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ORIGINAL ARTICLE

Application of a comprehensive metabolomics approach for the selection of flaxseed varieties with the highest nutritional and medicinal attributes

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Abstract

Flaxseed is considered an indispensable generally recognized as safe (GRAS) source of polyphenolic lignans, polyunsaturated fatty acids (PUFA), fibers as well as minerals and vitamins. The metabolite content of flaxseed reflects its nutritional and medicinal value. Therefore, the selection of flaxseed variety for food industry is dependent on its metabolome. A metabolomics approach based on liquid or gas chromatography coupled to mass spectrometry has been applied to discriminate different flaxseed cultivars that are commercially available in Egypt. The available Sakha cultivars were subjected to a comprehensive metabolomics and lipidomics approach for investigation of their metabolomes. Our results showed that among the screened cultivars, Sakha 6, with its yellow-colored testa, showed marked metabolic discrimination. This yellow cultivar showed high accumulation of essential amino acids. Additionally, the oil of this cultivar accumulated the highest content of the two essential PUFA: alpha-linolenic acid (an ω-3 fatty acid) and linoleic acid (an ω-6 fatty acid). Interestingly, the content of the main antinutritional cyanogenic glycosides such as linustatin and neolinustatin was lower, while, the content of medicinally-important secondary metabolites was higher in Sakha 6 cultivar. These results support the use of this cultivar for human consumption owing to its high nutritional and medicinal value.

Keywords: Linseed cultivars, Metabolomics, Seed color, Triacylglycerols, ω-fatty acids

1. Introduction

Flaxseed (*Linum usitatissimum* L.) has long been known as valuable nutrient for humans and animals [1,2]. Flaxseed is also considered as an important industrial crop for linen and oil production, its oil is used in painting, oleochemicals and stains [3]. Flaxseeds have high contents of protein (20−30%), dietary fibers (20%), fats (30−40%), and ash (4%) [4,5]. The detection of bioactive constituents in flaxseeds such as mucilage, secoisolariciresinol diglucoside (SDG) and α-linolenic acid (ALA) makes it also a medicinally important component in nutraceuticals [6]. Additionally, the amino acid profile of flaxseed has a great similarity to soybeans which is a very important source of proteins [2,7]. However, there are many undesired compounds in flaxseeds just like its cyanogenic glycosides, linatine, which is considered as goitrogen, phytic acid, trypsin inhibitors, anti-pyridoxine factor and allergens [8,9]. Because of all these attributes,

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selection of the suitable variety of flax seed appears to be an important issue for supporting its use.

The nutritional value of the different varieties of flaxseeds is dependent on its primary and secondary metabolites. The primary metabolites directly affect the growth and reproductive functions of the plant, while its secondary metabolites have an important role in the defense mechanism of the plant and in various facultative operations.

Flaxseed is a valuable source of nutritional oil rich in α-linolenic acid ($\omega-3$ fatty acid) [2]. Among the well-documented biological activities for ω-3 poly unsaturated fatty acids (PUFAs) are anti-inflammatory actions particularly in asthma, rheumatoid arthritis, inflammatory bowel disease [10]. Flaxseed is considered the richest source of lignans [2]. Lignans, which are phenolic compounds, formed of two cinnamic acid residues, have been considered phytoestrogens [2]. Phytoestrogens are natural products that have weak estrogen agonists/antagonists properties and have been introduced as a natural alternative to estrogen replacement therapy with numerous health benefits in menopausal disorders, osteoporosis, heart diseases and breast cancer [11]. Secoisolariciresinol diglucoside (SDG) is the predominant lignan in flaxseeds [12]. SDG has been reported to exist in oligomers rather than the free form, particularly forming ester-linked with organic acid such as 3-hydroxy-3-methyl glutaric acid (HMGA), *p*-coumaric and ferulic acids [13]. SDG has been reported to have cancer-protective potential against various types of cancer such as breast, lung and colon owing to its antioxidant as well as anti-proliferative activities [14].

Linseed is grown in Egypt for two products, seeds and fibers. Flax was cultivated in ancient Egypt for linen, painting and medicinal purposes [15]. Several cultivars have been released in the last two decades for production of linseed oil and fiber [16,17]. We have shown previously that among the different cultivars, Giza 9 contains the highest concentration of SDG and showed very potent cytotoxic activity against ER-receptor positive breast cancer cell lines MCF7 and T47D [17]. We have also reported that using optimum agronomic practice, flaxseed lignan content has been optimized in this cultivar to enhance its anticancer, health-promoting effect [16]. Moreover, our previous studies revealed that Giza 9 and 10 cultivars exhibited significant antidepressant-like effect in rat model of postpartum depression (PPD) without affecting the locomotor activity [18]. However, there are several cultivars (Sakha) that have not been deeply investigated for their metabolic content.

Metabolomics have been developed in the last decade as systems biology approach for comprehensive analysis of small molecules for understanding biological process [19]. Metabolomic studies have been used for numerous applications in the field of drug discovery, quality control and chemotaxonomy [20]. The goal of this study was to optimize a comprehensive metabolomics approach for MS-based analysis of flaxseed metabolites for better selection of high quality cultivar. For this purpose, the available Sakha cultivars were subjected to a comprehensive metablomics analysis to investigate their metabolic contents. We provide a systematic protocol for extraction, analysis and identification of flaxseed metabolites. Our results showed that among the screened cultivars, Sakha 6, which has yellow color of testa, showed marked metabolic discrimination represented by higher content of nutritional and medicinally-important metabolites, while, the antinutritional metabolites were decreased.

2. Materials and methods

2.1. Plant material

Seeds of *Linum usitatisimum* L. cultivars, were collected in winter 2017/2018 from different localities in Egypt *viz*. Qaliubiya, Sharkia, Gahrbia and Kafr El-Shaikh and Giza Governorates, and identified by staff members of Fiber Crops Research Institute, Giza. Cairo, Egypt. The flaxseed cultivars were identified namely; Sahka 1, Sakha 2, Sakha 3, Sakha 4, Sakha 6.

2.2. Metabolite extraction

Seeds were subjected to extraction using our previously published protocol with minor modifications [21,22]. Briefly, aliquots (10 mg/biological replicate) were extracted with 1000 μL of methanol: methyl-*tert*-butyl-ether (1:3, [v:v]) mixture. The samples were kept on a shaker for 30 min followed by a 10-min sonication. Liquid-liquid separation was achieved by adding 500 μL of methanol: water (1:3, [v:v]) mixture. The samples were kept on a shaker for 5 min followed by centrifugation for 20 min at 15,000 g. For LC/MS lipids analysis, 200 μL aliquots were collected from upper phase. For analysis of semi-polar to polar metabolites, 200 μL and 400 μL aliquots from the lower phase were collected for GC/MS and LC/MS analysis, respectively. The collected aliquots were dried down in a speed-vacuum concentrator without heating (28–30 °C). The precipitated pellets were used for

spectrophotometric analysis of starch and proteins [21,22]. Blank samples (empty tubes that were subjected to the whole sample preparation, extraction and analysis) as well as quality control (QC) samples, a pool that were collected from all tested samples were included for quality assessment. QC samples were analyzed prior to sample acquisition and after every five samples, were used to confirm the stability and reproducibility of the analysis. The run order effect was excluded by randomizing all the samples prior to analysis.

2.3. Analysis of lipids by LC/MS

The dried lipid samples from the organic upper phase were re-suspended in 400 μL of LC-grade isopropanol: acetonitrile (3:7 [v:v]) mixture. Two μL from each sample were injected and separated on an Acquity UPLC system (Waters, Machester, UK). Chromatographic separations were performed on a C8 column (100 mm × 2.1 mm containing 1.7 μm diameter particles, Waters, Machester, UK) at a column oven temperature of 40 °C. The flow rate was set to 400 μL/min and the auto-sampler was maintained at 10 °C. The mobile phases consisted of 1% 1 M ammonium acetate, 0.1% acetic acid in UPLC MS grade water (solvent A) and 1% 1 M ammonium acetate, 0.1% acetic acid in acetonitrile/isopropanol 7:3 (v:v) (solvent B). The steps of the gradient profile were 45% A from 0 to 1 min, 45% A to 35% A from 1 to 4 min, 35% A to 11% A from 4 to 12 min, 11% A to 1% A from 12 to 15 min, 1% A from 15 to 18 and 45% A from 18 to 22 min. The mass spectra were acquired using high resolution mass spectrometer (Orbitrap XL, Thermo Scientific, Bremen, Germany) in both positive and negative ionization mode covering the mass range between 150 and 1500 *m*/*z*. The MS data were collected in the full scan mode [21,22].

2.4. Analysis of primary metabolites by GC/MS

The dried samples from the polar phase (200 μL aliquots from the aqueous lower phase) were derivatized using methoxyamine hydrochloride (in pyridine) and *N*-trimethylsilyl-*N*-methyl trifluoroacetamide (MSTFA) [21]. One μL of the derivatized sample was injected onto the GC column (DB-35MS, 30 m × 0.32 mm internal diameter, 0.25 μm film thickness, Agilent) in a splitless mode. The GC/MS system consisted of GC-TOF-MS (Agilent 6890 gas chromatograph coupled to Leco Pegasus 2 mass spectrometer). The injector temperature was set at 230 °C. The GC was operated at a constant flow rate of 2 mL/min carrier gas (helium). The analysis was performed using the following run temperature program: isothermal heating at 70 °C for 5 min, followed by a 5 °C/min oven temperature ramp to 350 °C and a final heating at 350 °C for 5 min. The interface and ion source were set at 225 °C and 200 °C, respectively.

2.5. Analysis of secondary metabolites by LC/MS

The dried samples from the polar phase (400 μL aliquots from the aqueous lower phase) were re-suspended in 300 μL of methanol: water (50%) mixture. Two μL of the sample were injected and separated on an Acquity UPLC system (Waters, Machester, UK). Chromatographic separations were performed on a reversed phase C_{18} column (High Strength Silica (HSS) T3, 100 mm × 2.1 mm containing 1.7 μm diameter particles, Waters) at a column oven temperature of 30 °C. The flow rate was set to 400 μL/min and the auto-sampler was maintained at 10 °C. The mobile phases consisted of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The steps of the gradient profile were 99% A from 0 to 1 min, 99% A to 65% A from 1 to 14 min, 65% A to 30% A from 14 to 14.5 min, 30% A to 1% A from 14.5 to 15.5 min, 1% A from 15.5 to 17 and 99% A from 17 to 20 min. The samples were measured in positive and negative ionization mode. The mass spectra were acquired using high resolution mass spectrometer (Exactive, Thermo Scientific, Bremen, Germany) in both positive and negative ionization mode covering the mass range between 150 and 1500 *m*/*z*. The MS data were collected in the full scan as well as all-ion-fragmentation modes covering a mass range from 100 to 1500 *m*/*z* [21,22]. The resolution was set to 10.000, with 10 scans/sec, restricting the loading time to 100 ms. The capillary voltage was set to 3.5 kV with a sheath gas flow value of 60 and an auxiliary gas flow of 35 (values are in arbitrary units). The capillary temperature was set to 150 °C, whereas the drying gas in the heated electrospray source was set to 350 °C. The skimmer voltage was set to 25 V, whereas the tube lens was set to a value of 130 V.

2.6. Data analysis

The obtained data resulted from LC/MS analysis were subjected to automated spectra processing using Progenesis QI software (Nonlinear Dynamics, Newcastle upon Tyne, UK) for peak extraction, alignment and generation of a visual data matrix. GC/MS data were analyzed using the Target Search R package [23]. Briefly, GC-MS chromatograms

were exported to NetCDF file format by using Leco ChromaTOF. Baseline correction and smoothing were performed using the algorithms implemented in TargetSearch. A reference in-house developed library containing metabolite name, expected RI, selective abundant masses and RI deviation, that was generated based on the Glom Metabolome database, was used for cross-referencing of the mass spectra. For metabolites identification, selective masses for every metabolite are searched for in a given time window around the expected RI. The intensities of the selected masses are extracted and finally normalized. Only the molecular entities detected in at least 80% of the samples belonging to the same group were considered for further analysis. The obtained data were normalized by dry weight and internal standard. Corticosterone (50 μL of a 1 mg/mL stock solution in methanol was added for the preparation of 100 mL extraction solvent) and ampicillin (25 μL of a 1 mg/mL stock solution in methanol was added for the preparation of 100 ml extraction solvent) were used as internal standards for analysis of secondary metabolites. 1,2-diheptadecanoyl-sn-glycero-3-phosphocholine (50 μL of a 1 mg/mL stock solution in chloroform was added for the preparation of 100 mL extraction solvent) was added as internal standard for the analysis of lipids. ^{13}C sorbitol and ribitol (50 μL of a 1 mg/mL stock solution in water was added for the preparation of 100 mL extraction solvent) were added as internal standards for the analysis of primary metabolites. Data presentation and experimental details are provided in Supplemental Data Sets 1 and 2 (the full data sets are located at the following URLs rather than try to file each data set int the article PDF. The data set URLs are as follows: Supplemental Data Set 1 https://www.jfda-online.com/cgi/viewcontent.cgi?filename=1&article=3347&context=journal&type=additional Supplemental Data Set 2 https://www.jfda-online.com/cgi/viewcontent.cgi?filename=2&article=3347&context=journal&type=additional) following the suggested recommendations for reporting metabolite data [24]. The filtered and normalized data matrices were imported into the Metaboanalyst 3.0 [25] for multivariate statistical analysis including principal component analysis (PCA) and heat map generation. All data were expressed by means ± SD for three independent biological replicates. The statistical significance was tested using One Way ANOVA and Tukey post hoc test. Differences were considered to be significant when p-values were <0.05.

3. Results

3.1. Flaxseed extraction and metabolite analysis

The available Sakha flaxseed cultivars (Sakha 1, 2, 3, 4 and 6) have been collected for the comprehensive analysis from different localities in Egypt (See Materials and method section). All the collected samples have characteristic brown seed color, however, only the Sakha 6 cultivar has yellow seed color (Fig. 1A). We aimed at covering the primary and secondary metabolites as well as the oil contents among the flaxseed cultivars. Selection of the best variety for the phtyoestrogenic content of lignans has been extensively described in our recent study [17]. Lignans require special alkaline treatment to release them from the ester-linked macromolecular complexes [17]. The experimental procedure applied for the current approach was based on our comprehensive liquid-liquid extraction

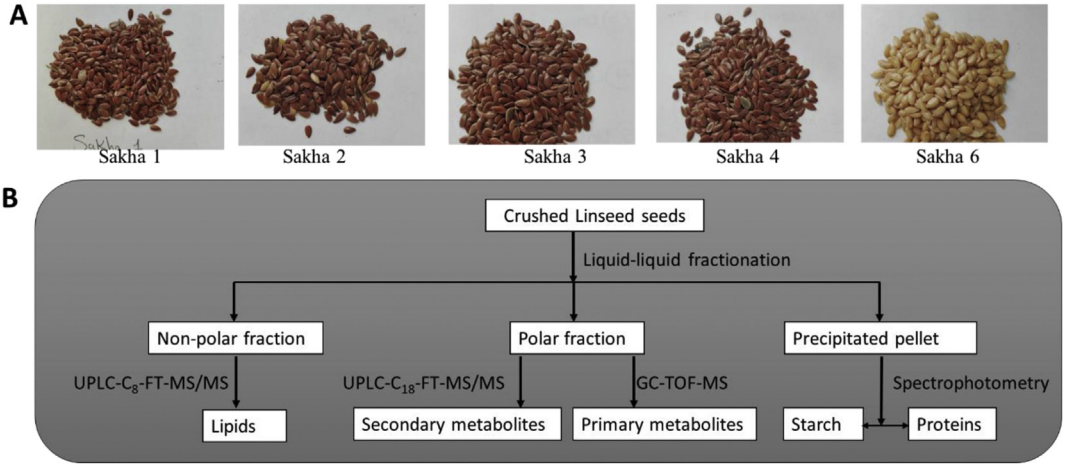

Fig. 1. Flaxseed Sakha cultivars and analytical approach used in metabolites analysis. A. Flaxseed Sakha cultivars available in Egypt for investigation of the metabolic content. B. Schematic representation of the extraction method and analytical platforms used for flaxseed metabolomics analysis.

method [21,22]. This comprehensive fractionation is based on distribution of metabolites between two immiscible phases, consisting of methyl-*tert*-butyl-ether/methanol and water/methanol [21,22] (Fig. 1B). Macromolecules such as proteins and starch can be analyzed from the precipitated pellets. The respective fractions from lipids and metabolites have been subjected to liquid or gas chromatography coupled to mass spectrometry. Lipids, which are enriched in the methyl-*tert*-butyl-ether/methanol non-polar phase, were subjected to ultra performance liquid chromatography-tandem mass spectrometry (UPLC-C8-FT-MS/MS) analysis, while, polar to semi-polar secondary metabolites, which are enriched in the water/methanol phase, were subjected to UPLC-C18-FT-MS/MS analysis. Primary metabolites such as sugars and amino acids were subjected to gas chromatography time-of-flight mass spectrometry (GC-TOF-MS) analysis after derivatization. The total protein content was measured spectrophotometrically from the precipitated pellet. After enzymatic hydrolysis and colorimetric measurement, starch was not detected in the collected varieties (Fig. 1B).

3.2. Identification of metabolites by MS-based analysis

Flaxseed lipids and polar metabolites were tentatively identified by using liquid chromatography coupled with mass spectrometry (LC/MS/MS) with both positive and negative ionization modes (Fig. S1). Identification of the metabolites was based on their retention times and accurate mass spectra derived in full scan (MS) as well as all-ion-fragmentation (MS/MS) modes [26–28]. For the first time, complete list of polar and non-polar metabolites of Flaxseed was provided. An exemplary description for the identification of compounds from selected lipid and polar metabolites is provided in the following paragraphs. The presented results were acquired from chromatograms and mass spectra of a pool sample that was prepared by aliquoting the same volume from the analyzed samples from all cultivars. The pool sample was subjected to LC/MS analysis every five analysis from the tested cultivars.

The extracted ion chromatograms (EIC) of a protonated compound from the polar fraction at *m/z* 424.18188 $[M+H]^+$ and a retention time (Rt) of 4.11 min was detected (Fig. 2A). The predicted molecular formula for this adduct was $C_{17}H_{30}NO_{11}$. Searching the molecular formula to common MS databases such as METLIN (http://metlin.scripps.edu), MassBank (www.massbank.jp), the Human Metabolome Database (http://www.hmdb.ca/), FooDB (https://foodb.ca/) and ChemSpider (http://www.chemspider.com/) showed that the formula could be assigned to a protonated neolinustatin. The MS spectra of the compound showed sodiated $[M+Na]^+$ adduct that were also detected at *m/z* 446.17290 (Fig. 2A). In negative ionization mode, a deprotonated adduct at *m/z* 422.16623 $[M-H]^-$ and a deprotonation followed by addition of formic acid $[M+FA-H]^-$ at *m/z* 468.17090 were detected at the same Rt (Fig. 2B). Additionally, highly intense peaks for fragment ions characteristic for neolinustatin were nicely co-eluted as their precursor adduct at the same Rt (Fig. 2A, B and S2).

Using the same identification protocol, another major cyanogenic glycoside was also identified from the polar fraction. The EIC of a protonated compound at *m/z* 410.16623 $[M+H]^+$ and a retention time (Rt) of 3.32 min was detected (Fig. 2C). The predicted molecular formula for this adduct was $C_{16}H_{28}NO_{11}$. In negative ionization mode, a deprotonated adduct at *m/z* 408.15058 $[M-H]^-$ and a deprotonation followed by addition of formic acid $[M+FA-H]^-$ at *m/z* 454.16 were detected at the same (Fig. 2D). Consistent with the fragmentation pattern, this compound was assigned to linustatin (Fig. S2).

The proper peak annotation of fatty acids from flaxseed lipid fraction was based on molecular formula annotation supported by using authentic fatty acid standards. For instance, in negative ionization mode, a deprotonated adduct at *m/z* 283.26456 $[M-H]^-$ was detected at Rt of 5.18 min (Fig. 3). The predicted molecular formula for this adduct was $C_{18}H_{35}O_2$. After searching the molecular formula to common MS databases, the formula could be assigned to a deprotonated stearic acid (a saturated fatty acid with an 18-carbon chain, FA 18:0). Three other peaks with retention time shift of $\approx$0.5 min were detected at 4.60, 4.06 and 3.56 min showing deprotonated peaks at *m/z* 281.24881, 279.23325 and 277.21753, respectively. The peaks were assigned to oleic acid (FA 18:1), linoleic acid (FA 18:2) and linolenic acid (FA 18:3), respectively (Fig. 3).

Another example is given for the identification of triacylglycerols (TAG). Here, different TAG species show a systematic RT shift according to the degree of unsaturation (Fig. 4A). High energy analysis of neutral losses allowed us to determine the acyl chain composition. For instance, the extracted ion chromatograms (EIC) of a protonated compound at *m/z* 873.69531 $[M+H]^+$ and a retention time (Rt) of 13.62 min was detected. The predicted molecular formula for this adduct was $C_{57}H_{93}O_6$. Searching the molecular formula to common MS databases

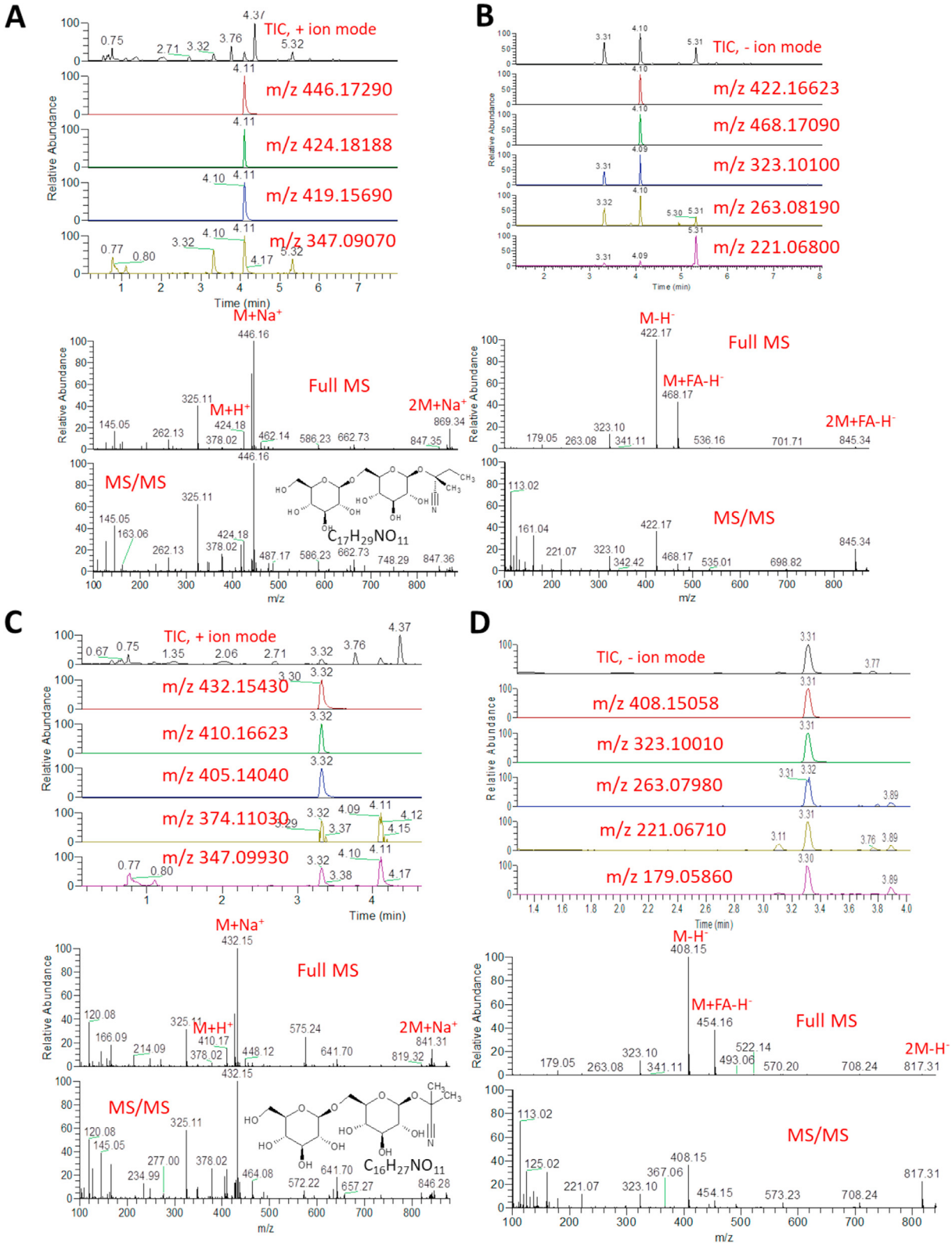

Fig. 2. Total ion chromatogram (TIC) and extracted ion chromatograms (EIC) of the peak at m/z representing neolinustatin (A and B) and linustatin (C and D) from Flaxseed measured by UPLC/MS of the polar fraction in positive (A and C) and negative (B and D) ionization mode. Full MS and MS/MS spectra are shown.

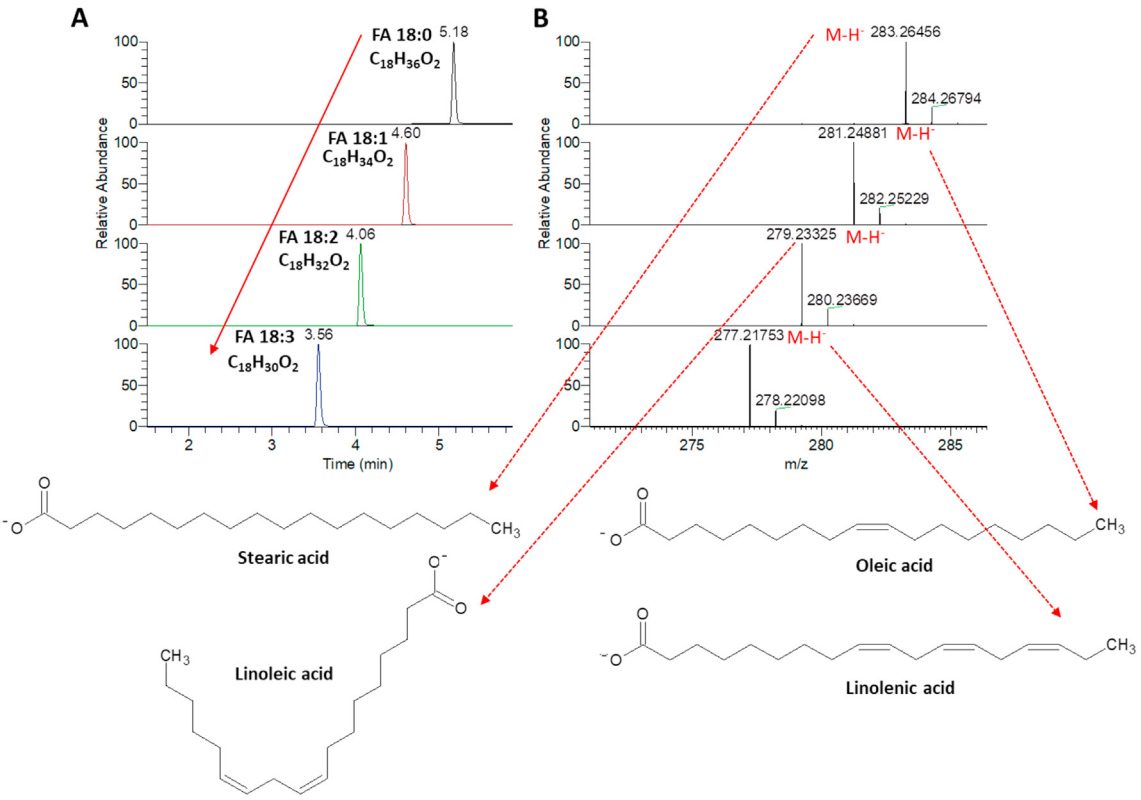

Fig. 3. Extracted ion chromatograms (EIC) (A) of the peak at m/z representing fatty acids from flaxseed non-polar fraction measured by UPLC/MS in negative ionization mode. Full MS spectra are shown (B). Chemical structures of the corresponding fatty acids are shown. For fatty acids 18: x, the first number denotes the sum of carbons in the acyl chains, while the second number denotes the sum of double bonds.

showed that the formula could be assigned to a protonated TAG 54:9 (the first number denotes the sum of carbons in the acyl chains, while the second number denotes the sum of double bonds)(Fig. 4B). The MS spectra of the compound showed a predominant ammoniated $[M+NH_4]^+$ adduct that were also detected at *m/z* 890.71973 (Fig. 4B and C). This ammonium adduct was fragmented resulting in the identification of a neutral loss of 295.251 ([M(FA 18:3)+NH3]) and the product ion was detected at *m/z* 595.472 (Fig. 4C). Other fragments were detected at *m/z* 335.25742, 317.24692 and 243.21070 which represented the acyl chain ($[RC = O +74]^+$), ($[RC = O +74]^+$ with loss of H_2O) and ($[RC = O]^+$ with loss of H_2O), respectively (Fig. 4D). Therefore, MS/MS fragmentation and co-elution pattern allowed us to confirm that the detected TAG has acyl chain composition containing 18:3/18:3/18:3.

3.3. Metabolite contents of brown and yellow flaxseed of the Sakha cultivars

In Egypt, the commercial local cultivars of the Sakha genotype have been released to be used for production of oil and fibers. Five Sakha cultivars have been subjected to comprehensive metabolite profiling. All the Sakha cultivars have characteristic dark brown seed color, and can not be differentiated by size or color, however, Sakha 6 cultivar has yellow seed color and was distinguishable. While PCA score plot shows the clusters of samples based on their similarity, the loading plot shows how strongly each metabolite influences the principal component. Analysis of primary metabolites from different brown and yellow flaxseed Sakha cultivars have been performed by GC-TOF-MS analysis (Table S1). Principal component analysis (PCA) of primary metabolites from all Sakha cultivars revealed the presence of two main clusters represents the analyzed cultivars (Fig. 5A). The first cluster represent the cultivars 1, 2, 3, 4, while, the second cluster contains Sakha 6. PCA loading plots and heat maps showed that several metabolites contributed to the discrimination of Sakha 6, the yellow cultivar, from other cultivars (Fig. 5B and C).

Several amino acids such as histidine, alanine, glycine, arginine, serine, methionine, valine,

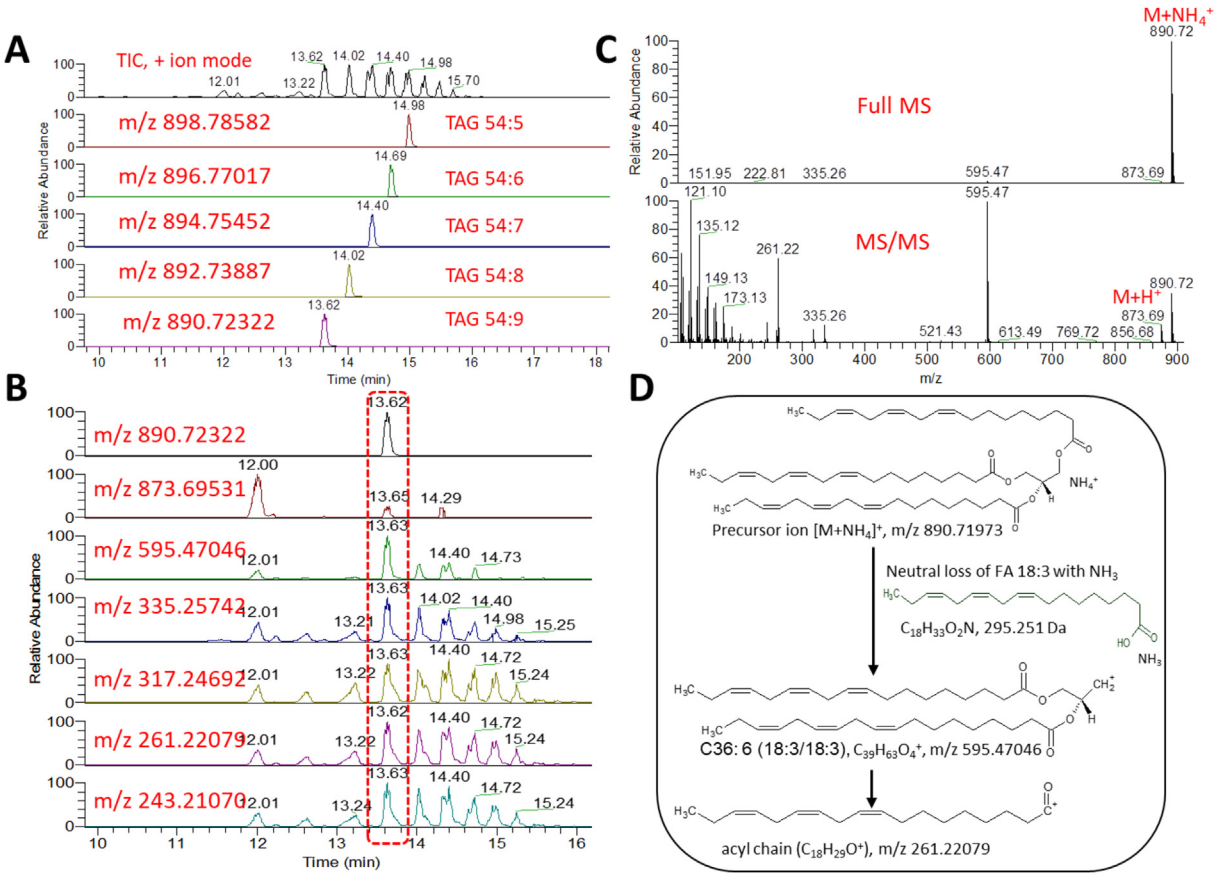

Fig. 4. Identification of triacylglycerols (TAG) from flaxseed oil measured by UPLC/MS in positive ionization mode. A. Total ion chromatogram (TIC) and extracted ion chrmotograms (EIC) of the peaks representing the ammoniated adduct of TAG 54:5–9. B. EIC of the peak at m/z representing TAG 54:9 (18:3/18:3/18:3). C. Full MS and MS/MS spectra of TAG 54:9. D. Determination of the acyl chain composition of TAG 54: 9 (18:3/18:3/18:3) after MS/MS fragementaion. For TAG 54:9, the first number denotes the sum of carbons in the acyl chains, while the second number denotes the sum of double bonds.

tyrosine, phenylalanine and glutamine have been significantly accumulated in Sakha 6 seeds. However, the total protein content was not significantly changed among the screened cultivars (Fig. S3). Additionally, the yellow Sakha seeds showed high level of free organic acids such as citric, glutaric, pyruvic, and fumaric acids. Several sugars such as glucose, fructose, rhamnose, xylose and raffinose showed low levels in the Sakha 6 cultivar compared to other seeds (Fig. 5C).

Analysis of lipids from different brown and yellow flaxseed Sakha cultivars have been performed by UPLC-FT-MS analysis (Table S2). PCA of lipids from all Sakha cultivars discriminated also Sakha 6 from other analyzed cultivars (Fig. 6A). Several phospholipid classes such phosphatidylglycerol (PG), phosphatidylcholine (PC) and phosphatidylethanolamine (PE) as well as fatty acids have been significantly accumulated in Sakha 6 seeds (Fig. 6B and C). The major forms of detected species of TAG were the 54:5 to 54:9 (The first number denotes the sum of carbons in the acyl chains, while the second number denotes the sum of double bonds) (Table 1). The major forms observed were LnOO, OLLn, LnLL, LnLnL and LnLnLn, for TAG 54:5, 54:6, 54:7, 54:8 and 54:9, respectively (O, L and Ln represents oleic, linoleic and linolenic acid, respectively). Additionally, the yellow Sakha seeds showed similar level of total triacylglycerol compared to other seeds (Fig. S4). From the identified fatty acids, linoleic acid (LA, FA 18:2) and α-Linolenic acid (ALA, FA 18:3) were significantly accumulated in Sakha 6 seeds (Fig. 4D).

For the secondary metabolites of different brown and yellow flaxseed Sakha cultivars (Table S3), PCA revealed the presence of two main clusters representing the analyzed cultivars (Fig. S5). The first cluster represents the cultivars 1, 2, 3, 4, while, the second cluster contains Sakha 6. Several metabolites

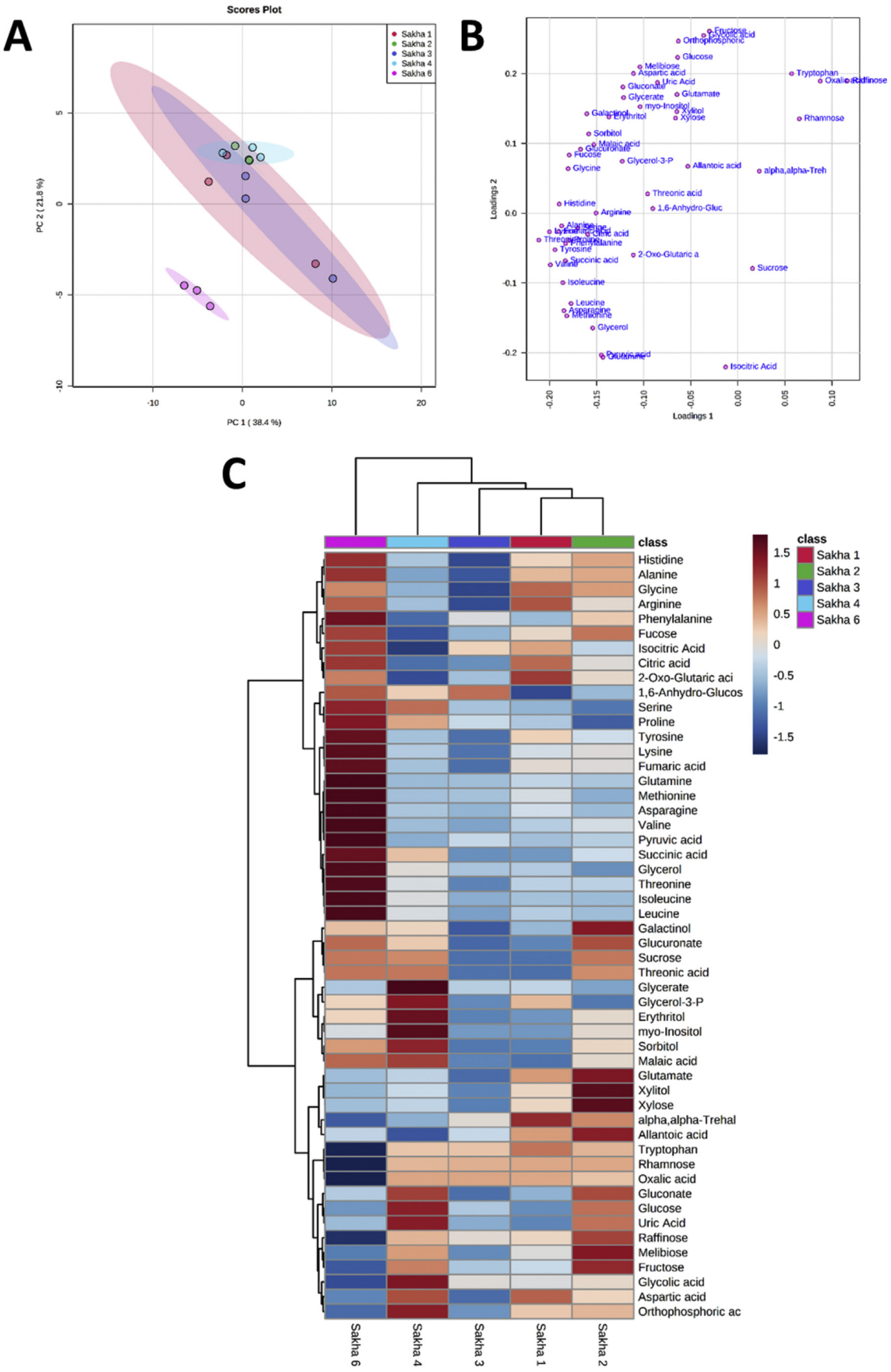

Fig. 5. Primary metabolites identified by GC/MS analysis from brown and yellow flaxseed Sakha cultivars. A. Principal component analysis (PCA) score plots of metabolites analyzed by GC/MS. B. PCA loading plots of metabolites contributing to separation of different cultivars. C. Heat map of the level of identified metabolites in different cultivars.

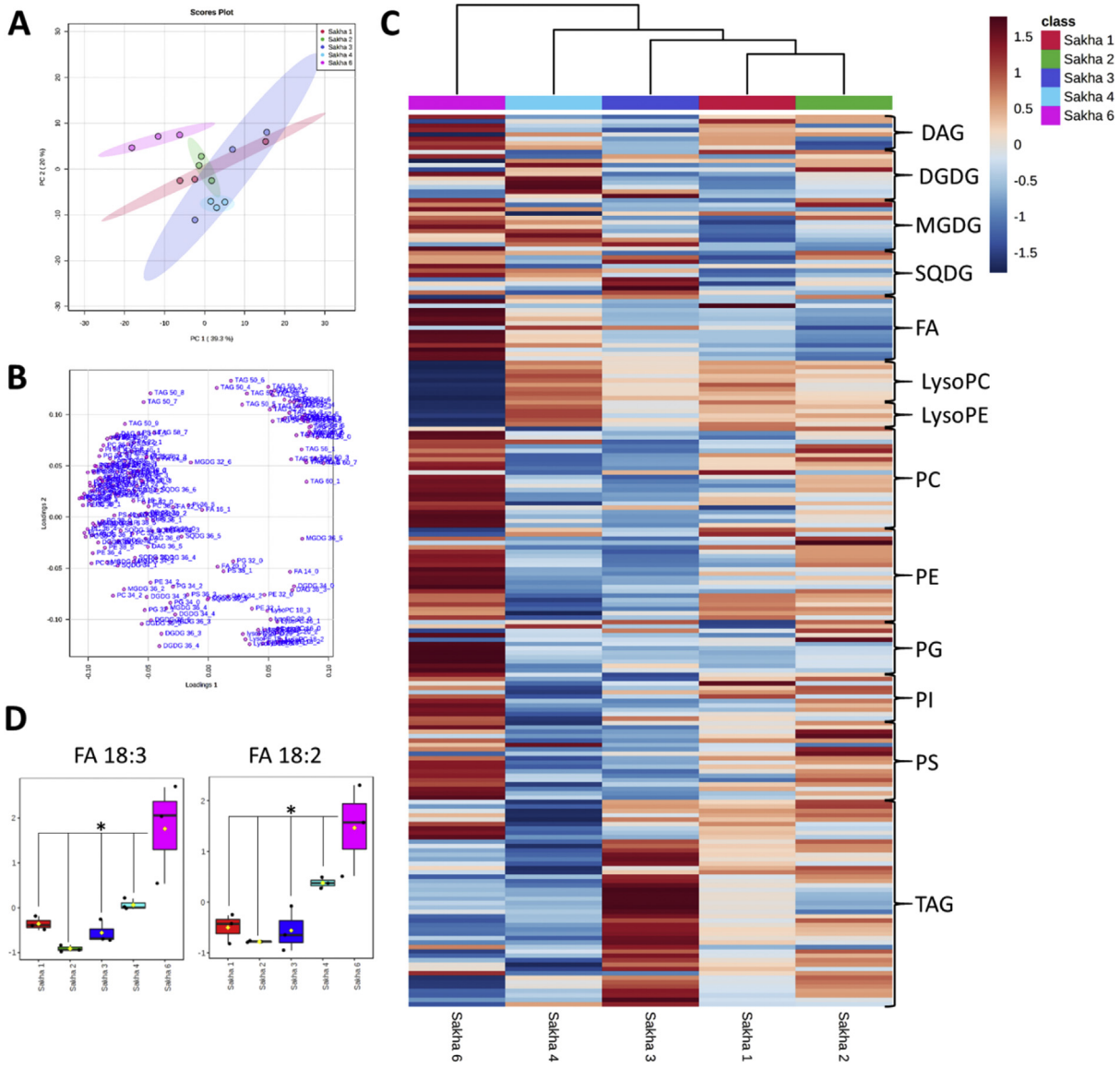

*Fig. 6. Lipids identified by LC/MS analysis from brown and yellow flaxseed Sakha cultivars. A. Principal component analysis (PCA) score plots of lipids analyzed by LC/MS. B. PCA loading plots of lipids contributing to separation of different cultivars. C. Heat map of the level of identified lipids in different cultivars. D. The level of the essential fatty acids (FA) in different linseed cultivars. The y-axis represents the log_2 values of metabolite abundance for three biological replicates. * indicates that the difference is significant at $p < 0.05$ by One Way ANOVA and Tukey post hoc test.*

such as riboflavin, pathothenic acid, glutathione, sinapic acid, cinnamic acid and benzoic acid have been significantly accumulated in Sakha 6 seeds. Linustatin and neolinuststin showed low levels in the Sakha 6 cultivar compared to other seeds (Fig. 7).

Table 1. The predominating triacylglycerols (TAG) identified from different flaxseed of Sakha cultivars.

Name	Formula	ECN	Detected Form	monoisotopic mass	RT (min)	M+H	Predominant adduct	Mass Error (ppm)	Difference between cultivars
TAG 54:5	$C_{57}H_{100}O_6$	44	LnOO	880.75	14.98	881.76	M+NH4	−7.43	Non-significant
TAG 54:6	$C_{57}H_{98}O_6$	42	OLLn	878.74	14.69	879.74	M+NH4	−2.47	Non-significant
TAG 54:7	$C_{57}H_{96}O_6$	40	LnLL	876.72	14.4	877.73	M+NH4	−0.04	Non-significant
TAG 54:8	$C_{57}H_{94}O_6$	38	LnLnL	874.71	14.02	875.71	M+NH4	−1.35	Non-significant
TAG 54:9	$C_{57}H_{92}O_6$	36	LnLnLn	872.69	13.62	873.7	M+NH4	−2.76	Non-significant

ECN, equivalent carbon number, which is defined as the total number of carbons in acyl chains minus two times the number of double bonds. Fatty acids abbreviation: L, linoleic acid; Ln, linolenic acid; O, oleic acid. The order of fatty acyls in the detected forms is arbitrary. The same TAG species were detected from all cultivars.

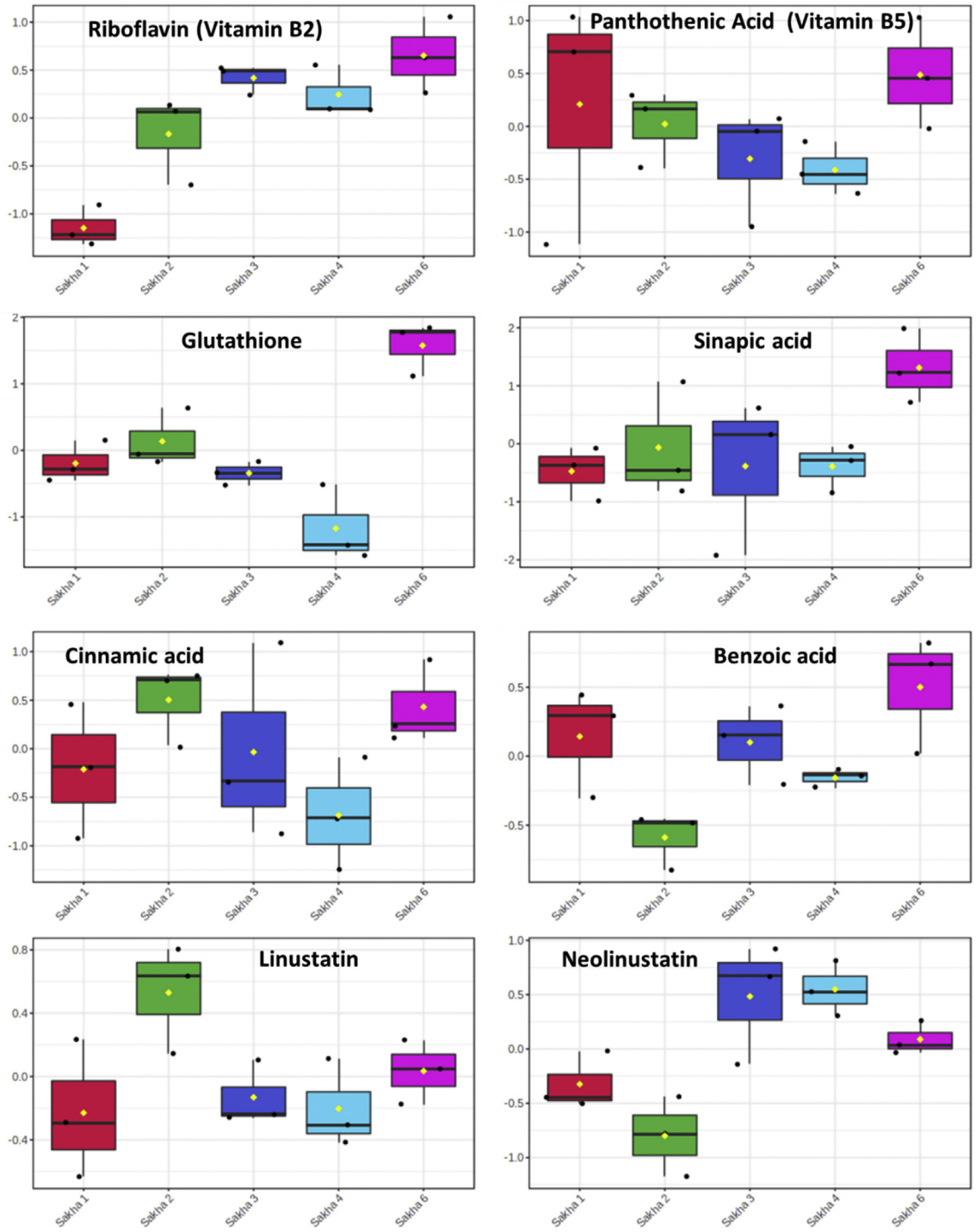

Fig. 7. The levels of secondary metabolites identified by LC/MS analysis from brown and yellow flaxseed Sakha cultivars. The y-axis represents the log_2 values of metabolite abundance.

4. Discussion

Flax (*Linum usitatissimum* L. family Linaceae) is a globally important crop that has been cultivated since ancient times for whole seed as well as its oil production [2]. The seeds of flax, which have 4–6 mm size, are flat and oval in shape with prominent pointed tip, and have a light golden yellow to deep brown or reddish brown color [29]. The color of flaxseed is determined by the pigment

content and tannins, in the pigment cells of the seed coat. The brown flaxseeds contain more pigments compared to yellow-seeded flax [30]. Flaxseeds are consumed for its nutritional qualities. Several health-promoting compounds have been found in flaxseeds such as lignans, proteins, mucilages, cyclic peptides, dietary fibers as well as polysaccharides, glycosides, vitamins and minerals [31]. Additionally, the oil has many industrial applications such as linoleum, stains and painting industries [32].

Most flaxseed available varieties cannot be differentiated by size or shape; however, some varieties can be distinguished by its yellow seed color. Therefore, it is necessary to precisely investigate the metabolic content in flaxseeds for better selection of high quality cultivar. In spite of the nutritional and medicinal importance of flaxseed, there are few studies that have been directed toward the comprehensive understanding of flaxseed metabolome [33–35]. Herein, we optimized the extraction and analysis protocol of flaxseed metabolites, taking into account its high oil and mucilage contents, which can lead to a cloudy unclear extract. The cultivars that are available have similar seed size and shape, while, only one cultivar (Sakha 6) has yellow colored seed coat. Among the studied Egyptian Sakha cultivars, the high content of nutritional and health-promoting metabolites discriminates the yellow Sakha 6 cultivar.

Oil represents about 45% of flaxseed composition. Flaxseed is a good source of PUFAs, representing about 73% of the flax fatty acid composition, of which ω-3-α-linolenic acid (ALA) constitutes about half of fatty acid composition [36]. Most of the oil is stored as triacylglycerols (TAG) in the oil bodies located in the embryo [37]. Phospholipids and the oleosin protein constitute the outer membrane of the oil bodies [38]. Interestingly, the major detected TAG species in the Sakha cultivars were in agreement with the predominating flaxseed TAG reported in the literature [39,40]. In our study, large proportion of the flaxseed oil contains TAG rich in the ALA, in agreement with reported data [39,40]. Previous studies have shown that the mature flaxseeds from different cultivars have different TAG levels during maturation; however, the mature seeds accumulated the same amount of TAG at complete maturity [39]. In agreement with these reports, the detected TAG species accumulated at similar levels in the mature Sakha seeds from all cultivars.

Interestingly, the Sakha 6 cultivar accumulated the highest levels of ω-6-LA and ω-3-ALA, the only two fatty acids that are known to be essential for humans and must obtain them through their diet. ALA is converted to the long-chain ω-3 fatty acids; eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), important structural components in cell membrane phospholipid bilayer. In addition of being important as structural components and energy source, ω-6 and ω-3 fatty acids exert anti-inflammatory, anti-hyperlipidemic, atherosclorosis-protecting activities as well as reducing the risk of cancer and cardiovascular diseases [41,42]. However, the high content of ω-3 fatty acids makes flax oil readily oxidized, and thus affecting its shelf life stability [43].

Phospholipids from PG, PC and PE classes significantly accumulated in Sakha 6 seeds. Phospholipids contribute to the quality and stability of the flaxseed oils through their antioxidant activities [5]. Previous studies also showed that antioxidant compounds greatly increase flaxseed oil stability [43]. Riboflavin [44], pathothenic acid, glutathione [45], sinapic acid [46], cinnamic acid [47] and benzoic acid [48] have been reported as antioxidant metabolites. These metabolites, along with phospholipids, contribute to the stabilization of the oil of Sakha 6, which have high ALA and similar TAG.

Flaxseed contains also high levels of phytotoxic undesirable antinutritional cyanogenic glycosides [49]. Epidemiological studies have shown that the consumption of these compounds can lead to poorly bioavailable essential nutrients [31]. Cyanogenic glycosides are glycosides of 2-hydroxynitriles that are widely distributed in more than 2500 plant species. Cyanogenic glycosides play crucial roles in plant-insect interaction [50]. After human intake, cyanogenic glycosides are degraded by intestinal β–glycosidase to yield the respiratory inhibitor, hydrogen cyanide, which in turn is converted to thiocyanates. Long term exposure to thiocyanates causes iodine-deficiency disorders such as goiter and cretinism [3]. Previous studies have shown that cyanogenic monoglucosides such as linamarin and its methylated relative lotaustralin as well as diglucosides such as linustatin and neolinustatina accumulate in young developing flaxseeds soon after anthesis [51,52]. Upon maturation, only the diglucosides accumulate in the mature seeds [51,52]. In the mature Sakha cultivar seeds, we detected both linustatin and neolinustatin. The low levels detected in the Sakha 6 compared to other Sakha cultivars

will encourage its use for human consumption and animal feed.

Brown and golden flaxseeds collected from Brazil showed non-significant differences in the total protein or lipid contents, however, the authors did not investigate the total chemical profile [53]. Yellow flaxseeds accumulated higher ω-3 and −6, consistent with literature data [54]. Previous studies showed also that the amino acid content did not change significantly between yellow and brown flaxseeds; however, there was a tendency of higher accumulation of this compound class in the yellow-coated seeds [32]. In our study, the Egyptian yellow cultivar accumulated several fold higher concentrations of all essential amino acids. Next to genetic variations, the cultivation methods and geographical aspects contribute also to amino acid content. In agreement to other studies, no differences between the studied flax cultivars were noticed regarding their total protein content.

Our optimized protocol can be regarded as a suitable tool to characterize new cultivars, and thus speed up selection within the breeding processes. Currently, the conventional breeding processes use molecular markers or single nucleotide polymorphisms (SNPs) to monitor the desired effects in the progeny and subsequent generations [33]. Marker metabolites are closely related to phenotypes and do not require priori knowledge of genetic information or plant species. Flaxseeds have been extensively used in the last years as nutraceuticals or in food industry; therefore, it is necessary to select the quality of the seeds based not only on oil characteristics, but also to have a snapshot of the nutritional and antinutritional metabolites. Flaxseeds are gluten free [55] and can be desirable in both baking and cooking for people having celiac disease or gluten-sensitive enteropathy to partially replace wheat, barely or rye.

The objective of our study was the optimization of a comprehensive metabolomics approach for better selection of high-quality cultivar of flaxseed. The principle for selection was mainly based on their nutritional values together with the presence of the lowest amount of the antinutritional principles which are represented by the cyanophoric glycosides. Sakha 6 showed the highest levels of ω-6-LA and ω-3-ALA, moreover Phospholipids from PG, PC and PE classes significantly accumulated in Sakha 6 seeds. On the other hand, both linustatin and neolinustatin, the methylated diglucoside cyanophoric glycosides were present in Sakha 6 at a concentration lower than other Sakha cultivars. Accordingly, Sakha 6 was proved to be the most preferable cultivar for human consumption.

5. Conclusion

To the best of our knowledge, this is the first complete report with detailed screening of the metabolic content of Egyptian Sakha linseed cultivars. This study allowed the application of a comprehensive metabolomics approach for metabolites analysis. Multivariate analysis using PCA led to satisfactory discrimination of the yellow Sakha 6 cultivar from other cultivars. The yellow seeded cultivar was characterized by a high content of nutritional and medicinally important metabolites. A low level of the antinutritional cyanogenic glycosides was notable in this cultivar. These results contribute to the estimation of nutritionally rich flaxseed quality that is important for food processing. Additionally, the provided data will allow the establishment guidelines for the selection of better quality of new cultivars. Flaxseeds have been used as dietary supplement and as a feed source; therefore, the quality state can be easily assessed using the introduced data. Lastly, flaxseeds can be subjected to varying growth conditions including fertilizers, biotic or abiotic stresses for optimum production of bio-active metabolites.

Conflict of interest

The authors have no conflict to declare.

Appendix A. Supplementary material

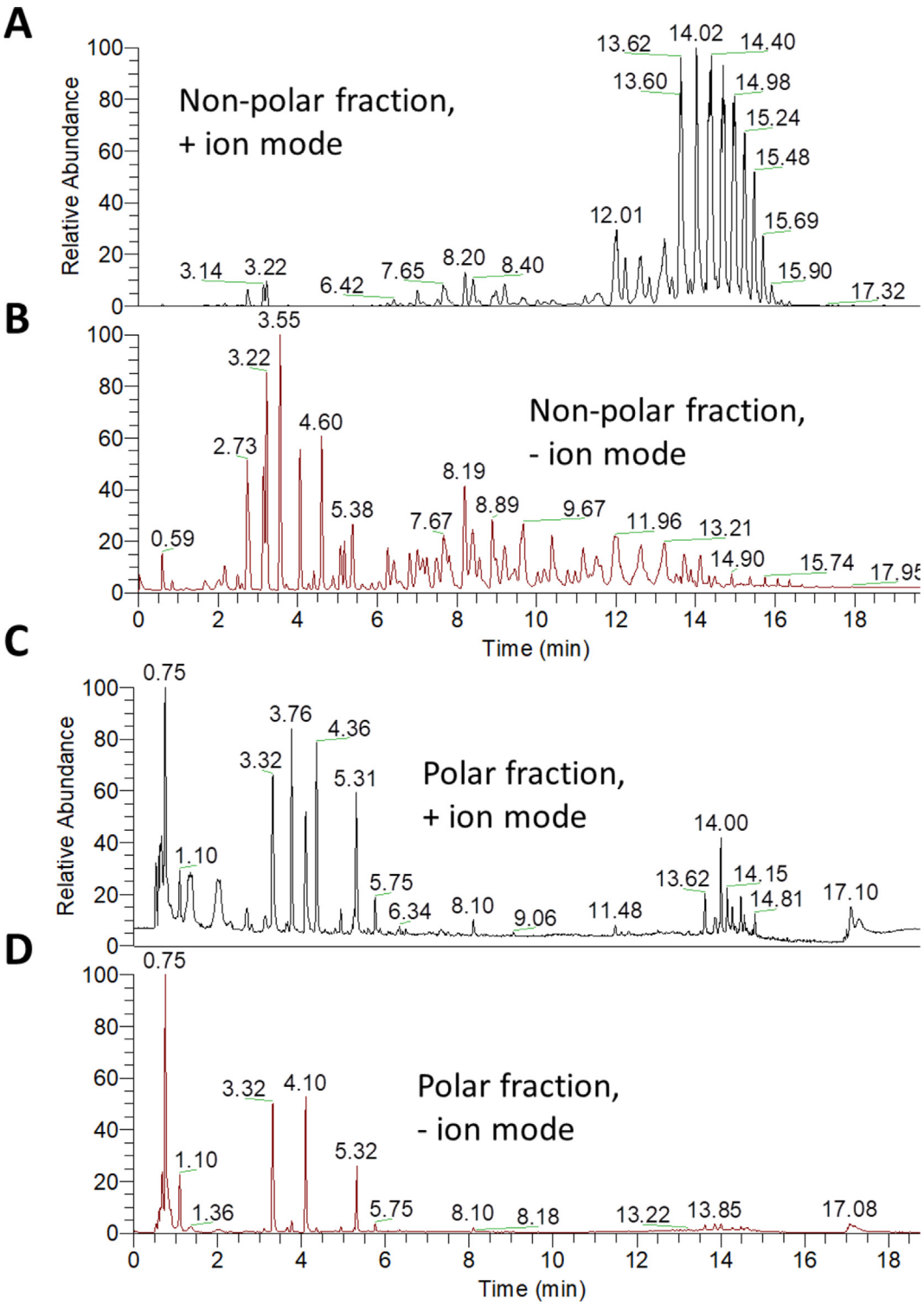

Fig. S1. Total ion chromatogram (TIC) of non-polar (A and B) and polar fractions (C and D) of Flaxseed analyzed in positive and negative ionization modes.

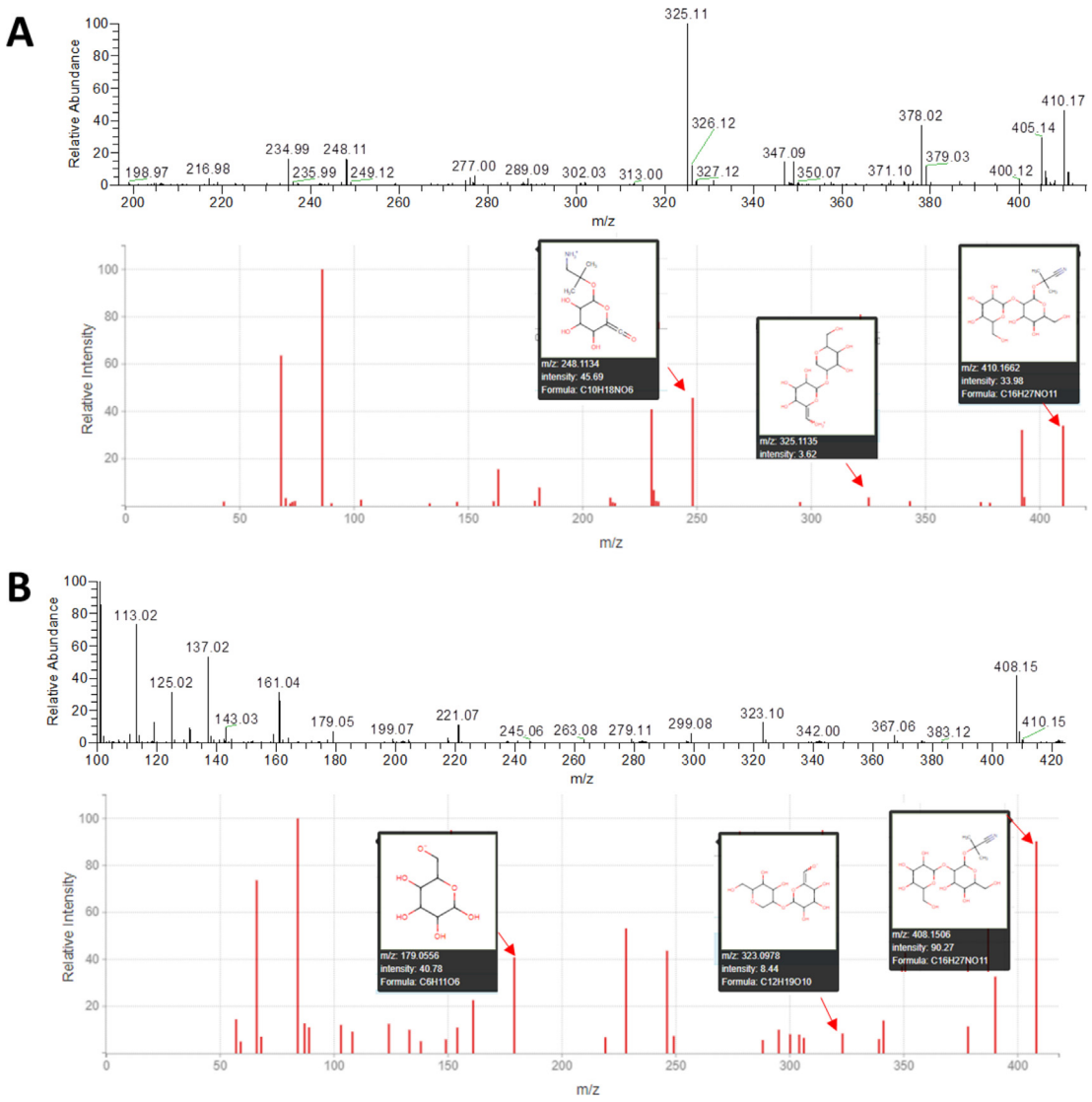

Fig. S2. Identification of linustatin from flaxseed polar extract measured in positive (A) and negative (B) ionization modes based on comparison to FooDB database. The upper panel represents MS spectrum obtained from our analysis; the lower panel represents the data in FooDB database online (https://foodb.ca/)(Allen et al., 2015).

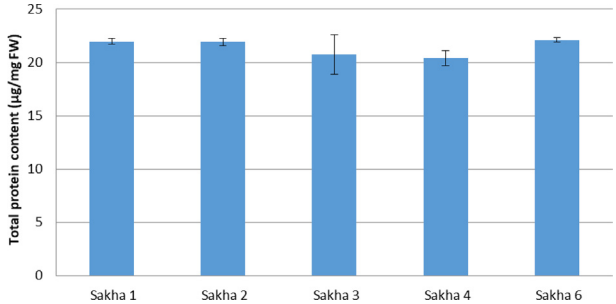

Fig. S3. Total protein level in brown and yellow flaxeed Sakha cultivars.

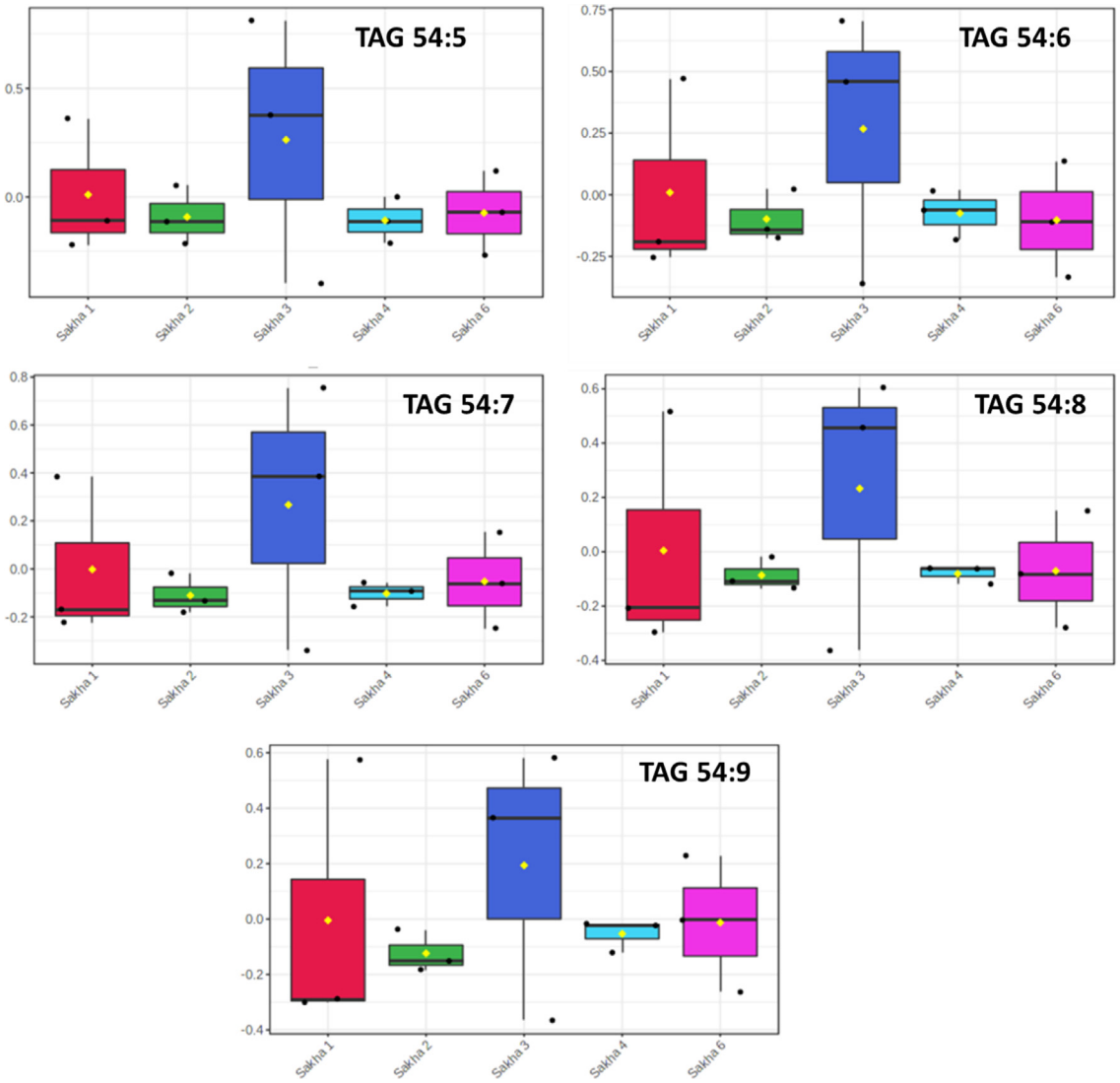

Fig. S4. The levels of major triacylglycerols (TAG) in different flaxseed cultivars showed no significant difference. The y-axis represents the $\log_2$ values of metabolite abundance.

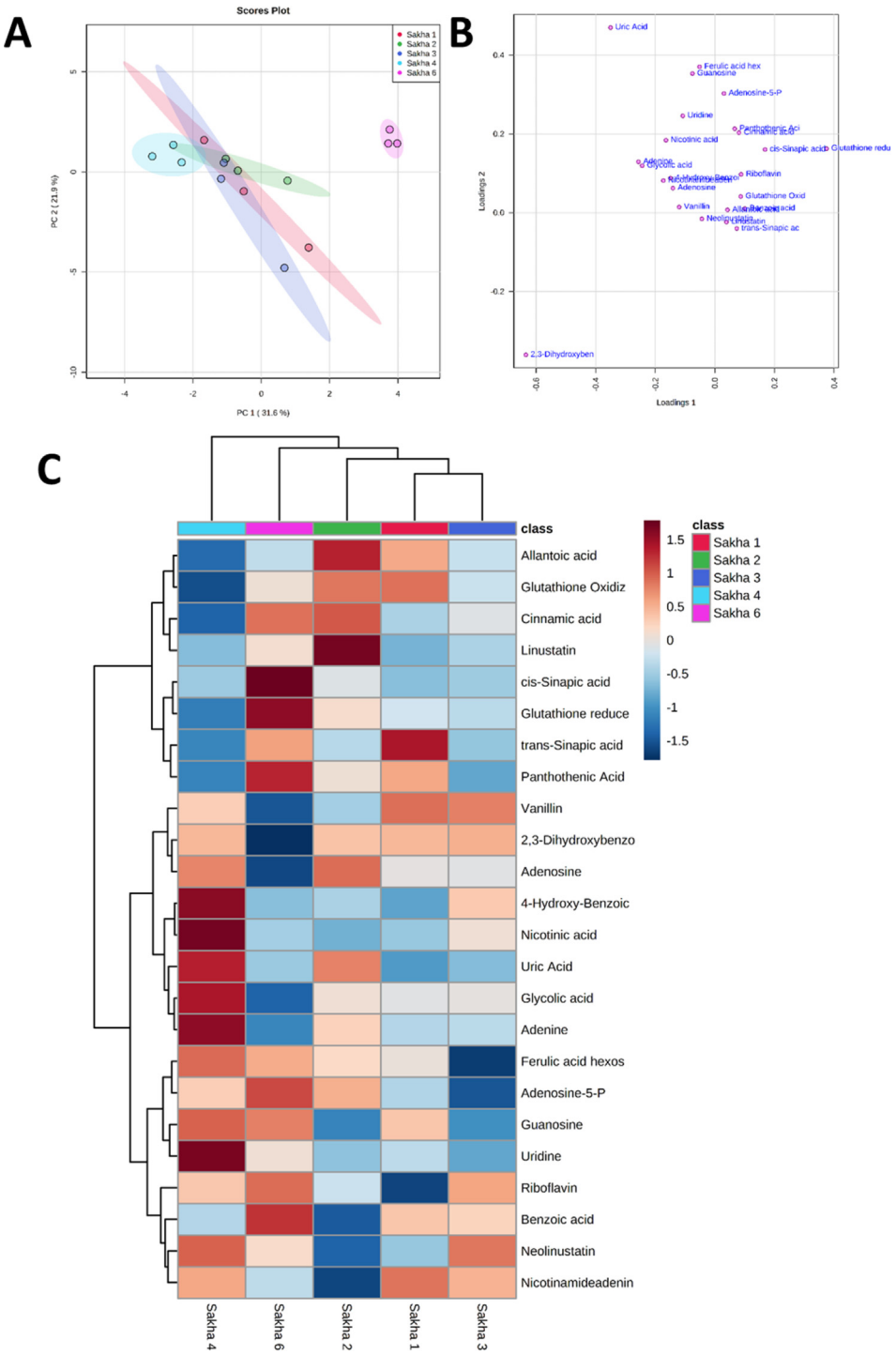

Fig. S5. Secondary metabolites identified by LC/MS analysis from brown and yellow flaxseed Sakha cultivars. A. Principal component analysis (PCA) score plots of metabolites analyzed by LC/MS. B. PCA loading plots of metabolites contributing to separation of different cultivars. C. Heat map of the level of identified metabolites in different cultivars.

Table S1. Analysis of primary metabolites from different brown and yellow flaxseed Sakha cultivars by GC-TOF-MS.

Sample name	Sakha 1_1	Sakha 1_2	Sakha 1_3	Sakha 2_1	Sakha 2_2	Sakha 2_3	Sakha 3_1	Sakha 3_2	Sakha 3_3	Sakha 4_1	Sakha 4_2	Sakha 4_3	Sakha 6_1	Sakha 6_2	Sakha 6_3
Threonine	1.37E+04	1.87E+04	8.18E+03	1.22E+04	1.26E+04	1.36E+04	1.59E+04	6.38E+03	1.26E+04	1.19E+04	1.78E+04	1.19E+04	3.28E+04	2.01E+04	1.68E+04
Isoleucine	1.29E+04	1.59E+04	9.95E+03	1.04E+04	1.54E+04	1.19E+04	1.94E+04	7.43E+03	1.19E+04	1.21E+04	2.26E+04	1.13E+04	3.17E+04	2.91E+04	2.06E+04
Leucine	9.57E+03	1.27E+04	7.93E+03	8.78E+03	1.17E+04	7.83E+03	1.11E+04	5.73E+03	9.51E+03	8.80E+03	1.75E+04	8.67E+03	1.93E+04	3.11E+04	2.12E+04
Lysine	1.67E+04	1.68E+04	2.21E+03	6.91E+03	1.54E+04	7.00E+03	1.09E+04	2.81E+03	4.81E+03	6.11E+03	1.04E+04	6.77E+03	4.00E+04	1.59E+04	1.57E+04
Serine	2.34E+04	2.83E+04	1.39E+04	1.99E+04	1.39E+04	2.11E+04	2.06E+04	1.25E+04	4.12E+04	2.93E+04	4.38E+04	3.22E+04	4.18E+04	4.54E+04	3.60E+04
Phenylalanine	2.11E+04	2.52E+04	1.14E+04	2.36E+04	1.94E+04	2.41E+04	3.50E+04	1.20E+04	2.04E+04	1.21E+04	2.15E+04	1.48E+04	3.66E+04	2.91E+04	2.62E+04
Proline	1.53E+05	2.74E+05	8.21E+04	1.12E+05	9.11E+04	1.14E+05	2.10E+05	6.75E+04	3.03E+05	1.72E+05	2.76E+05	2.33E+05	5.27E+05	2.65E+05	2.60E+05
Tyrosine	4.36E+04	1.03E+05	1.94E+04	3.95E+04	3.84E+04	3.44E+04	5.57E+04	1.63E+04	2.02E+04	2.75E+04	4.84E+04	2.83E+04	9.18E+04	8.07E+04	5.72E+04
Methionine	3.05E+03	4.02E+03	1.61E+03	1.46E+03	2.93E+03	1.76E+03	4.14E+03	1.17E+03	2.08E+03	1.58E+03	3.05E+03	2.03E+03	1.07E+04	8.61E+03	6.70E+03
Histidine	5.89E+04	3.66E+04	9.00E+03	4.18E+04	2.33E+04	3.17E+04	1.80E+04	8.54E+03	1.29E+04	1.91E+04	2.28E+04	1.88E+04	5.08E+04	4.82E+04	3.49E+04
Glutamate	4.07E+04	4.44E+04	3.64E+04	4.74E+04	4.44E+04	4.84E+04	3.21E+04	2.61E+04	3.27E+04	3.50E+04	3.41E+04	3.52E+04	3.46E+04	3.37E+04	3.22E+04
Glutamine	1.60E+04	1.52E+04	1.27E+04	1.31E+04	1.44E+04	1.30E+04	1.27E+04	1.18E+04	1.48E+04	1.33E+04	1.24E+04	1.26E+04	3.28E+04	4.90E+04	3.99E+04
Valine	3.23E+04	5.21E+04	2.00E+04	2.99E+04	3.76E+04	3.84E+04	5.10E+04	1.60E+04	2.80E+04	2.72E+04	4.12E+04	2.54E+04	1.08E+05	6.12E+04	4.87E+04
Alanine	1.99E+05	2.76E+05	1.04E+05	1.54E+05	2.27E+05	1.82E+05	1.11E+05	7.19E+04	1.28E+05	1.30E+05	1.26E+05	1.16E+05	2.63E+05	2.49E+05	2.01E+05
Asparagine	6.78E+04	2.95E+05	3.90E+04	6.83E+04	5.47E+04	8.39E+04	1.13E+05	3.60E+04	6.64E+04	8.65E+04	1.18E+05	3.96E+04	6.80E+05	3.30E+05	3.58E+05
Glycine	8.75E+04	8.99E+04	3.79E+04	6.19E+04	5.42E+04	6.88E+04	3.84E+04	2.33E+04	5.08E+04	3.33E+04	6.12E+04	4.39E+04	7.57E+04	6.63E+04	5.06E+04
Arginine	8.46E+03	5.81E+03	1.05E+03	2.07E+03	5.03E+03	8.72E+02	2.00E+03	4.93E+02	5.75E+02	1.03E+03	2.36E+03	1.36E+03	6.62E+03	2.39E+03	2.89E+03
Tryptophan	1.50E+05	1.26E+05	1.02E+05	1.42E+05	1.03E+05	8.78E+04	1.84E+05	7.13E+04	8.88E+04	1.28E+05	8.89E+04	1.00E+05	3.83E+04	7.99E+04	4.34E+04
Aspartic acid	5.44E+05	4.34E+05	2.84E+05	3.44E+05	3.93E+05	3.62E+05	3.71E+05	2.03E+05	3.68E+05	3.59E+05	5.02E+05	3.90E+05	3.77E+05	2.44E+05	3.28E+05
Galactinol	6.47E+05	5.50E+05	3.18E+05	6.20E+05	7.11E+05	7.59E+05	5.51E+05	3.13E+05	4.43E+05	5.32E+05	5.54E+05	5.80E+05	6.85E+05	5.28E+05	5.17E+05
Gluconate	4.20E+04	4.70E+04	1.90E+04	4.18E+04	3.67E+04	4.66E+04	3.18E+04	2.49E+04	3.92E+04	4.23E+04	3.91E+04	4.44E+04	2.69E+04	4.54E+04	3.36E+04
Glucose	5.25E+04	4.13E+04	3.97E+04	7.23E+04	6.16E+04	6.63E+04	5.98E+04	2.45E+04	8.23E+04	7.33E+04	7.59E+04	7.77E+04	4.35E+04	4.80E+04	4.28E+04
1,6-Anhydro-Glucose	7.82E+03	6.34E+03	7.58E+03	7.14E+03	8.43E+03	8.06E+03	1.07E+04	5.99E+03	1.13E+04	7.42E+03	8.83E+03	9.31E+03	9.18E+03	8.99E+03	9.08E+03
Glucuronate	1.67E+03	2.37E+03	9.79E+02	1.81E+03	1.71E+03	2.21E+03	1.52E+03	1.31E+03	1.81E+03	1.46E+03	2.01E+03	1.86E+03	1.64E+03	2.13E+03	1.88E+03
Xylitol	1.49E+04	1.04E+04	1.18E+04	1.44E+04	2.25E+04	1.40E+04	1.47E+04	6.31E+03	1.02E+04	9.82E+03	1.55E+04	9.52E+03	1.11E+04	1.02E+04	1.02E+04
Xylose	4.92E+04	3.37E+04	3.92E+04	4.67E+04	7.35E+04	4.49E+04	4.69E+04	2.12E+04	3.39E+04	3.21E+04	5.03E+04	3.09E+04	3.69E+04	3.52E+04	3.39E+04
Sucrose	0.00E+00	1.30E+06	1.39E+06	1.22E+06	1.34E+06	1.19E+06	1.21E+06	1.36E+06	0.00E+00	9.81E+05	1.09E+06	1.05E+06	1.21E+06	1.26E+06	1.24E+06
Glycerate	6.14E+03	7.48E+03	3.37E+03	5.25E+03	4.77E+03	5.07E+03	6.24E+03	3.32E+03	7.23E+03	8.34E+03	6.92E+03	6.70E+03	4.88E+03	6.07E+03	4.94E+03
Glycerol	1.45E+05	1.51E+05	8.09E+04	8.35E+04	8.67E+04	9.27E+04	1.39E+05	7.94E+04	1.39E+05	1.72E+05	1.42E+05	1.53E+05	3.10E+05	6.62E+05	5.55E+05
Glycerol-3-P	2.12E+04	5.57E+04	1.47E+04	2.53E+04	1.57E+04	1.69E+04	2.23E+04	1.52E+04	2.15E+04	3.13E+04	3.45E+04	3.22E+04	3.23E+04	2.17E+04	2.16E+04
Rhamnose	1.05E+04	9.62E+03	4.14E+03	7.26E+03	7.28E+03	7.91E+03	7.99E+03	4.67E+03	9.39E+03	6.18E+03	8.24E+03	6.27E+03	0.00E+00	6.30E+03	6.33E+03
alpha,alpha-Trehalose	2.17E+03	0.00E+00	4.82E+02	0.00E+00	8.64E+02	5.60E+02	4.08E+02	0.00E+00	4.94E+02	1.34E+02	0.00E+00	6.30E+02	0.00E+00	0.00E+00	1.86E+03

(continued on next page)

Table S1. (continued)

Sample name	Sakha 1_1	Sakha 1_2	Sakha 1_3	Sakha 2_1	Sakha 2_2	Sakha 2_3	Sakha 3_1	Sakha 3_2	Sakha 3_3	Sakha 4_1	Sakha 4_2	Sakha 4_3	Sakha 6_1	Sakha 6_2	Sakha 6_3
Sorbitol	6.12E+04	5.13E+04	2.64E+04	5.13E+04	6.69E+04	6.26E+04	4.25E+04	2.76E+04	6.75E+04	5.65E+04	1.12E+05	9.30E+04	8.29E+04	5.74E+04	6.55E+04
Melibiose	9.06E+03	8.40E+03	5.03E+03	7.85E+03	7.29E+03	1.11E+04	6.55E+03	5.38E+03	8.07E+03	6.21E+03	8.45E+03	9.02E+03	7.74E+03	6.59E+03	5.38E+03
Erythritol	1.29E+04	1.09E+04	5.08E+03	7.45E+03	8.63E+03	1.31E+04	7.94E+03	6.37E+03	1.38E+04	1.21E+04	1.02E+04	8.97E+03	1.12E+04	8.87E+03	8.69E+03
Fructose	1.55E+05	1.52E+05	1.01E+05	1.87E+05	2.04E+05	2.08E+05	1.59E+05	8.34E+04	1.55E+05	1.72E+05	1.68E+05	1.80E+05	7.99E+04	1.46E+05	8.71E+04
Fucose	3.70E+04	4.33E+04	1.91E+04	3.38E+04	2.69E+04	3.88E+04	3.40E+04	2.11E+04	3.67E+04	2.80E+04	3.15E+04	2.55E+04	3.51E+04	3.24E+04	3.31E+04
Raffinose	2.15E+07	1.79E+07	2.05E+07	1.99E+07	2.49E+07	2.15E+07	2.05E+07	2.06E+07	1.79E+07	2.03E+07	2.04E+07	2.08E+07	1.68E+07	1.59E+07	1.64E+07
Threonic acid	0.00E+00	1.46E+04	6.07E+03	1.00E+04	9.67E+03	8.60E+03	7.96E+03	0.00E+00	1.22E+04	9.35E+03	1.26E+04	1.04E+04	1.10E+04	8.88E+03	1.33E+04
myo-Inositol	1.98E+05	1.46E+05	4.53E+04	9.82E+04	1.67E+05	1.52E+05	8.71E+04	1.05E+05	1.44E+05	2.23E+05	1.97E+05	1.92E+05	1.34E+05	1.56E+05	1.07E+05
Orthophosphoric acid	5.60E+05	6.84E+05	4.10E+05	5.59E+05	5.56E+05	5.34E+05	4.30E+05	3.59E+05	5.71E+05	6.67E+05	7.01E+05	5.87E+05	4.72E+05	3.91E+05	4.01E+05
Oxalic acid	2.43E+03	1.80E+03	1.91E+03	1.62E+03	1.65E+03	1.51E+03	1.94E+03	1.81E+03	2.56E+03	1.69E+03	2.23E+03	2.38E+03	1.64E+03	0.00E+00	0.00E+00
Malaic acid	4.17E+04	3.60E+04	1.66E+04	4.92E+04	1.57E+04	6.04E+04	5.17E+04	1.25E+04	4.18E+04	3.37E+04	4.96E+04	4.94E+04	4.10E+04	3.92E+04	4.50E+04
Pyruvic acid	4.61E+02	4.46E+02	2.14E+02	4.27E+02	4.20E+02	4.00E+02	4.13E+02	3.43E+02	6.95E+02	4.33E+02	2.78E+02	2.17E+02	4.82E+03	4.41E+03	5.01E+03
Succinic acid	2.33E+03	2.53E+03	1.76E+03	2.40E+03	2.78E+03	3.05E+03	2.24E+03	1.38E+03	3.16E+03	3.07E+03	3.40E+03	3.63E+03	5.54E+03	5.04E+03	5.51E+03
Citric acid	1.20E+06	1.00E+06	7.51E+05	8.89E+05	8.43E+05	8.93E+05	7.86E+05	7.06E+05	8.99E+05	7.13E+05	8.68E+05	7.39E+05	9.34E+05	9.84E+05	1.09E+06
Fumaric acid	7.22E+03	7.37E+03	5.04E+03	5.72E+03	5.11E+03	8.94E+03	6.08E+03	4.04E+03	6.41E+03	5.30E+03	6.48E+03	6.14E+03	7.63E+03	9.04E+03	8.14E+03
Allantoic acid	6.66E+04	4.86E+04	2.31E+04	5.00E+04	7.30E+04	4.97E+04	3.64E+04	3.28E+04	2.54E+04	1.20E+04	3.09E+04	2.44E+04	4.05E+04	2.73E+04	2.61E+04
Glycolic acid	2.13E+03	1.78E+03	9.50E+02	1.66E+03	1.46E+03	1.65E+03	1.62E+03	1.02E+03	2.25E+03	2.65E+03	1.91E+03	2.10E+03	1.21E+03	1.01E+03	1.11E+03
2-Oxo-Glutaric acid	2.29E+03	1.72E+03	1.61E+03	1.44E+03	1.36E+03	1.44E+03	1.39E+03	8.86E+02	1.48E+03	8.51E+02	1.09E+03	9.61E+02	1.62E+03	1.63E+03	1.76E+03
Isocitric Acid	1.79E+04	1.82E+04	1.80E+04	1.72E+04	1.56E+04	1.63E+04	1.58E+04	1.93E+04	1.70E+04	1.44E+04	1.42E+04	1.34E+04	1.71E+04	1.93E+04	2.21E+04

Table S2. Analysis of lipids from different brown and yellow flaxseed Sakha cultivars by UPLC-FT-MS analysis.

Sample name	Sakha 1_1	Sakha 1_2	Sakha 1_3	Sakha 2_1	Sakha 2_2	Sakha 2_3	Sakha 3_1	Sakha 3_2	Sakha 3_3	Sakha 4_1	Sakha 4_2	Sakha 4_3	Sakha 6_1	Sakha 6_2	Sakha 6_3
DAG 34:1	1.27E+06	1.18E+06	1.11E+06	1.20E+06	1.32E+06	1.25E+06	9.82E+05	9.06E+05	8.06E+05	1.03E+06	1.05E+06	8.44E+05	1.65E+06	1.45E+06	1.32E+06
DAG 34:2	1.71E+06	2.01E+06	2.02E+06	2.08E+06	1.81E+06	1.48E+06	1.81E+06	1.24E+06	1.93E+06	1.67E+06	1.81E+06	1.58E+06	1.49E+06	1.46E+06	1.43E+06
DAG 34:3	3.15E+06	3.52E+06	2.97E+06	2.55E+06	2.35E+06	2.11E+06	2.61E+06	2.34E+06	2.71E+06	2.81E+06	2.76E+06	2.48E+06	3.52E+06	4.37E+06	3.58E+06
DAG 34:4	6.33E+04	7.19E+04	5.12E+04	6.30E+04	6.33E+04	5.20E+04	4.91E+04	4.44E+04	4.49E+04	5.04E+04	5.89E+04	4.94E+04	7.57E+04	7.28E+04	6.45E+04
DAG 36:3	8.96E+06	9.23E+06	1.04E+07	9.69E+06	9.90E+06	9.25E+06	9.68E+06	7.96E+06	8.88E+06	1.00E+07	1.05E+07	8.74E+06	6.37E+06	7.94E+06	6.95E+06
DAG 36:4	2.13E+07	2.35E+07	2.00E+07	2.01E+07	1.95E+07	1.64E+07	1.94E+07	1.68E+07	2.08E+07	1.95E+07	2.18E+07	1.90E+07	2.19E+07	2.54E+07	2.30E+07
DAG 36:5	1.72E+07	2.23E+07	1.62E+07	1.59E+07	1.50E+07	1.38E+07	1.76E+07	1.33E+07	1.83E+07	1.85E+07	1.90E+07	1.65E+07	1.74E+07	2.20E+07	1.94E+07
DAG 36:6	2.44E+07	2.88E+07	2.13E+07	1.93E+07	1.78E+07	1.61E+07	2.01E+07	1.71E+07	2.69E+07	3.11E+07	2.86E+07	2.36E+07	2.45E+07	4.12E+07	3.46E+07
DGDG 30:2	3.31E+06	4.40E+06	2.60E+06	2.94E+06	3.25E+06	3.24E+06	2.76E+06	2.21E+06	2.77E+06	2.28E+06	2.59E+06	2.12E+06	3.23E+06	2.28E+06	2.90E+06
DGDG 32:5	4.17E+02	6.80E+02	2.24E+02	3.95E+02	3.46E+02	9.22E+02	3.78E+02	5.30E+02	8.02E+02	7.64E+02	2.74E+02	2.46E+02	8.29E+02	3.91E+02	7.94E+02
DGDG 34:0	3.45E+04	2.95E+04	3.57E+04	3.11E+04	3.55E+04	3.49E+04	3.67E+04	2.47E+04	3.31E+04	3.84E+04	4.05E+04	3.32E+04	6.81E+03	2.93E+04	1.38E+04
DGDG 34:3	1.39E+04	1.88E+04	9.04E+03	1.58E+04	1.55E+04	1.22E+04	1.15E+04	1.30E+04	1.51E+04	1.37E+04	1.57E+04	1.67E+04	1.94E+04	1.16E+04	1.19E+04
DGDG 34:4	1.38E+03	7.79E+02	1.48E+02	1.15E+03	1.62E+03	6.55E+02	3.44E+02	3.87E+02	1.10E+03	4.58E+02	7.84E+02	8.56E+02	7.85E+02	1.03E+02	3.14E+02
DGDG 36:1	2.67E+03	3.43E+03	8.91E+02	2.12E+03	2.75E+03	2.79E+03	2.24E+03	1.11E+03	2.26E+03	2.68E+03	3.12E+03	2.46E+03	1.21E+04	2.08E+03	2.56E+03
DGDG 36:2	3.16E+04	3.27E+04	2.45E+04	3.59E+04	3.90E+04	2.98E+04	3.12E+04	2.36E+04	4.12E+04	4.19E+04	4.48E+04	4.67E+04	4.77E+04	2.98E+04	3.34E+04
DGDG 36:3	1.08E+05	7.17E+04	6.54E+04	1.03E+05	1.04E+05	9.35E+04	8.19E+04	6.60E+04	1.32E+05	1.53E+05	1.70E+05	1.53E+05	1.18E+05	8.07E+04	1.13E+05
DGDG 36:4	4.50E+05	3.47E+05	2.17E+05	4.30E+05	3.71E+05	3.18E+05	3.47E+05	2.84E+05	5.22E+05	4.59E+05	5.07E+05	5.55E+05	4.18E+05	2.80E+05	3.85E+05
DGDG 36:5	1.06E+06	9.14E+05	4.59E+05	9.00E+05	8.26E+05	6.41E+05	8.91E+05	6.51E+05	1.00E+06	8.20E+05	9.88E+05	8.64E+05	9.04E+05	5.85E+05	7.71E+05
DGDG 36:6	7.90E+05	1.10E+06	6.89E+05	9.67E+05	8.85E+05	7.72E+05	1.04E+06	9.72E+05	1.37E+06	8.67E+05	8.79E+05	1.02E+06	9.47E+05	5.66E+05	9.12E+05
MGDG 32:1	1.22E+04	1.64E+03	2.72E+02	7.98E+03	1.33E+04	1.83E+04	2.77E+03	9.89E+02	5.41E+03	5.24E+03	7.06E+03	4.21E+03	3.73E+04	1.20E+04	2.51E+04

MGDG 32:2	8.64E+02	0.00E+00	0.00E+00	5.36E+02	1.54E+03	9.36E+02	0.00E+00	2.41E+02	0.00E+00	0.00E+00	0.00E+00	0.00E+00	1.65E+03	0.00E+00	1.16E+03
MGDG 32:4	1.98E+03	1.40E+03	3.62E+02	7.87E+02	7.52E+02	9.61E+02	7.27E+02	5.52E+02	2.25E+03	3.20E+03	2.47E+03	2.89E+03	3.93E+03	7.03E+03	6.65E+03
MGDG 32:6	9.09E+03	2.14E+04	1.07E+04	1.25E+04	1.15E+04	1.11E+04	1.26E+04	1.14E+04	1.05E+04	8.54E+03	6.61E+03	9.89E+03	1.15E+04	1.04E+04	1.36E+04
MGDG 34:1	1.31E+04	8.68E+03	5.56E+03	1.38E+04	1.45E+04	1.33E+04	8.04E+03	8.36E+03	1.23E+04	1.40E+04	1.24E+04	1.02E+04	1.72E+04	1.14E+04	1.31E+04
MGDG 34:2	4.80E+03	6.33E+03	4.47E+03	7.95E+03	6.71E+03	7.86E+03	7.07E+03	5.58E+03	1.31E+04	9.75E+03	9.34E+03	1.06E+04	1.12E+04	1.07E+04	1.29E+04
MGDG 36:1	3.62E+03	3.53E+03	1.07E+03	3.50E+03	3.42E+03	3.34E+03	3.29E+03	2.25E+03	3.37E+03	3.65E+03	4.07E+03	3.70E+03	8.23E+03	4.10E+03	5.39E+03
MGDG 36:2	4.22E+04	3.00E+04	2.57E+04	4.05E+04	4.57E+04	3.77E+04	3.28E+04	2.41E+04	6.05E+04	6.80E+04	6.22E+04	5.65E+04	5.70E+04	5.37E+04	6.97E+04
MGDG 36:3	5.36E+04	3.99E+04	3.83E+04	5.59E+04	5.46E+04	5.12E+04	5.61E+04	4.09E+04	8.76E+04	8.82E+04	8.83E+04	8.92E+04	5.96E+04	5.41E+04	7.59E+04
MGDG 36:4	9.23E+04	8.84E+04	6.33E+04	9.06E+04	9.88E+04	9.34E+04	1.18E+05	9.38E+04	1.70E+05	1.26E+05	1.30E+05	1.50E+05	1.18E+05	9.81E+04	1.24E+05
MGDG 36:5	1.28E+04	1.73E+04	1.82E+04	1.42E+04	1.44E+04	1.51E+04	2.96E+04	4.11E+04	2.41E+04	2.37E+04	2.52E+04	2.86E+04	1.16E+04	1.95E+04	1.48E+04
MGDG 36:6	1.78E+05	1.87E+05	7.88E+04	1.13E+05	1.39E+05	1.35E+05	1.46E+05	1.39E+05	1.75E+05	1.32E+05	1.56E+05	1.59E+05	2.72E+05	1.64E+05	1.97E+05
SQDG 34:1	7.69E+03	6.20E+03	3.67E+03	8.26E+03	8.49E+03	7.16E+03	7.42E+03	3.43E+03	6.34E+03	6.54E+03	7.44E+03	7.96E+03	1.37E+04	6.17E+03	5.30E+03
SQDG 34:2	1.92E+04	4.15E+04	1.57E+04	3.35E+04	3.29E+04	3.46E+04	4.55E+04	2.43E+04	3.90E+04	2.28E+04	2.35E+04	3.42E+04	5.51E+04	2.49E+04	2.59E+04
SQDG 34:3	4.29E+04	7.73E+04	3.35E+04	4.61E+04	6.54E+04	5.85E+04	1.05E+05	5.08E+04	9.55E+04	4.17E+04	5.91E+04	6.30E+04	8.78E+04	6.51E+04	5.22E+04
SQDG 36:0	3.05E+02	1.96E+03	7.41E+01	2.96E+02	4.50E+02	3.79E+02	1.12E+03	0.00E+00	8.93E+02	5.69E+01	1.58E+02	5.42E+02	1.64E+03	7.28E+02	3.76E+02
SQDG 36:1	1.70E+03	1.29E+03	0.00E+00	6.32E+02	2.81E+02	5.89E+02	1.94E+03	6.57E+02	9.95E+02	1.13E+03	9.26E+02	2.30E+03	3.54E+03	1.30E+03	8.29E+02
SQDG 36:2	3.36E+04	4.09E+04	2.18E+04	3.51E+04	3.79E+04	3.28E+04	5.04E+04	2.58E+04	3.95E+04	3.94E+04	3.85E+04	5.70E+04	7.37E+04	4.09E+04	4.68E+04
SQDG 36:3	8.07E+04	1.08E+05	3.61E+04	7.12E+04	9.77E+04	8.14E+04	1.77E+05	7.51E+04	1.76E+05	6.84E+04	9.79E+04	1.13E+05	8.11E+04	5.97E+04	4.27E+04
SQDG 36:4	3.98E+04	5.13E+04	2.17E+04	4.91E+04	4.84E+04	4.29E+04	7.13E+04	4.15E+04	6.38E+04	3.47E+04	4.63E+04	5.48E+04	7.33E+04	3.70E+04	3.80E+04
SQDG 36:5	2.07E+04	3.85E+04	1.34E+04	1.95E+04	2.65E+04	2.29E+04	5.55E+04	2.18E+04	3.82E+04	1.66E+04	2.35E+04	2.30E+04	3.06E+04	2.01E+04	2.02E+04
SQDG 36:6	3.24E+04	3.71E+04	2.83E+04	1.87E+04	3.83E+04	3.46E+04	4.50E+04	2.60E+04	4.30E+04	2.16E+04	3.45E+04	2.53E+04	3.74E+04	3.49E+04	3.54E+04
FA 14:0	1.33E+03	1.36E+03	1.68E+03	1.79E+03	2.09E+03	2.05E+03	1.87E+03	2.23E+03	1.85E+03	1.87E+03	1.61E+03	2.14E+03	1.47E+03	9.10E+02	9.17E+02
FA 16:0	1.87E+05	1.99E+05	1.64E+05	1.52E+05	1.57E+05	1.63E+05	1.55E+05	1.58E+05	1.92E+05	2.56E+05	2.26E+05	2.35E+05	2.44E+05	7.38E+05	4.73E+05
FA 16:1	1.28E+04	1.12E+04	5.67E+03	5.49E+03	7.10E+03	6.00E+03	4.47E+03	7.38E+03	4.93E+03	7.03E+03	5.19E+03	5.00E+03	4.02E+03	8.40E+03	6.30E+03
FA 18:0	2.02E+05	2.83E+05	1.50E+05	1.40E+05	1.54E+05	1.73E+05	1.75E+05	1.65E+05	2.13E+05	3.08E+05	2.77E+05	2.65E+05	6.17E+05	2.01E+06	1.20E+06
FA 18:1	1.84E+06	1.79E+06	1.28E+06	1.33E+06	1.40E+06	1.35E+06	1.28E+06	1.44E+06	1.64E+06	2.69E+06	2.34E+06	2.30E+06	3.56E+06	1.29E+07	7.77E+06
FA 18:2	1.72E+06	1.93E+06	1.33E+06	1.35E+06	1.36E+06	1.38E+06	1.49E+06	1.22E+06	2.16E+06	3.14E+06	2.72E+06	2.91E+06	3.18E+06	1.04E+07	6.41E+06
FA 18:3	2.95E+06	3.44E+06	2.72E+06	1.98E+06	2.14E+06	1.92E+06	2.30E+06	2.37E+06	3.24E+06	4.57E+06	3.85E+06	3.96E+06	5.85E+06	2.86E+07	1.78E+07
FA 20:0	1.83E+05	1.36E+05	9.61E+04	1.06E+05	1.15E+05	1.09E+05	1.73E+05	1.35E+05	1.44E+05	1.62E+05	1.65E+05	1.46E+05	9.01E+04	1.77E+05	1.29E+05
FA 20:1	2.52E+04	2.95E+04	1.62E+04	1.56E+04	1.92E+04	2.02E+04	2.19E+04	2.23E+04	2.63E+04	3.72E+04	3.37E+04	3.16E+04	5.65E+04	1.72E+05	1.04E+05
FA 20:2	5.22E+03	5.66E+03	3.07E+03	3.19E+03	3.57E+03	3.66E+03	4.13E+03	3.81E+03	5.67E+03	8.28E+03	6.76E+03	6.57E+03	9.21E+03	2.99E+04	1.92E+04
FA 20:3	3.94E+03	3.65E+03	3.13E+03	2.09E+03	3.06E+03	2.53E+03	3.46E+03	3.10E+03	4.93E+03	6.81E+03	5.69E+03	5.48E+03	6.35E+03	3.68E+04	2.20E+04
FA 22:0	2.60E+05	1.96E+05	1.58E+05	1.72E+05	1.74E+05	2.07E+05	2.24E+05	2.37E+05	2.11E+05	2.66E+05	2.43E+05	2.38E+05	2.02E+05	3.74E+05	2.60E+05
FA 22:1	5.79E+03	6.17E+03	5.69E+03	4.68E+03	6.43E+03	4.67E+03	5.41E+03	6.22E+03	5.42E+03	6.51E+03	6.14E+03	6.01E+03	1.02E+04	1.63E+04	1.17E+04
FA 24:0	3.74E+05	3.75E+05	4.04E+05	3.40E+05	3.30E+05	3.81E+05	4.47E+05	4.97E+05	4.48E+05	5.38E+05	4.85E+05	4.45E+05	6.03E+05	8.65E+05	7.26E+05
FA 24:1	1.37E+04	1.43E+04	8.53E+03	8.29E+03	1.11E+04	1.04E+04	1.49E+04	1.19E+04	1.59E+04	1.31E+04	1.40E+04	1.26E+04	1.39E+04	2.83E+04	2.60E+04
LysoPC 16:0	3.53E+05	3.95E+05	2.27E+05	3.03E+05	2.57E+05	2.66E+05	2.34E+05	1.99E+05	2.90E+05	3.47E+05	3.35E+05	3.48E+05	7.23E+04	1.09E+05	8.63E+04
LysoPC 16:1	4.36E+03	5.35E+03	2.49E+03	5.11E+03	4.11E+03	3.56E+03	2.87E+03	2.65E+03	3.54E+03	4.06E+03	4.10E+03	4.45E+03	7.49E+02	1.06E+03	7.21E+02
LysoPC 18:0	1.26E+05	2.97E+05	9.57E+04	1.15E+05	1.13E+05	1.24E+05	1.29E+05	9.56E+04	1.44E+05	2.03E+05	1.40E+05	1.97E+05	4.80E+04	4.04E+04	4.77E+04
LysoPC 18:1	2.07E+06	1.91E+06	1.13E+06	1.62E+06	1.68E+06	1.57E+06	1.22E+06	1.13E+06	1.81E+06	2.65E+06	2.17E+06	2.05E+06	4.90E+05	7.82E+05	6.72E+05
LysoPC 18:2	8.98E+05	1.04E+06	6.16E+05	7.79E+05	6.42E+05	6.52E+05	7.01E+05	5.67E+05	1.09E+06	1.17E+06	1.03E+06	1.16E+06	1.67E+05	2.62E+05	2.27E+05
LysoPC 18:3	2.97E+05	4.42E+05	1.71E+05	1.93E+05	1.64E+05	1.90E+05	1.99E+05	1.78E+05	1.99E+05	2.61E+05	2.39E+05	2.29E+05	6.95E+04	1.19E+05	9.19E+04
lysoPC 20:0	2.82E+03	5.36E+03	1.63E+03	2.22E+03	2.39E+03	2.09E+03	2.32E+03	1.92E+03	2.87E+03	3.40E+03	2.38E+03	3.84E+03	1.52E+03	9.91E+02	9.57E+02
lysoPC 20:1	5.04E+03	8.66E+03	3.00E+03	4.08E+03	4.01E+03	5.13E+03	4.36E+03	3.83E+03	5.00E+03	7.94E+03	5.16E+03	7.03E+03	2.11E+03	1.54E+03	1.72E+03
lysoPC 20:2	7.71E+02	1.46E+03	3.51E+02	8.41E+02	4.87E+02	7.21E+02	7.35E+02	6.51E+02	1.05E+03	1.56E+03	9.40E+02	1.25E+03	1.25E+02	1.39E+02	5.81E+01
lysoPC 22:0	1.00E+03	1.32E+03	1.37E+03	8.82E+02	1.19E+03	8.17E+02	7.31E+02	6.60E+02	1.15E+03	1.19E+03	8.81E+02	1.38E+03	5.84E+02	3.88E+02	2.92E+02
LysoPE 16:0	5.97E+04	6.09E+04	4.01E+04	5.81E+04	4.72E+04	5.10E+04	4.00E+04	3.16E+04	5.68E+04	7.06E+04	6.82E+04	6.44E+04	1.52E+04	2.21E+04	1.92E+04

(continued on next page)

Table S2. (continued)

Sample name	Sakha 1_1	Sakha 1_2	Sakha 1_3	Sakha 2_1	Sakha 2_2	Sakha 2_3	Sakha 3_1	Sakha 3_2	Sakha 3_3	Sakha 4_1	Sakha 4_2	Sakha 4_3	Sakha 6_1	Sakha 6_2	Sakha 6_3
LysoPE 18:0	1.40E+04	2.87E+04	9.64E+03	1.38E+04	1.27E+04	1.47E+04	1.44E+04	8.50E+03	1.75E+04	2.58E+04	1.72E+04	2.23E+04	5.67E+03	5.35E+03	6.78E+03
LysoPE 18:1	1.91E+05	1.57E+05	8.92E+04	1.47E+05	1.58E+05	1.66E+05	1.06E+05	8.83E+04	1.35E+05	2.31E+05	1.96E+05	1.70E+05	5.16E+04	7.94E+04	6.51E+04
LysoPE 18:2	1.87E+05	1.83E+05	1.25E+05	1.71E+05	1.46E+05	1.52E+05	1.39E+05	1.11E+05	2.15E+05	2.42E+05	2.36E+05	2.35E+05	3.69E+04	5.77E+04	5.28E+04
LysoPE 18:3	5.48E+04	6.81E+04	2.94E+04	3.65E+04	3.41E+04	3.83E+04	3.51E+04	3.11E+04	4.07E+04	5.17E+04	4.89E+04	4.60E+04	1.57E+04	2.16E+04	1.83E+04
PC 32:0	3.61E+05	4.29E+05	2.67E+05	3.78E+05	3.71E+05	3.12E+05	3.44E+05	2.16E+05	3.03E+05	1.79E+05	2.73E+05	2.49E+05	3.94E+05	2.56E+05	2.99E+05
PC 32:1	3.18E+05	1.81E+05	9.73E+04	2.41E+05	3.40E+05	2.96E+05	1.85E+05	2.08E+05	2.09E+05	1.50E+05	3.61E+05	2.88E+05	6.61E+05	4.51E+05	7.31E+05
PC 32:2	4.40E+05	2.75E+05	1.86E+05	4.18E+05	4.49E+05	4.45E+05	3.25E+05	3.53E+05	4.09E+05	2.59E+05	5.69E+05	5.16E+05	8.81E+05	6.25E+05	1.00E+06
PC 32:3	7.28E+02	0.00E+00	0.00E+00	0.00E+00	0.00E+00	3.65E+02	0.00E+00	0.00E+00	0.00E+00	6.85E+02	2.93E+02	3.62E+02	3.40E+03	0.00E+00	1.23E+03
PC 34:1	2.85E+07	1.75E+07	1.53E+07	2.34E+07	2.92E+07	2.68E+07	1.57E+07	1.54E+07	1.80E+07	1.65E+07	1.84E+07	1.66E+07	3.10E+07	2.33E+07	2.70E+07
PC 34:2	3.33E+07	2.76E+07	2.24E+07	3.16E+07	3.17E+07	2.88E+07	2.59E+07	1.93E+07	3.74E+07	2.71E+07	2.91E+07	2.74E+07	3.03E+07	2.62E+07	3.30E+07
PC 34:3	1.29E+06	1.06E+06	7.82E+05	9.10E+05	1.06E+06	9.80E+05	8.87E+05	8.05E+05	7.68E+05	6.94E+05	8.15E+05	7.96E+05	1.32E+06	9.37E+05	1.09E+06
PC 34:4	5.09E+04	4.72E+04	2.88E+04	4.89E+04	5.72E+04	5.32E+04	3.29E+04	3.37E+04	2.69E+04	2.47E+04	2.77E+04	2.93E+04	6.84E+04	3.74E+04	4.34E+04
PC 34:5	1.33E+04	2.40E+04	8.67E+03	1.42E+04	1.46E+04	1.67E+04	1.17E+04	1.09E+04	9.95E+03	9.51E+03	8.36E+03	1.09E+04	2.80E+04	1.21E+04	2.07E+04
PC 34:6	2.74E+03	6.45E+03	8.58E+02	2.18E+03	2.49E+03	3.25E+03	1.75E+03	1.90E+03	9.03E+02	1.33E+03	1.32E+03	1.29E+03	6.83E+03	3.04E+03	4.38E+03
PC 36:0	3.04E+04	5.08E+04	2.40E+04	2.77E+04	2.87E+04	2.54E+04	3.16E+04	2.78E+04	3.12E+04	2.19E+04	2.92E+04	2.64E+04	3.74E+04	2.61E+04	2.44E+04
PC 36:1	9.96E+06	8.00E+06	3.75E+06	5.60E+06	8.17E+06	8.41E+06	5.85E+06	4.45E+06	5.23E+06	5.76E+06	6.90E+06	5.50E+06	1.38E+07	6.72E+06	7.83E+06
PC 36:2	4.79E+07	3.14E+07	2.56E+07	3.64E+07	5.23E+07	4.52E+07	2.94E+07	2.95E+07	3.50E+07	3.75E+07	4.08E+07	3.44E+07	6.09E+07	4.81E+07	6.24E+07
PC 36:3	6.02E+07	4.07E+07	4.07E+07	5.29E+07	6.15E+07	5.69E+07	4.45E+07	3.98E+07	5.80E+07	5.18E+07	5.68E+07	5.22E+07	7.27E+07	5.66E+07	7.38E+07
PC 36:4	5.60E+07	4.45E+07	3.73E+07	4.81E+07	5.49E+07	4.91E+07	4.32E+07	3.68E+07	5.28E+07	4.35E+07	4.73E+07	4.60E+07	6.16E+07	4.83E+07	5.89E+07
PC 36:5	2.46E+07	2.30E+07	1.61E+07	2.05E+07	2.09E+07	2.03E+07	2.07E+07	1.66E+07	2.03E+07	1.82E+07	1.96E+07	1.86E+07	2.71E+07	2.18E+07	2.54E+07
PC 36:6	6.87E+05	8.51E+05	4.04E+05	5.00E+05	5.33E+05	5.39E+05	5.23E+05	4.47E+05	3.84E+05	4.62E+05	4.18E+05	4.36E+05	1.00E+06	7.51E+05	8.66E+05
PC 38:0	4.68E+03	3.39E+03	0.00E+00	2.24E+03	9.03E+02	1.63E+02	2.42E+02	0.00E+00	3.21E+03	0.00E+00	2.03E+02	0.00E+00	4.96E+03	5.02E+02	3.10E+03
PC 38:2	1.86E+04	1.88E+04	1.55E+04	3.02E+04	2.34E+04	2.25E+04	2.56E+04	2.79E+04	3.38E+04	2.65E+04	2.35E+04	2.75E+04	3.26E+04	3.75E+04	3.64E+04
PC 38:3	3.69E+05	2.95E+05	1.54E+05	2.57E+05	2.97E+05	3.29E+05	2.62E+05	2.08E+05	3.04E+05	2.58E+05	2.88E+05	2.60E+05	4.36E+05	2.85E+05	3.42E+05
PC 38:4	2.60E+05	2.34E+05	9.85E+04	1.70E+05	1.88E+05	2.16E+05	1.67E+05	1.46E+05	1.96E+05	1.74E+05	1.85E+05	1.61E+05	3.21E+05	1.87E+05	2.28E+05
PC 38:5	7.36E+04	6.70E+04	3.70E+04	5.87E+04	5.72E+04	6.66E+04	5.80E+04	4.63E+04	6.82E+04	6.32E+04	6.17E+04	5.65E+04	8.34E+04	5.71E+04	6.96E+04
PC 38:6	2.51E+04	2.88E+04	1.14E+04	1.67E+04	1.72E+04	2.18E+04	1.80E+04	1.36E+04	1.54E+04	1.68E+04	1.84E+04	1.43E+04	3.45E+04	2.38E+04	2.84E+04
PE 32:0	6.12E+04	7.07E+04	6.09E+04	6.81E+04	4.59E+04	5.45E+04	4.04E+04	3.48E+04	4.39E+04	5.73E+04	5.92E+04	3.86E+04	2.28E+04	3.03E+04	2.90E+04
PE 32:1	1.62E+04	3.10E+04	1.68E+04	1.37E+04	9.83E+03	1.23E+04	1.22E+04	1.00E+04	1.35E+04	1.65E+04	1.87E+04	1.47E+04	8.44E+03	8.48E+03	7.80E+03
PE 34:1	3.86E+06	2.57E+06	1.95E+06	3.35E+06	4.11E+06	4.13E+06	2.16E+06	1.84E+06	2.12E+06	2.28E+06	2.46E+06	1.97E+06	4.15E+06	3.03E+06	3.62E+06
PE 34:2	6.02E+06	4.61E+06	4.61E+06	6.93E+06	6.29E+06	5.74E+06	4.83E+06	3.63E+06	6.72E+06	4.57E+06	5.54E+06	4.75E+06	5.24E+06	4.18E+06	5.84E+06
PE 34:3	2.88E+06	2.44E+06	1.90E+06	2.28E+06	2.34E+06	2.27E+06	2.00E+06	1.77E+06	1.86E+06	1.82E+06	2.14E+06	1.75E+06	2.75E+06	1.98E+06	2.31E+06
PE 36:1	5.17E+05	4.11E+05	2.02E+05	3.09E+05	4.51E+05	5.10E+05	2.92E+05	2.12E+05	2.39E+05	3.19E+05	3.33E+05	2.53E+05	7.52E+05	3.66E+05	4.44E+05
PE 36:2	3.22E+06	2.22E+06	1.49E+06	2.41E+06	3.27E+06	3.37E+06	1.92E+06	1.53E+06	2.17E+06	2.27E+06	2.39E+06	1.99E+06	4.56E+06	2.96E+06	3.95E+06
PE 36:3	7.67E+06	4.71E+06	3.88E+06	6.30E+06	7.51E+06	8.22E+06	4.72E+06	3.95E+06	5.47E+06	5.75E+06	6.27E+06	5.21E+06	1.08E+07	6.87E+06	9.22E+06
PE 36:4	1.05E+07	7.15E+06	6.10E+06	8.93E+06	1.00E+07	9.39E+06	7.29E+06	5.70E+06	1.01E+07	7.85E+06	8.93E+06	8.12E+06	1.15E+07	8.04E+06	1.10E+07
PE 36:5	4.59E+06	3.36E+06	2.36E+06	3.06E+06	3.49E+06	3.50E+06	3.15E+06	2.52E+06	3.25E+06	2.87E+06	3.27E+06	2.92E+06	5.03E+06	3.63E+06	4.52E+06
PE 36:6	1.03E+06	9.72E+05	4.09E+05	5.02E+05	6.45E+05	6.72E+05	5.54E+05	4.63E+05	4.51E+05	5.45E+05	5.79E+05	4.77E+05	1.49E+06	8.36E+05	9.58E+05
PE 38:2	3.53E+04	2.94E+04	1.42E+04	2.51E+04	3.45E+04	3.09E+04	2.53E+04	1.63E+04	2.83E+04	2.01E+04	2.58E+04	2.08E+04	5.90E+04	3.15E+04	4.13E+04
PE 38:3	1.88E+04	1.70E+04	3.62E+03	8.23E+03	1.18E+04	1.09E+04	8.85E+03	5.95E+03	7.05E+03	5.83E+03	9.02E+03	5.33E+03	3.97E+04	1.65E+04	1.84E+04
PE 38:4	9.66E+04	8.43E+04	4.34E+04	6.09E+04	7.51E+04	7.52E+04	6.76E+04	5.84E+04	8.48E+04	5.89E+04	7.12E+04	6.05E+04	1.27E+05	8.40E+04	9.87E+04
PE 38:5	9.20E+03	7.57E+03	5.24E+03	6.82E+03	8.34E+03	8.79E+03	8.19E+03	5.44E+03	8.73E+03	6.44E+03	7.71E+03	7.05E+03	9.97E+03	6.25E+03	8.69E+03
PE 40:1	1.02E+04	7.25E+03	6.24E+03	5.18E+03	9.01E+03	7.77E+03	2.89E+03	4.92E+03	4.07E+03	3.84E+03	3.36E+03	3.30E+03	1.13E+04	5.35E+03	8.30E+03
PE 40:2	7.54E+04	6.37E+04	7.28E+04	6.62E+04	7.05E+04	5.57E+04	5.57E+04	2.71E+04	6.71E+04	2.99E+04	3.80E+04	3.29E+04	9.47E+04	5.16E+04	7.64E+04
PE 40:3	3.94E+04	3.24E+04	2.12E+04	2.10E+04	2.43E+04	2.35E+04	1.86E+04	1.30E+04	1.53E+04	9.78E+03	1.19E+04	1.09E+04	5.23E+04	2.71E+04	3.54E+04
PE 42:1	1.33E+04	9.49E+03	8.69E+03	7.52E+03	1.25E+04	1.02E+04	4.51E+03	4.52E+03	5.48E+03	4.49E+03	5.08E+03	3.91E+03	1.37E+04	5.97E+03	1.11E+04

PE 42:2	1.19E+05	9.62E+04	1.09E+05	1.22E+05	1.21E+05	8.26E+04	1.09E+05	5.44E+04	1.46E+05	5.87E+04	8.91E+04	6.21E+04	1.09E+05	9.07E+04	1.26E+05
PE 42:3	6.96E+04	6.71E+04	3.81E+04	4.20E+04	4.46E+04	3.83E+04	4.26E+04	2.85E+04	4.10E+04	2.52E+04	3.42E+04	2.54E+04	7.75E+04	5.09E+04	5.99E+04
PG 32:0	1.75E+04	1.63E+04	2.29E+04	3.46E+04	2.50E+04	2.10E+04	2.04E+04	2.67E+04	3.48E+04	2.27E+04	2.64E+04	2.18E+04	2.25E+04	2.06E+04	2.27E+04
PG 32:1	2.77E+03	2.53E+03	1.90E+03	3.78E+03	3.15E+03	3.28E+03	2.71E+03	2.33E+03	3.80E+03	5.02E+03	3.46E+03	3.64E+03	4.31E+03	2.56E+03	3.31E+03
PG 34:0	3.64E+04	2.77E+04	1.92E+04	3.07E+04	3.28E+04	2.83E+04	2.53E+04	2.62E+04	3.85E+04	2.52E+04	3.14E+04	2.35E+04	3.05E+04	1.72E+04	2.41E+04
PG 34:1	2.04E+05	1.55E+05	1.81E+05	2.18E+05	2.31E+05	2.15E+05	1.71E+05	1.78E+05	2.41E+05	2.15E+05	2.30E+05	1.72E+05	2.79E+05	2.85E+05	3.41E+05
PG 34:2	4.77E+05	4.68E+05	4.27E+05	4.87E+05	5.34E+05	4.79E+05	4.57E+05	3.97E+05	5.06E+05	4.53E+05	4.54E+05	4.52E+05	4.53E+05	3.57E+05	4.97E+05
PG 34:3	1.57E+05	1.48E+05	1.15E+05	1.19E+05	1.29E+05	1.22E+05	1.33E+05	1.31E+05	1.42E+05	1.31E+05	1.29E+05	1.06E+05	2.09E+05	1.71E+05	2.06E+05
PG 36:1	1.73E+04	1.31E+04	1.06E+04	1.15E+04	1.55E+04	1.87E+04	1.50E+04	1.09E+04	1.30E+04	1.83E+04	1.51E+04	1.33E+04	3.57E+04	2.54E+04	3.11E+04
PG 36:2	1.29E+05	8.94E+04	8.80E+04	9.46E+04	1.30E+05	1.31E+05	1.07E+05	8.70E+04	1.32E+05	1.33E+05	1.15E+05	1.02E+05	2.25E+05	2.43E+05	2.82E+05
PG 36:3	1.82E+05	1.26E+05	1.23E+05	1.39E+05	1.76E+05	1.79E+05	1.53E+05	1.13E+05	1.70E+05	1.74E+05	1.48E+05	1.29E+05	2.84E+05	2.82E+05	3.37E+05
PG 36:4	1.78E+05	1.37E+05	1.17E+05	1.45E+05	1.67E+05	1.60E+05	1.56E+05	1.17E+05	1.83E+05	1.63E+05	1.30E+05	1.35E+05	2.54E+05	2.66E+05	2.81E+05
PG 36:5	5.95E+04	7.47E+04	5.58E+04	6.72E+04	6.82E+04	7.46E+04	8.58E+04	6.26E+04	8.15E+04	7.83E+04	4.30E+04	6.06E+04	1.11E+05	8.23E+04	1.17E+05
PG 36:6	2.46E+04	3.61E+04	2.14E+04	2.32E+04	2.51E+04	2.66E+04	3.10E+04	2.60E+04	2.82E+04	3.00E+04	1.42E+04	2.12E+04	6.37E+04	4.71E+04	6.43E+04
PI 36:1	7.15E+05	5.92E+05	4.43E+05	4.97E+05	6.28E+05	6.59E+05	4.32E+05	3.68E+05	4.04E+05	5.77E+05	6.26E+05	4.41E+05	9.07E+05	6.81E+05	7.68E+05
PI 36:2	2.88E+06	2.83E+06	2.59E+06	2.71E+06	3.13E+06	2.97E+06	2.31E+06	2.25E+06	2.61E+06	2.54E+06	2.72E+06	2.32E+06	2.76E+06	2.80E+06	3.24E+06
PI 36:3	1.90E+06	2.69E+06	9.73E+05	1.02E+06	1.20E+06	1.28E+06	1.32E+06	1.03E+06	1.24E+06	9.55E+05	1.15E+06	8.50E+05	1.76E+06	9.60E+05	1.14E+06
PI 36:4	2.02E+06	2.26E+06	1.57E+06	2.13E+06	2.28E+06	2.12E+06	1.81E+06	1.38E+06	1.90E+06	1.44E+06	1.36E+06	1.44E+06	2.24E+06	1.72E+06	2.28E+06
PI 36:5	5.78E+05	1.03E+06	6.06E+05	7.76E+05	7.32E+05	7.41E+05	8.12E+05	5.66E+05	7.11E+05	5.34E+05	4.28E+05	5.49E+05	7.16E+05	4.47E+05	6.85E+05
PI 36:6	3.19E+05	5.43E+05	2.60E+05	2.75E+05	2.67E+05	2.97E+05	3.06E+05	2.72E+05	2.55E+05	2.26E+05	2.15E+05	2.47E+05	3.65E+05	3.38E+05	3.37E+05
PI 38:1	5.72E+03	4.04E+03	1.00E+03	3.20E+03	7.07E+03	6.62E+03	2.64E+03	2.12E+03	2.85E+03	3.33E+03	3.57E+03	2.40E+03	8.92E+03	5.35E+03	6.69E+03
PI 38:2	1.27E+04	1.23E+04	1.03E+04	1.08E+04	1.79E+04	1.88E+04	9.71E+03	8.67E+03	1.02E+04	8.67E+03	1.38E+04	7.36E+03	2.00E+04	2.33E+04	3.05E+04
PI 38:3	2.02E+04	2.14E+04	1.97E+04	2.20E+04	2.56E+04	2.84E+04	2.20E+04	1.61E+04	2.62E+04	1.91E+04	2.12E+04	1.68E+04	2.48E+04	2.82E+04	3.56E+04
PI 38:4	3.36E+04	3.92E+04	2.32E+04	2.73E+04	3.06E+04	3.37E+04	3.18E+04	2.44E+04	3.33E+04	2.33E+04	2.48E+04	1.78E+04	4.80E+04	4.01E+04	4.96E+04
PI 38:5	5.26E+03	7.19E+03	4.19E+03	4.86E+03	5.04E+03	5.64E+03	6.73E+03	4.09E+03	6.88E+03	4.66E+03	5.10E+03	3.98E+03	6.29E+03	5.24E+03	6.02E+03
PS 34:1	1.95E+03	6.70E+02	9.10E+02	9.62E+02	1.47E+03	2.96E+03	2.04E+03	6.03E+02	1.04E+03	3.04E+02	3.85E+02	5.89E+02	2.90E+03	1.25E+03	2.02E+03
PS 34:2	1.61E+04	2.01E+04	2.12E+04	2.10E+04	1.85E+04	1.85E+04	1.54E+04	1.47E+04	1.95E+04	2.07E+04	1.52E+04	1.60E+04	2.58E+04	1.70E+04	2.78E+04
PS 36:0	1.01E+05	7.18E+04	5.57E+04	1.06E+05	1.24E+05	1.16E+05	6.06E+04	6.29E+04	5.65E+04	4.62E+04	5.75E+04	6.31E+04	1.17E+05	6.85E+04	8.01E+04
PS 36:1	5.00E+04	3.86E+04	3.09E+04	4.51E+04	4.89E+04	5.31E+04	3.75E+04	3.00E+04	3.41E+04	2.91E+04	3.41E+04	3.65E+04	4.42E+04	2.87E+04	3.66E+04
PS 36:2	2.03E+04	1.76E+04	9.13E+03	1.22E+04	1.51E+04	1.74E+04	1.11E+04	1.05E+04	8.62E+03	8.61E+03	9.06E+03	9.27E+03	1.99E+04	1.37E+04	1.47E+04
PS 36:3	1.05E+02	1.35E+03	2.12E+02	8.68E+01	1.18E+02	1.11E+02	1.32E+02	7.52E+01	5.53E+02	5.00E+03	2.98E+03	3.35E+03	2.63E+02	9.35E+02	1.28E+03
PS 38:0	4.64E+06	3.28E+06	3.35E+06	4.50E+06	5.43E+06	4.95E+06	3.21E+06	3.27E+06	3.33E+06	3.08E+06	3.26E+06	3.20E+06	5.06E+06	3.72E+06	4.57E+06
PS 38:1	4.46E+06	3.81E+06	4.54E+06	5.08E+06	5.23E+06	4.55E+06	4.21E+06	3.64E+06	4.85E+06	3.69E+06	4.11E+06	4.26E+06	3.96E+06	3.31E+06	3.79E+06
PS 38:2	2.39E+06	2.20E+06	1.81E+06	2.01E+06	2.30E+06	2.12E+06	1.87E+06	1.76E+06	1.60E+06	1.47E+06	1.60E+06	1.64E+06	2.69E+06	1.84E+06	2.11E+06
PS 38:4	2.19E+03	2.74E+03	8.81E+02	2.01E+03	1.79E+03	2.66E+03	1.91E+03	1.34E+03	1.22E+03	1.43E+03	1.10E+03	1.42E+03	3.73E+03	2.10E+03	2.37E+03
PS 38:5	3.63E+02	1.48E+03	4.23E+01	1.57E+02	1.44E+02	5.41E+02	1.27E+02	5.38E+01	2.52E+02	3.87E+01	5.95E+01	8.29E+01	1.67E+03	4.14E+02	7.55E+02
PS 40:0	1.97E+06	1.78E+06	1.05E+06	1.38E+06	1.90E+06	1.88E+06	1.41E+06	1.22E+06	1.26E+06	1.32E+06	1.48E+06	1.33E+06	2.72E+06	1.44E+06	1.69E+06
PS 40:1	7.91E+06	5.69E+06	5.53E+06	6.82E+06	9.58E+06	8.32E+06	5.79E+06	5.88E+06	6.45E+06	6.69E+06	7.23E+06	6.30E+06	9.84E+06	7.92E+06	9.68E+06
PS 40:2	9.55E+06	7.77E+06	8.26E+06	9.76E+06	1.09E+07	1.02E+07	8.61E+06	8.09E+06	9.95E+06	8.86E+06	9.39E+06	9.14E+06	1.12E+07	8.89E+06	1.14E+07
PS 40:4	4.12E+06	4.11E+06	3.41E+06	4.05E+06	4.05E+06	3.80E+06	3.97E+06	3.28E+06	3.88E+06	3.27E+06	3.46E+06	3.49E+06	4.49E+06	3.48E+06	4.33E+06
PS 40:5	1.40E+06	1.68E+06	9.25E+05	1.06E+06	1.09E+06	1.20E+06	1.06E+06	9.46E+05	8.03E+05	8.86E+05	9.16E+05	8.83E+05	1.95E+06	1.41E+06	1.63E+06
PS 42:1	6.16E+04	5.78E+04	2.44E+04	4.49E+04	5.99E+04	6.46E+04	4.34E+04	3.87E+04	3.89E+04	4.26E+04	4.71E+04	4.02E+04	9.22E+04	5.77E+04	6.73E+04
PS 42:5	1.97E+03	1.90E+03	1.08E+03	1.17E+03	1.66E+03	2.98E+03	2.06E+03	8.81E+02	1.35E+03	1.70E+03	1.88E+03	1.34E+03	3.63E+03	2.05E+03	2.82E+03
TAG 50:1	1.36E+07	1.51E+07	2.54E+07	2.04E+07	2.21E+07	2.26E+07	2.42E+07	3.71E+07	7.52E+06	8.85E+06	1.05E+07	1.41E+07	1.34E+07	1.66E+07	1.73E+07
TAG 50:2	2.16E+07	2.73E+07	4.05E+07	3.07E+07	3.53E+07	3.82E+07	4.32E+07	4.04E+07	1.63E+07	1.83E+07	2.00E+07	2.05E+07	2.01E+07	2.34E+07	2.19E+07
TAG 50:3	5.39E+07	5.71E+07	8.88E+07	6.41E+07	6.69E+07	6.88E+07	8.08E+07	8.54E+07	3.66E+07	3.85E+07	4.29E+07	4.32E+07	5.00E+07	6.46E+07	5.56E+07
TAG 50:4	1.19E+07	1.17E+07	1.26E+07	1.30E+07	1.17E+07	1.44E+07	1.44E+07	1.30E+07	6.67E+06	7.23E+06	8.43E+06	8.91E+06	1.23E+07	1.30E+07	1.06E+07

(continued on next page)

Table S2. (continued)

Sample name	Sakha 1_1	Sakha 1_2	Sakha 1_3	Sakha 2_1	Sakha 2_2	Sakha 2_3	Sakha 3_1	Sakha 3_2	Sakha 3_3	Sakha 4_1	Sakha 4_2	Sakha 4_3	Sakha 6_1	Sakha 6_2	Sakha 6_3
TAG 50:5	6.77E+06	7.65E+06	8.71E+06	7.04E+06	6.91E+06	1.03E+07	1.12E+07	8.43E+06	5.48E+06	6.01E+06	6.54E+06	5.84E+06	7.75E+06	7.76E+06	6.73E+06
TAG 50:6	1.42E+07	1.46E+07	1.95E+07	1.33E+07	1.33E+07	1.75E+07	1.97E+07	1.62E+07	1.04E+07	1.19E+07	1.43E+07	1.22E+07	1.54E+07	1.87E+07	1.58E+07
TAG 50:7	3.71E+05	4.93E+05	2.83E+05	2.28E+05	3.32E+05	3.96E+05	3.68E+05	4.04E+05	1.94E+05	2.57E+05	2.47E+05	2.32E+05	6.71E+05	5.36E+05	4.18E+05
TAG 50:8	3.24E+05	3.61E+05	3.27E+05	2.40E+05	3.46E+05	3.71E+05	3.33E+05	3.20E+05	2.22E+05	2.56E+05	2.55E+05	2.35E+05	4.79E+05	4.18E+05	3.42E+05
TAG 50:9	3.11E+05	3.61E+05	1.24E+05	1.32E+05	2.02E+05	2.12E+05	1.96E+05	2.20E+05	9.50E+04	1.52E+05	1.34E+05	1.56E+05	4.86E+05	4.93E+05	3.31E+05
TAG 52:1	1.93E+07	2.16E+07	2.68E+07	2.65E+07	2.95E+07	2.95E+07	3.67E+07	6.08E+07	1.14E+07	1.33E+07	1.59E+07	1.85E+07	1.66E+07	1.59E+07	2.04E+07
TAG 52:2	9.02E+07	8.62E+07	1.29E+08	1.04E+08	1.09E+08	1.08E+08	1.27E+08	2.01E+08	5.38E+07	6.39E+07	7.21E+07	8.50E+07	8.66E+07	9.66E+07	9.82E+07
TAG 52:3	1.17E+08	1.38E+08	2.19E+08	1.47E+08	1.71E+08	1.73E+08	2.18E+08	2.42E+08	1.03E+08	1.16E+08	1.26E+08	1.30E+08	1.23E+08	1.48E+08	1.34E+08
TAG 52:4	2.57E+08	2.56E+08	3.91E+08	2.82E+08	2.94E+08	2.90E+08	3.79E+08	4.82E+08	2.17E+08	2.41E+08	2.55E+08	2.59E+08	2.46E+08	3.06E+08	2.66E+08
TAG 52:5	2.32E+08	2.58E+08	3.88E+08	2.74E+08	2.82E+08	3.05E+08	3.99E+08	4.14E+08	2.25E+08	2.49E+08	2.54E+08	2.57E+08	2.47E+08	2.95E+08	2.47E+08
TAG 52:6	3.48E+08	3.57E+08	6.19E+08	3.93E+08	4.09E+08	4.19E+08	5.50E+08	6.08E+08	3.14E+08	3.54E+08	3.78E+08	3.79E+08	3.60E+08	4.69E+08	4.05E+08
TAG 52:7	2.07E+07	2.44E+07	3.32E+07	2.76E+07	2.58E+07	2.99E+07	3.42E+07	2.63E+07	1.54E+07	1.80E+07	2.01E+07	1.93E+07	2.46E+07	2.82E+07	2.19E+07
TAG 52:8	1.99E+06	2.89E+06	4.75E+06	2.87E+06	2.87E+06	3.46E+06	4.19E+06	2.94E+06	1.82E+06	2.05E+06	2.27E+06	2.13E+06	2.50E+06	2.96E+06	2.37E+06
TAG 54:1	1.40E+07	1.86E+07	1.49E+07	1.77E+07	2.54E+07	2.11E+07	3.68E+07	5.80E+07	8.90E+06	1.14E+07	1.34E+07	1.55E+07	8.89E+06	8.77E+06	1.38E+07
TAG 54:2	9.64E+07	9.34E+07	9.14E+07	9.96E+07	1.18E+08	9.69E+07	1.49E+08	2.32E+08	5.48E+07	6.56E+07	6.94E+07	8.51E+07	7.75E+07	7.75E+07	9.16E+07
TAG 54:3	2.06E+08	1.94E+08	2.44E+08	2.06E+08	2.26E+08	1.96E+08	3.01E+08	4.28E+08	1.37E+08	1.73E+08	1.72E+08	2.10E+08	1.72E+08	2.15E+08	2.09E+08
TAG 54:4	2.97E+08	2.99E+08	4.29E+08	3.03E+08	3.60E+08	3.09E+08	4.69E+08	6.25E+08	2.56E+08	2.86E+08	3.11E+08	3.40E+08	2.71E+08	3.62E+08	3.19E+08
TAG 54:5	4.57E+08	4.22E+08	6.41E+08	4.55E+08	5.14E+08	4.23E+08	6.49E+08	8.87E+08	3.71E+08	4.24E+08	4.56E+08	4.95E+08	4.07E+08	5.39E+08	4.70E+08
TAG 54:6	5.42E+08	5.69E+08	9.17E+08	5.88E+08	6.63E+08	5.74E+08	9.09E+08	1.09E+09	5.02E+08	5.71E+08	6.24E+08	6.60E+08	5.12E+08	7.19E+08	6.02E+08
TAG 54:7	6.64E+08	6.40E+08	9.70E+08	6.80E+08	7.36E+08	6.59E+08	9.71E+08	1.25E+09	5.91E+08	6.69E+08	7.00E+08	7.17E+08	6.30E+08	8.27E+08	7.15E+08
TAG 54:8	5.68E+08	6.04E+08	9.97E+08	6.36E+08	6.89E+08	6.48E+08	9.57E+08	1.06E+09	5.42E+08	6.43E+08	6.69E+08	6.68E+08	5.75E+08	7.74E+08	6.59E+08
TAG 54:9	7.06E+08	7.11E+08	1.31E+09	7.66E+08	8.48E+08	7.85E+08	1.13E+09	1.31E+09	6.75E+08	8.01E+08	8.61E+08	8.58E+08	7.24E+08	1.02E+09	8.69E+08
TAG 56:0	6.17E+04	6.13E+04	1.05E+05	7.23E+04	7.96E+04	7.26E+04	8.68E+04	1.34E+05	5.72E+04	6.03E+04	6.93E+04	7.54E+04	5.40E+04	6.45E+04	7.07E+04
TAG 56:1	1.19E+06	1.45E+06	2.72E+06	2.44E+06	2.92E+06	2.63E+06	3.29E+06	9.55E+06	6.77E+05	1.19E+06	1.30E+06	1.83E+06	7.45E+05	6.91E+05	1.92E+06
TAG 56:2	5.64E+06	6.05E+06	7.96E+06	6.98E+06	7.59E+06	7.65E+06	1.25E+07	2.40E+07	3.04E+06	4.45E+06	4.81E+06	6.04E+06	3.71E+06	3.80E+06	6.68E+06
TAG 56:3	1.05E+07	1.16E+07	2.42E+07	1.27E+07	1.57E+07	1.63E+07	2.32E+07	2.95E+07	8.03E+06	1.05E+07	1.17E+07	1.32E+07	9.18E+06	1.16E+07	1.40E+07
TAG 56:4	1.61E+07	1.73E+07	2.20E+07	1.72E+07	1.93E+07	2.01E+07	2.49E+07	3.49E+07	1.33E+07	1.48E+07	1.61E+07	1.56E+07	1.27E+07	1.56E+07	1.60E+07
TAG 56:5	1.91E+07	2.03E+07	2.66E+07	2.02E+07	2.19E+07	2.30E+07	2.89E+07	3.51E+07	1.68E+07	1.79E+07	1.94E+07	1.90E+07	1.78E+07	2.31E+07	2.15E+07
TAG 56:6	2.44E+07	2.86E+07	4.25E+07	2.62E+07	2.98E+07	3.39E+07	4.14E+07	4.14E+07	2.53E+07	2.72E+07	2.94E+07	2.74E+07	2.50E+07	3.05E+07	2.71E+07
TAG 56:7	2.23E+07	2.11E+07	2.91E+07	2.29E+07	2.21E+07	2.44E+07	2.92E+07	3.79E+07	2.13E+07	2.43E+07	2.49E+07	2.38E+07	2.39E+07	3.07E+07	2.69E+07
TAG 58:1	5.46E+05	5.95E+05	1.75E+06	1.33E+06	1.58E+06	1.32E+06	1.72E+06	5.42E+06	2.86E+05	6.02E+05	7.44E+05	1.23E+06	2.74E+05	2.59E+05	1.20E+06
TAG 58:3	3.95E+06	5.19E+06	1.70E+07	6.60E+06	8.92E+06	9.94E+06	1.77E+07	2.46E+07	3.21E+06	5.10E+06	6.11E+06	7.84E+06	3.55E+06	4.47E+06	8.40E+06
TAG 58:4	8.56E+06	9.25E+06	1.82E+07	1.10E+07	1.32E+07	1.50E+07	1.82E+07	2.85E+07	5.39E+06	7.61E+06	8.09E+06	9.94E+06	7.81E+06	9.66E+06	1.29E+07
TAG 58:5	5.89E+06	8.07E+06	1.84E+07	7.87E+06	9.88E+06	1.40E+07	1.69E+07	1.29E+07	5.44E+06	6.62E+06	6.88E+06	7.51E+06	7.81E+06	9.73E+06	1.06E+07
TAG 58:6	1.24E+07	1.50E+07	3.75E+07	1.35E+07	1.72E+07	2.43E+07	3.17E+07	2.80E+07	1.12E+07	1.44E+07	1.49E+07	1.69E+07	1.48E+07	2.18E+07	1.96E+07
TAG 58:7	1.28E+06	1.12E+06	1.64E+06	1.53E+06	1.19E+06	1.61E+06	1.24E+06	9.70E+05	1.11E+06	1.10E+06	1.18E+06	9.82E+05	1.61E+06	1.53E+06	1.59E+06
TAG 60:1	2.04E+05	1.91E+05	5.93E+05	5.47E+05	6.23E+05	4.18E+05	5.11E+05	2.26E+06	8.56E+04	2.33E+05	3.20E+05	5.31E+05	6.68E+04	5.94E+04	3.95E+05
TAG 60:2	9.67E+05	9.95E+05	2.88E+06	2.23E+06	2.28E+06	1.85E+06	2.67E+06	9.87E+06	4.47E+05	1.05E+06	1.33E+06	2.16E+06	4.32E+05	4.39E+05	2.11E+06
TAG 60:3	1.60E+06	2.09E+06	8.23E+06	3.77E+06	4.75E+06	4.65E+06	6.86E+06	1.69E+07	1.15E+06	2.56E+06	3.24E+06	4.20E+06	9.65E+05	1.22E+06	3.97E+06
TAG 60:4	3.87E+06	4.44E+06	1.08E+07	6.84E+06	7.43E+06	7.54E+06	1.25E+07	2.83E+07	2.44E+06	4.35E+06	5.09E+06	6.35E+06	2.57E+06	3.55E+06	7.49E+06
TAG 60:5	3.56E+06	4.94E+06	1.35E+07	6.35E+06	7.33E+06	9.35E+06	1.35E+07	1.66E+07	3.21E+06	5.59E+06	6.03E+06	6.78E+06	3.34E+06	4.96E+06	7.48E+06
TAG 60:6	6.78E+06	8.69E+06	2.83E+07	1.04E+07	1.16E+07	1.46E+07	2.35E+07	2.60E+07	6.82E+06	1.04E+07	1.11E+07	1.22E+07	7.37E+06	1.23E+07	1.36E+07
TAG 60:7	1.37E+06	1.37E+06	3.42E+06	1.53E+06	1.86E+06	2.31E+06	3.40E+06	3.88E+06	1.30E+06	2.22E+06	2.19E+06	2.20E+06	1.12E+06	1.73E+06	1.70E+06

Table S3. Analysis of secondary metabolites from different brown and yellow flaxseed Sakha cultivars by UPLC-FT-MS analysis.

Sample name	Sakha 1_1	Sakha 1_2	Sakha 1_3	Sakha 2_1	Sakha 2_2	Sakha 2_3	Sakha 3_1	Sakha 3_2	Sakha 3_3	Sakha 4_1	Sakha 4_2	Sakha 4_3	Sakha 6_1	Sakha 6_2	Sakha 6_3
Benzoic acid	1.74E+03	1.38E+03	1.66E+03	1.30E+03	1.31E+03	1.17E+03	1.59E+03	1.42E+03	1.70E+03	1.47E+03	1.45E+03	1.41E+03	1.52E+03	1.96E+03	1.87E+03
4-Hydroxy-Benzoic acid	3.00E+03	2.09E+03	1.62E+03	1.74E+03	2.46E+03	3.09E+03	2.69E+03	2.19E+03	3.68E+03	3.52E+03	3.70E+03	3.71E+03	2.30E+03	2.24E+03	2.27E+03
Allantoic acid	6.66E+04	4.86E+04	2.31E+04	5.00E+04	7.30E+04	4.97E+04	3.64E+04	3.28E+04	2.54E+04	1.20E+04	3.09E+04	2.44E+04	4.05E+04	2.73E+04	2.61E+04
Glycolic acid	2.13E+03	1.78E+03	9.50E+02	1.66E+03	1.46E+03	1.65E+03	1.62E+03	1.02E+03	2.25E+03	2.65E+03	1.91E+03	2.10E+03	1.21E+03	1.01E+03	1.11E+03
Uric Acid	2.33E+02	1.90E+03	3.84E+01	4.38E+03	5.97E+02	1.06E+03	8.33E+02	1.29E+02	3.53E+02	3.28E+03	2.38E+03	1.86E+03	3.07E+02	4.85E+02	3.77E+02
2,3-Dihydroxybenzoic acid	4.53E+03	3.48E+03	2.50E+03	2.45E+03	3.60E+03	2.44E+03	4.30E+03	2.71E+03	4.81E+03	3.03E+03	4.29E+03	3.02E+03	2.29E+01	2.19E+01	2.49E+01
Cinnamic acid	2.18E+04	3.03E+04	1.51E+04	3.51E+04	2.44E+04	3.41E+04	4.12E+04	1.56E+04	2.02E+04	1.28E+04	2.29E+04	1.67E+04	3.79E+04	2.72E+04	2.53E+04
Vanillin	5.88E+02	4.03E+02	3.38E+02	3.01E+02	3.95E+02	3.94E+02	4.60E+02	3.05E+02	5.49E+02	3.91E+02	4.42E+02	3.66E+02	3.21E+02	2.95E+02	3.36E+02
Ferulic acid hexose	2.12E+02	2.85E+02	5.60E+02	5.88E+02	2.83E+02	2.65E+02	3.08E+02	0.00E+00	7.98E+02	5.20E+02	4.73E+02	5.40E+02	2.26E+02	5.64E+02	5.86E+02
cis-Sinapic acid	1.17E+03	8.94E+02	1.07E+03	9.41E+02	1.64E+03	1.05E+03	1.25E+03	6.77E+02	1.43E+03	9.33E+02	1.10E+03	1.18E+03	1.48E+03	1.71E+03	2.15E+03
trans-Sinapic acid	1.00E+03	8.68E+02	1.09E+03	8.93E+02	1.04E+03	7.37E+02	9.60E+02	8.31E+02	8.19E+02	8.40E+02	8.05E+02	8.77E+02	9.14E+02	9.73E+02	9.19E+02
Panthothenic Acid	6.47E+03	7.51E+03	2.85E+03	3.95E+03	5.07E+03	5.38E+03	4.62E+03	3.08E+03	4.86E+03	3.54E+03	4.41E+03	3.84E+03	7.48E+03	4.66E+03	5.77E+03
Riboflavin	3.48E+03	3.66E+03	4.26E+03	4.72E+03	6.86E+03	7.08E+03	8.44E+03	7.48E+03	8.59E+03	6.98E+03	6.93E+03	8.71E+03	1.12E+04	9.05E+03	7.57E+03
Nicotinic acid	2.15E+03	2.54E+03	1.27E+03	1.72E+03	2.10E+03	1.71E+03	2.51E+03	1.50E+03	2.71E+03	3.55E+03	2.91E+03	2.73E+03	2.04E+03	1.80E+03	1.97E+03
Linustatin	1.03E+06	1.13E+06	1.30E+06	1.45E+06	1.27E+06	1.52E+06	1.14E+06	1.26E+06	1.15E+06	1.09E+06	1.13E+06	1.26E+06	1.30E+06	1.17E+06	1.24E+06
Neolinustatin	1.41E+06	1.18E+06	1.16E+06	1.03E+06	8.73E+05	1.19E+06	1.88E+06	2.09E+06	1.35E+06	2.00E+06	1.77E+06	1.62E+06	1.59E+06	1.41E+06	1.45E+06
Adenine	2.15E+03	2.35E+03	1.41E+03	2.18E+03	2.58E+03	2.45E+03	2.16E+03	1.42E+03	2.46E+03	3.64E+03	3.93E+03	3.63E+03	1.50E+03	1.54E+03	1.51E+03
Adenosine-5-P	9.91E+03	7.07E+03	3.40E+03	6.52E+03	8.41E+03	8.62E+03	5.97E+03	2.91E+03	5.96E+03	7.54E+03	7.26E+03	7.28E+03	9.65E+03	9.82E+03	7.87E+03
Adenosine	8.87E+04	9.10E+04	7.14E+04	8.92E+04	9.40E+04	9.18E+04	9.50E+04	7.16E+04	8.41E+04	8.91E+04	9.49E+04	8.72E+04	8.02E+04	6.48E+04	6.87E+04
Guanosine	4.84E+03	5.43E+04	2.67E+03	7.09E+03	7.86E+03	4.84E+03	1.44E+04	4.30E+03	4.73E+03	9.05E+03	1.56E+04	7.31E+03	2.34E+04	6.33E+03	6.28E+03
Uridine	1.50E+04	2.19E+04	1.15E+04	1.50E+04	1.45E+04	1.52E+04	1.93E+04	1.00E+04	1.54E+04	2.04E+04	2.39E+04	1.92E+04	1.86E+04	1.58E+04	1.53E+04
Nicotinamideadenine -dinucleotide (NAD)	1.13E+03	2.07E+03	9.61E+02	1.34E+03	0.00E+00	1.56E+03	1.68E+03	6.53E+02	1.02E+03	1.01E+03	1.48E+03	8.46E+02	7.62E+02	6.52E+02	5.16E+02
Glutathione (reduced)	6.47E+04	4.78E+04	4.29E+04	5.19E+04	9.02E+04	5.61E+04	5.20E+04	4.07E+04	4.63E+04	1.97E+04	4.10E+04	2.19E+04	1.96E+05	1.25E+05	2.06E+05
Glutathione (Oxidized)	3.29E+05	4.47E+05	2.82E+05	3.22E+05	3.72E+05	3.38E+05	3.09E+05	1.71E+05	2.37E+05	1.22E+05	1.88E+05	1.34E+05	2.69E+05	2.33E+05	2.77E+05

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