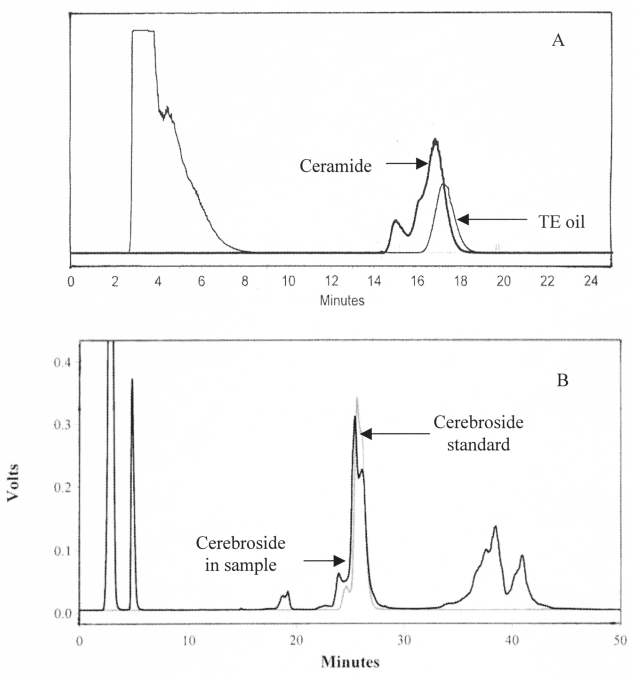

FIG. 1. HPLC chromatograms for (A) the ceramide standard vs. transesterified (TE) pure soybean oil, and (B) the cerebroside standard vs. the polar lipid extract from immature soybeans.

Effect of soybean genotype on GlcCer content. The 10 soybean genotypes grown near Ames, Iowa, were significantly different in GlcCer concentration (Table 2). The genotypes utilized for this study were not of a single genetic background, so differences in GlcCer content among them may not be due only to differences in their seed composition. The two genotypes with the highest palmitic contents, A97-877006 and A00-815004, had the highest GlcCer contents. A possible explanation for the greater GlcCer concentration in the genotypes with elevated palmitic acid is that the biosynthesis of sphingolipids begins with the condensation of serine and palmitoyl-CoA by serine palmitoyltransferase (19). The *de novo* studies by Merrill *et al.* (20) and Paumen *et al.* (21) showed that sphingoid or ceramide biosynthesis increased with palmitic acid in the medium compared with cells that received no exogenous FA or that were exposed to other types of FA (C_{15}–C_{18}). Their studies were not conducted with plant tissues, and cerebroside concentration was not measured; however, sphingolipid synthesis in plants is believed to closely simulate that of other cell types (19).

Few studies have reported the sphingolipid content in soybeans to which we could compare our 10 soybean genotypes. The most frequently cited value in the literature for sphingolipid content in soybeans is based on a qualitative study by Ohnishi and Yasuhiko (17), who analyzed one genotype and reported Cer (38 nmol/g) and GlcCer (91 nmol/g) amounts in the mature seeds. The authors reported only how the Cer and GlcCer structures were analyzed, not how they were quantified. Other authors have interpreted the results found by Ohnishi and Yasuhiko (17) and reported soybeans to have a sphingolipid content of 2,400 nmol/g (2,3,22), in that the gram unit (demoninator) actually represents the total glycolipid content found in soybeans. Therefore, this is not the sphingolipid content in soybeans. However, this value is often cited as the soybean sphingolipid content.

Effect of maturity stage on sphingolipid content. The immature seed of Pioneer 3981 was harvested on August 29, 2002, and the mature seeds on September 24, 2002. The moisture content was 68% for the immature seed and 14% for the mature seed. The GlcCer content for the immature Pioneer 3981 seeds (378 nmol/g, dry wt basis) was greater than the GlcCer content for the mature seeds (209 nmol/g, dry wt basis), but the difference between these two seed types was not statistically significant ($P > 0.05$). The effect of seed maturity was investigated because sphingolipids are primarily membrane lipids, so it was expected that sphingolipid concentration would be higher in immature seeds because other seed components, such as TAG and protein, would not have been fully synthesized and deposited in the seed as in the mature seeds. Ohnishi and Yasuhiko (17) reported immature beans to have a GlcCer concentration of 612 nmol/g, whereas the GlcCer content in the mature seeds of the same genotype was 128 nmol/g (as-is basis). The magnitude of difference between the GlcCer contents of the immature and mature seeds in our study may have varied from that by Ohnishi and Yasuhiko due to differences in the genotype, growing location, and stage of maturity that were evaluated.

TABLE 2
Mean Composition and Cerebroside (GlcCer) Content of 10 Soybean Genotypes Grown near Ames, Iowa

Genotype and selectively modified trait(s)	Protein[a] (%)	Oil[a] (%)	Palmitic (%)	Stearic (%)	Oleic (%)	Linoleic (%)	Linolenic (%)	GlcCer (nmol/g dry wt basis)
IA1008 Conventional	36.0	18.5	10.8	4.4	26.3	51.0	7.4	142
IA2021 Low-protein	36.0	19.3	10.6	4.7	25.4	52.4	6.8	283
IA2041 High-protein	41.0	16.3	9.9	4.4	25.8	52.5	7.3	201
A00-815004 High-palmitic	34.0	14.6	41.3	4.5	10.0	34.6	9.6	389
A97-877006 Mid-palmitic	34.0	15.0	27.0	4.6	14.1	45.3	9.3	493
FA22 High-oleic	38.8	16.7	8.1	3.4	51.8	31.8	4.9	306
B0147B013 Low-palmitic	36.4	16.7	3.4	3.0	24.6	60.1	8.9	168
AX7019-12 Mid-palmitic/stearic	33.6	15.0	20.6	24.0	8.7	39.2	7.5	246
A97-552013 Low-linolenic	37.0	17.4	10.1	5.0	27.9	55.6	1.3	229
A99-144085 High-stearic	35.1	16.9	8.0	28.1	20.3	40.8	2.9	197
MSD[b]	1.3	0.9	2.1	2.8	4.7	4.5	0.9	122

[a]Protein and oil based on 13% seed moisture content
[b]MSD = minimum significant difference determined by Tukey–Kramer's mean comparison test ($P = 0.05$).

Effect of environment on sphingolipid content. The GlcCer content of the genotype IA1008 (142 nmol/g, dry wt basis) when grown in Ames, Iowa, was not significantly different from its content when grown in Isabela, Puerto Rico (208 nmol/g, dry wt basis) ($P > 0.05$). The protein and oil contents were significantly different between the IA1008 seeds grown at different locations, but their FA compositions were not ($P > 0.05$). The IA1008 seeds from Ames were composed of 36.0% protein and 18.5% oil, whereas the seeds collected from Puerto Rico contained 34.4% protein and 20.0% oil (13% moisture basis).

Compared to the commonly used sphingolipid quantification methods, our method does not involve derivatization and complicated instrumentation. The sphingolipid quantification methods used most frequently include radiolabeling and fluorophor derivatization of the sphingolipids, enzymatic assay, and hydrolysis (23). Ahn and Schroeder (24) quantified sphingolipids in soybean and dairy products by acid hydrolysis and subsequent measurement of the base backbone. Tandem (triple quadrupole) MS was also used to characterize (by precursor ion scans) and quantify (by multiple reaction monitoring) sphingolipids (23). Highly advanced and specialized instrumentation, a skilled operator, and various synthetic internal standards are needed for such analyses.

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