Lipid fingerprinting of yellow mealworm *Tenebrio molitor* by untargeted liquid chromatography-mass spectrometry

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Abstract

Insects such as *Tenebrio molitor* have been considered an alternative source of nutrition for animals and have also been adopted as human food throughout history, especially in Asia and Africa. Lipids are the second most abundant component followed by proteins. However, studies focusing on comprehensive lipid composition analsysis of these widely reared species are limited. The untargeted lipidomic analysis of yellow mealworm larvae (*T. molitor*) led to the identification of several lipid molecular species from lipid classes such as: free fatty acids, sphingolipids, phospholipids, and triacylglycerols. The results revealed that polyunsaturated fatty acids (PUFAs) (45%) are the most abundant fatty acids, followed by monounsaturated fatty acids (MUFAs) (42%) and saturated fatty acids (13%). Fatty acids such as FA 18:1 and FA 18:2 are the most abundant fatty acids and are substantially enriched in other complex lipids in the form of esters. Moreover, functional lipids such as sphingomyelins, ceramides, cardiolipins, phosphatidylinositols, and phosphatidylethanolamines were characterised for the first time, with a large number of MUFAs and PUFAs as their main acyl chains. Overall, our data showed the occurrence of multiple structurally diverse lipids in *T. molitor,* suggesting that mealworms are not only enriched with proteins but also have several functional lipids, which are highly beneficial to human and animal health. Thus, the larvae of *T. molitor* could serve as a promising candidate for the development of functional food and feed products.

Keywords: UHPLC/LTQ Orbitrap MS, *T molitor* larvae, total lipids, PUFA, MS DIAL

1. Introduction

As endorsed by the Food and Agriculture Organization of the United Nations, edible insects are a traditional and readily available nutritive food resource for both animals and humans, (Van Huis *et al.*, 2013). Moreover, insects are increasingly becoming recognised as an alternative source of high-quality protein, along with other nutritionally valuable substances that are exploited by the food industry (Schlüter *et al.*, 2017). Edible insects are highly nutritive, because they are rich in protein and micronutrients such as minerals and vitamins (Grau *et al.*, 2017; Jajić *et al.*, 2019; Jensen *et al.*, 2019; Ravzanaadii *et al.*, 2012). Fat is the second most abundant constituent of insect nutrient composition, followed by proteins (Mlcek *et al.*, 2014). Many studies have focused on insects as a food source of protein content, but comprehensive lipid analysis studies were limited, although lipids are a major component of insects (Yi *et al.*, 2013).

Lipids are a source of energy and important macronutrients in the diet. Insect lipids can contribute significantly to human nutrition both as a source of energy and essential fatty acids (Ramos-Elorduy, 2008). Among insect species, the yellow mealworm (*Tenebrio molitor),* belonging to darkling beetles (family: *Tenebrionidae*), has high nutritional value and is easy to breed (Alves *et al.*, 2016). It is also enriched in protein (13.68-22.32 g/100 g edible portion) and fat (8.90-19.94 g/100 g edible portion) with significant amounts of polyunsaturated fatty acids (PUFAs)

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(Nowak *et al.*, 2016). The peptides derived from *T. molitor* have been shown to exert long-term immunological effects on cultured haemocytes along with high pro-apoptotic activities (Czarniewska *et al.*, 2018). Their larvae are reported to contain an even higher proportion of lipid content (50.15 g/100 g), (Alves *et al.*, 2016) in addition to proteins. *T. molitor* is the most widely reared mealworm and has great potential for use in sustainable food and feed products (Caparros Megido *et al.*, 2014; Van Huis, 2013, 2020). Mealworms are not only suitable as animal feed, but are also a good source for human nutrition and have even been suggested as a bio-regenerative life support system for space missions (Li *et al.*, 2013). Comparison of the nutritional profiles showed significantly higher nutritional value and energy yield of insects as compared to those of beef and chicken (Ramos-Elorduy, 2008). Insects are a good source of essential amino acids (Rumpold and Schlüter, 2013) and fatty acids (Jeon *et al.*, 2016). *T. molitor larvae* are reported to be a better food option than the adults (Gere *et al.*, 2019; Liu and Zhao, 2019). Furthermore, oil extracted from mealworms is consider to use as novel food ingredients (Son *et al.*, 2020).

Previous studies have profiled the protein content (Nowak *et al.*, 2016), minerals (Siemianowska *et al.*, 2013), and most importantly, lipid composition (such as essential fatty acids) in *T. molitor* (Costa *et al.*, 2020; Kröncke *et al.*, 2019; Wu *et al.*, 2020). However, comprehensive lipidomic studies are limited. Free fatty acid levels were comprehensively analysed in *T. molitor,* and palmitic acid, oleic acid, and linoleic acid were the major fatty acids found in most of the studies (Alves *et al.*, 2016; Dreassi *et al.*, 2017; Tzompa-Sosa *et al.*, 2014; Van Broekhoven *et al.*, 2015). However, the fatty acid composition largely depends on the nature of the diet fed to the *T molitor* (Dreassi *et al.*, 2017; Van Broekhoven *et al.*, 2015). An alteration in diet composition significantly changed the total fatty acid percentages, and with total monounsaturated fatty acids then being the most abundant in the larvae (Alves *et al.*, 2016). An effort to determine other lipid classes such as phospholipids, monoacylglycerol, diacylglycerol, and triacylglycerol by thin-layer chromatography using different types of solvent extraction methods has been demonstrated (Tzompa-Sosa *et al.*, 2014); however, exact compositions and molecular level characterisation studies have not been performed.

Gas-chromatography/chemical ionisation mass spectrometry (GC/MS) and gas chromatography/flame ionisation detection are widely used techniques for the determination of lipids, including total and free fatty acids in insects (Alves *et al.*, 2016; Cakmak *et al.*, 2007; Dreassi *et al.*, 2017; Siemianowska *et al.*, 2013). However, these techniques are limited by their poor sensitivity, are time-consuming, and require pre-derivatisation before analysis. Liquid chromatography/high resolution mass spectrometry is more sensitive, and a multiple lipid class analysis is feasible within a short period with the least available amount of sample (Wu *et al.*, 2014). There is a growing interest to study the nutritive properties of edible insects in order to select the species that could be used as an effective natural supplement to the human diet. In this study, we aimed to comprehensively profile the total lipid content in the yellow mealworm using ultra-high-performance liquid chromatography (UHPLC)-linear trap quadrupole (LTQ) Orbitrap mass spectrometry. The concentration of each lipid component was determined using the internal standard technique.

2. Materials and methods

Materials

Mealworm larvae in sawdust (moisture: 60.16%, ash: 2.4%, protein: 23.3%, lipid: 11%, carbohydrate: 3.2%) were purchased from Jupiter Co. Ltd., (Tokyo, Japan) and stored in a refrigerator in a state of asphyxia. The mobile phase and extraction solvents (such as methanol, isopropanol, chloroform, and ammonium acetate solution (1 M)) for liquid chromatography/mass spectrometry (LC/MS) grade were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The EquiSPLASH Lipidomix quantitative standard for mass spectrometry, oleic acid-d9, and deuterated internal standards were purchased from Avanti Polar Lipids (Alabaster, AL, USA).

Extraction of total lipids from mealworm

Total lipids were extracted from the larvae of the yellow mealworm using the Folch method (Folch *et al.*, 1957), with slight modifications established earlier in our lab (Gowda *et al.*, 2020a,b). The workflow of lipid extraction and analysis is shown in Figure 1A. In brief, 100 mg of live mealworms were weighed into a 1.5 ml Eppendorf tube with 5-6 ceramic beads (1.4 mm, catalogue no. 15-340-159, Fisherbrand, Pittsburgh, PA, USA) and homogenised (30 s × 2 cycles) using a Bead Mill 4 (Fisherbrand) homogeniser. Then, 1 ml of ice-cold methanol was added, and homogenisation was repeated for another 30 s. About 200 µl of the methanolic homogenate (20 mg) were transferred to an Eppendorf tube, followed by addition of 100 µl of the internal standard mixture in methanol (having each of the following labelled standards 13.2 µM of phosphatidylcholine (PC) (15:0-18:1(d7)), 14 µM of phosphatidylethanolamine (PE) (15:0-18:1(d7)), 12.5 µM of phosphatidylserine (PS) (15:0-18:1(d7)), 12.7 µM of phosphatidylglycerol (PG) (15:0-18:1(d7)), 11.5 µM of phosphatidylinositol (PI) (15:0-18:1(d7)), 18.9 µM of lysophosphatidylcholine (LPC) (18:1(d7)), 20.5 µM of lysophosphatidylethanolamine (LPE) (18:1(d7)), 13.5 µM of sphingomyelin (SM) (d18:1/18:0(d9)), 18.8 µM of ceramide (Cer) (d18:1/15:0 (d7)), 12.3 µM of triacylglycerol (TAG) (15:0-18:1(d7)-15:0), 17 µM of diacylglycerol (DAG) (15:0-18:1(d7)), 15.1 µM of cholesterol ester (18:1(d7)), 27.5

µM of monoacylglycerol (18:1(d7)), and 340 µM of oleic acid (d9) and the mixture was vortexed at 3,500 rpm for 30 s. Subsequently, 600 µl of chloroform were added and vortexed for 5 min, and 75 µl of milli-Q were added with an additional vortex for approximately 30 s. The biphasic extracts were centrifuged at 15,000 rpm for 10 min at 4 °C, the lower chloroform layer was transferred to a vial, while the upper layer was re-extracted with an additional 600 µl of chloroform. The chloroform extracts were combined and concentrated under vacuum. The dried total lipids were redissolved in 100 µl of methanol with gentle vortexing and centrifuged. Approximately 10 µl of each sample was injected into the LC/MS via an autosampler.

Lipidomic analysis by UHPLC/LTQ Orbitrap MS

The lipidomic analysis was performed using a prominence UHPLC system (Shimadzu Corp., Kyoto, Japan) coupled with an LTQ Orbitrap MS (Thermo-Fisher Scientific Inc., San Jose, CA) and an Atlantic T3 C18 column (2.1×150 mm, 3 µm, Waters, Milford, MA, USA) at 40 °C. The flow rate of the mobile-phase (A: aqueous 10 mM CH_3COONH_4, B: isopropanol, C: methanol) was set to 200 µl/min with a linear flow of: 30% B and 35% C (0-1 min), 80% B and 10% C (1-14 min), 85% B and 10% C (14-27 min) in negative mode and a linear gradient of 6% B and 90% C (0-1 min), 83% B and 15% C (1-10 min), 83% B and 15% C (10-19 min), 6% B and 90% C (19-19.5 min), 6% B and 90% C (19.5-22 min) in positive mode. The mass spectrometric analysis conditions were identical to those described in our previous report (Gowda *et al.*, 2020a,b). In brief, MS data were acquired in both electron spray ionisation (ESI)-positive and negative modes with the same capillary temperature (330 °C), sheath gas flow (50 units), and auxiliary gas (20 units). For the negative mode, the source voltage was set to 3 kV, and the capillary voltage was set to -10 V, whereas for the positive mode source voltage was set to 4 kV and capillary voltage was set at 25 V. A Fourier transform full scan range was set to *m/z* 150-1,700 and *m/z* 250-1,600 in the positive and negative modes to acquire MS^1 spectra for high-resolution masses. Low-resolution MS/MS spectra were obtained at a collision energy of 40 V in ion-trap mode. The raw data were processed using MS DIAL (version 4.2) software (RIKEN, Wako, Japan) for the alignment and identification of lipid species (Tsugawa *et al.*, 2015). Further, each identified lipid species was confirmed by MS/MS spectral matching with the built-in reference library of MS DIAL. The extracted ion chromatograms were obtained using Xcalibur 2.2 software (Thermo-Fisher Scientific Inc., San Jose, CA, USA).

The quantification of lipid molecules was carried out according to the definition of Lipidomics Standards Initiative level 2 and level 3, which was achieved if the lipid molecule was quantified by the labelled internal standard of the same lipid subclasses or representative lipid class category. The data were normalised by the weight

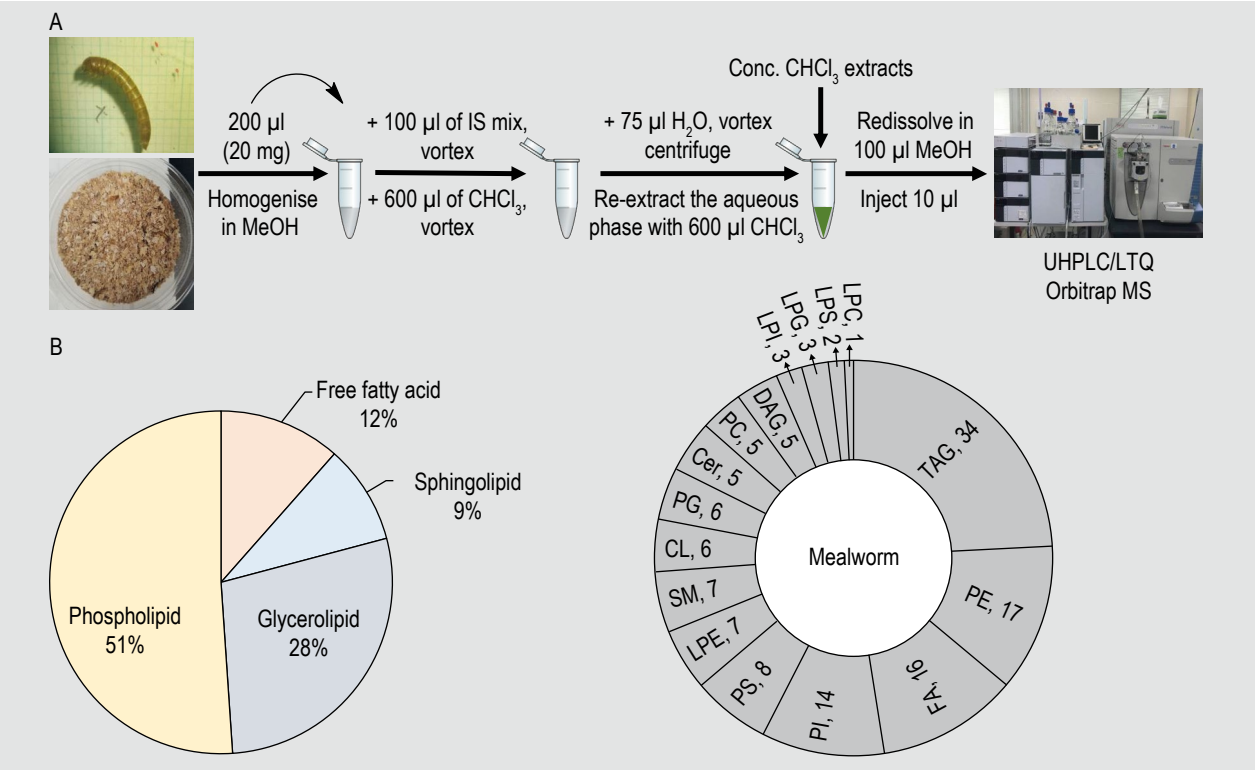

Figure 1. (A) Workflow of lipid extraction and analysis. (B) Lipid distributions of phospholipid, glycerolipid, free fatty acid, and sphingolipid (left) and number of molecular species characterised in yellow mealworm (right).

of the mealworm, and the concentration (in pmol) of the lipid molecular species was calculated by taking the peak intensity ratios of the analyte to the internal standard and multiplying it by the pmol of the added internal standard. The lipid percentage distributions were calculated based on the total number of molecular species identified in each class. The percentage distributions of free fatty acids, such as saturated, monounsaturated, and PUFAs, were calculated by taking the ratio of the sum of the concentrations of individual fatty acids of the respective type to their total sum. The graphs were plotted in Excel 2016 and GraphPad Prism 8 software (San Diego, CA, USA). The data were represented as the mean ± standard error (n=5).

3. Results and discussion

GC/MS based lipid analysis methods are widely applied to profile the lipid content in insects (Alves *et al.*, 2016; Cakmak *et al.*, 2007; Dreassi *et al.*, 2017; Siemianowska *et al.*, 2013), but these techniques are limited in terms of sensitivity, require pre-derivatisation, large sample amounts, and are not comprehensive. In other words, the LC/MS-based untargeted analysis technique employed in this study has many advantages: high sensitivity, direct analysis being possible without any pre-derivatisation, and a more comprehensive lipid profile that can be obtained with a smaller amount of sample. Figure 1A shows the workflow of total lipid extraction from yellow mealworm and analysis by UHPLC/ LTQ Orbitrap MS. The lipid percentage distributions of major lipid classes and the number of molecular species characterised from each lipid subclass are shown in Figure 1B. The results from an untargeted lipidomic analysis of the yellow mealworm showed the highest abundance of phospholipids followed by glycerolipids, free fatty acids, and sphingolipids. In terms of the number of molecular species identified, triacylglycerols comprised a higher diversity of molecular species, followed by phosphatidylethanolamines and free fatty acids.

The representative structures of each lipid subclass identified in the yellow mealworm are shown in Figure 2A, followed by the extracted ion chromatograms in Figure 2B. The lipid molecular species such as fatty acid (FA) 18:1, PE (16:0/18:2), PG (18:0/18:1), PI (18:0/18:1), PS (18:1/18:1), and LPE (18:0) eluted at 10.3 min, 16.8 min, 15.0 min, 14.9 min, 14.5 min, and 11.6 min, respectively, in negative analysis mode, whereas DAG (16:0/18:1) and TAG (16:0/18:0/18:1) were eluted at 10.7 min and 14.9 min, respectively, in the positive analysis mode. The MS/MS data of the representative lipids from each subclass are shown in Figure 3. In general, monounsaturated fatty acids (MUFA 18:1 of *m/z* 281) lose a molecule of water to give *m/z* 263 $[M\text{-}H\text{-}H_2O]^-$ as a major product ion, whereas polyunsaturated fatty acids lose a neutral molecule of CO_2 (Gowda, *et al.*, 2020b; Yang *et al.*, 2011). All the lipid species demonstrated in Figure 1 were identified based on their exact masses acquired from high-resolution MS^1 spectra and confirmed by their low-resolution MS/MS spectral match with the built-in computational library of MS DIAL. The detailed list of acquired and reference masses of each molecular species and their retention times obtained from MS DIAL processing are provided in the Supporting Information (Table S1). The phospholipids ionise in negative mode to give the $[M\text{-}H]^-$ or $[M+CH_3COO]^-$ ions, whereas the diacylglycerols and triacylglycerols ionise in positive mode to produce $[M+NH_4]^+$ as their molecular ions (Figure 3). The characteristic fragment ions of each lipid species are shown in the MS/MS spectra.

Phospholipids are the major lipids in insects (Fast, 1966) and have been previously characterised from *T. molitor* (Gilby, 1965; Tzompa-Sosa *et al.*, 2014). However, detailed lipidome analysis at the molecular species level has not yet been performed. In this study, we analysed their profiles comprehensively, and the concentrations of each type of phospholipid subclasses, identified in *T. molitor*, are shown in Figure 4. Among the phosphatidylinositol and phosphatidylserine subclass, they are mainly acylated or O-alkylated with either FA 18:1 or FA 18:2 (Figure 4A), with PI (18:1/18:1) being the most abundant molecular species. The concentrations (mean ± standard error) of PI and PS per milligram of mealworm ranged from 0.3±0.1 to 36.5±1.7 pmol, and 1.2±0.1 to 23.8±0.9 pmol respectively. Oral administration of these lipids such as dietary PIs, is known to show beneficial health such as, prevention of non-alcoholic fatty liver disease in rats (Shirouchi *et al.*, 2008), and while intake of PS normalises chronic stress in men (Hellhammer *et al.*, 2014). The occurrence of PI and PS in mealworms suggests that it is a valuable source of functional lipids. The concentrations of other phospholipid subclasses, such as phosphatidylcholine, phosphatidylethanolamine, and phosphatidylglycerol, are shown in Figure 4B. Phosphatidylethanolamines were higher in terms of the number of molecular species followed by phosphocholines, whereas phosphatidylglycerol concentrations were relatively low, with a predominance of FA 18:1 and FA 18:2 acyl chains. Among phosphatidylcholines, PC (18:2/18:2) was the most abundant species relative to concentration, followed by PC (16:0/18:2). The oral intake of phosphatidylethanolamines was shown to have an antineoplastic effect on the survival time of Ehrlich tumour-bearing mice, suggesting their importance as a functional food (De Arruda *et al.*, 2011).

Among the lysophospholipids identified, FA 18:1 and FA 18:2 acylated molecular species were highly abundant relative to concentration in all the subclasses (Figure 4C). In particular, the most abundant molecular species in terms of relative concentrations were LPC (18:2) and LPE (18:2). The concentration ranges of lysophospholipids were as follows: LPE (0.1±0.03 to 9.15±0.72 pmol), LPG (0.1±0.02 to 0.24±0.04 pmol), LPG (0.05±0.02 to 1.52±0.32 pmol), LPS (0.7±0.09 to 1.09±0.2 pmol) and LPC (18:2) 301±31

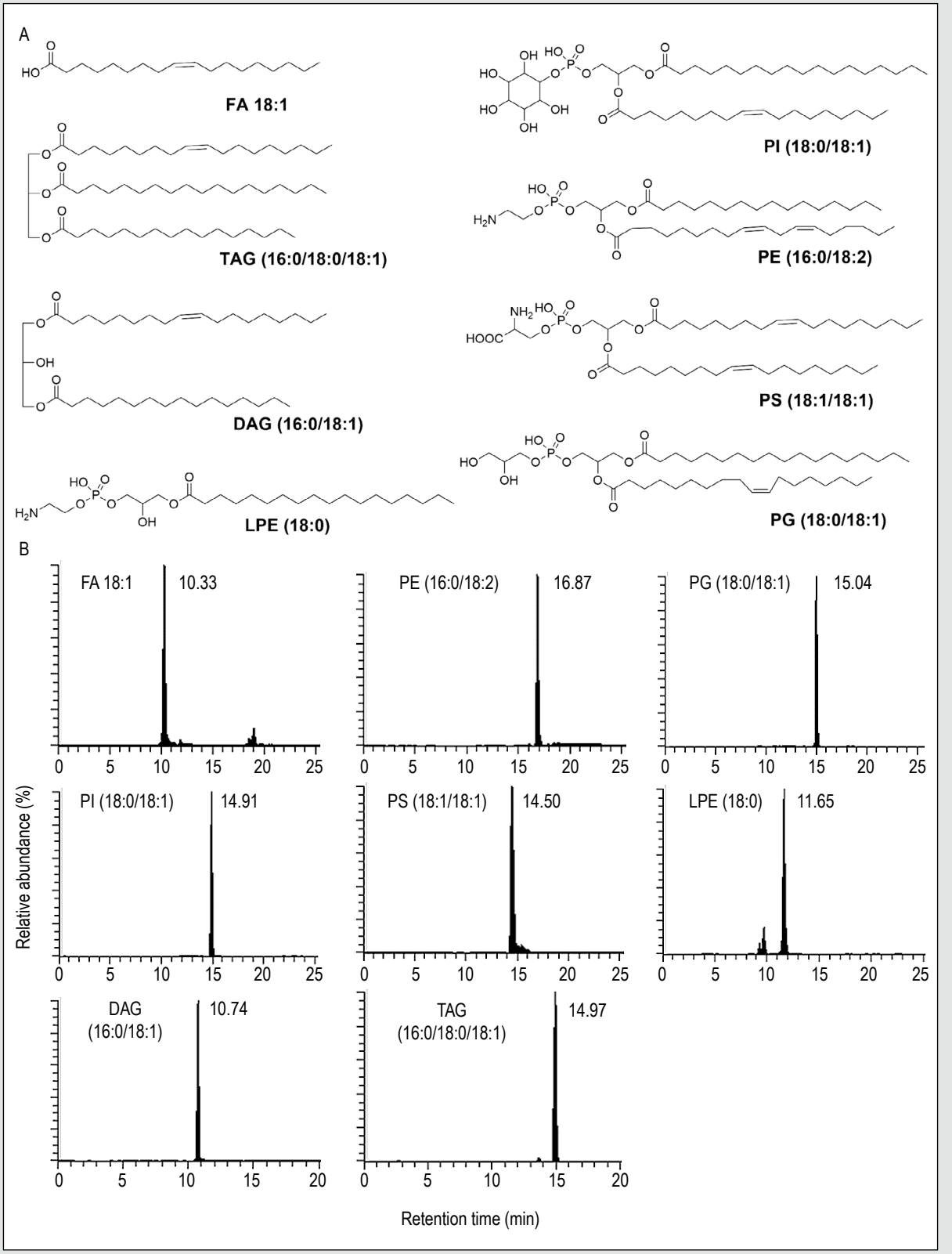

Figure 2. (A) Chemical structures of representative lipids from each class such as free fatty acid (FA 18:1), triacylglycerol (TAG 16:0/18:0/18:1), diacylglycerol (DAG 16:0/18:1), lysophosphatidylethanolamine (LPE 18:0), phosphatidylethanolamine (PE 18:0/18:1), phosphatidylinositol (PI 18:0/18:1), phosphatidylserine (PS 18:1/18:1) and phosphatidylglycerol (PG 18:0/18:1). (B) Extracted ion chromatograms of the representative lipids from each class.

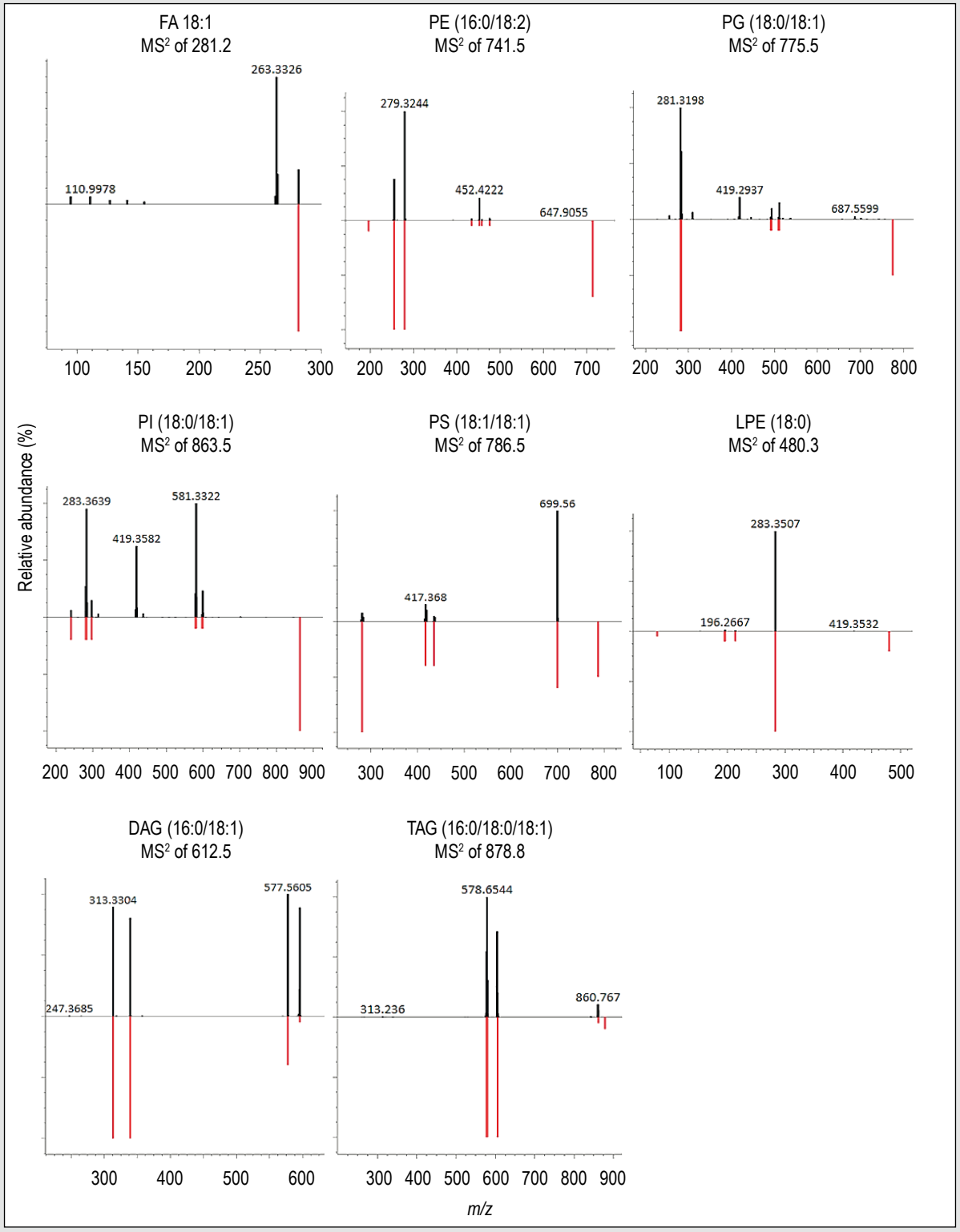

Figure 3. The MS/MS spectra of the representative lipids characterised in *Tenebrio molitor*.

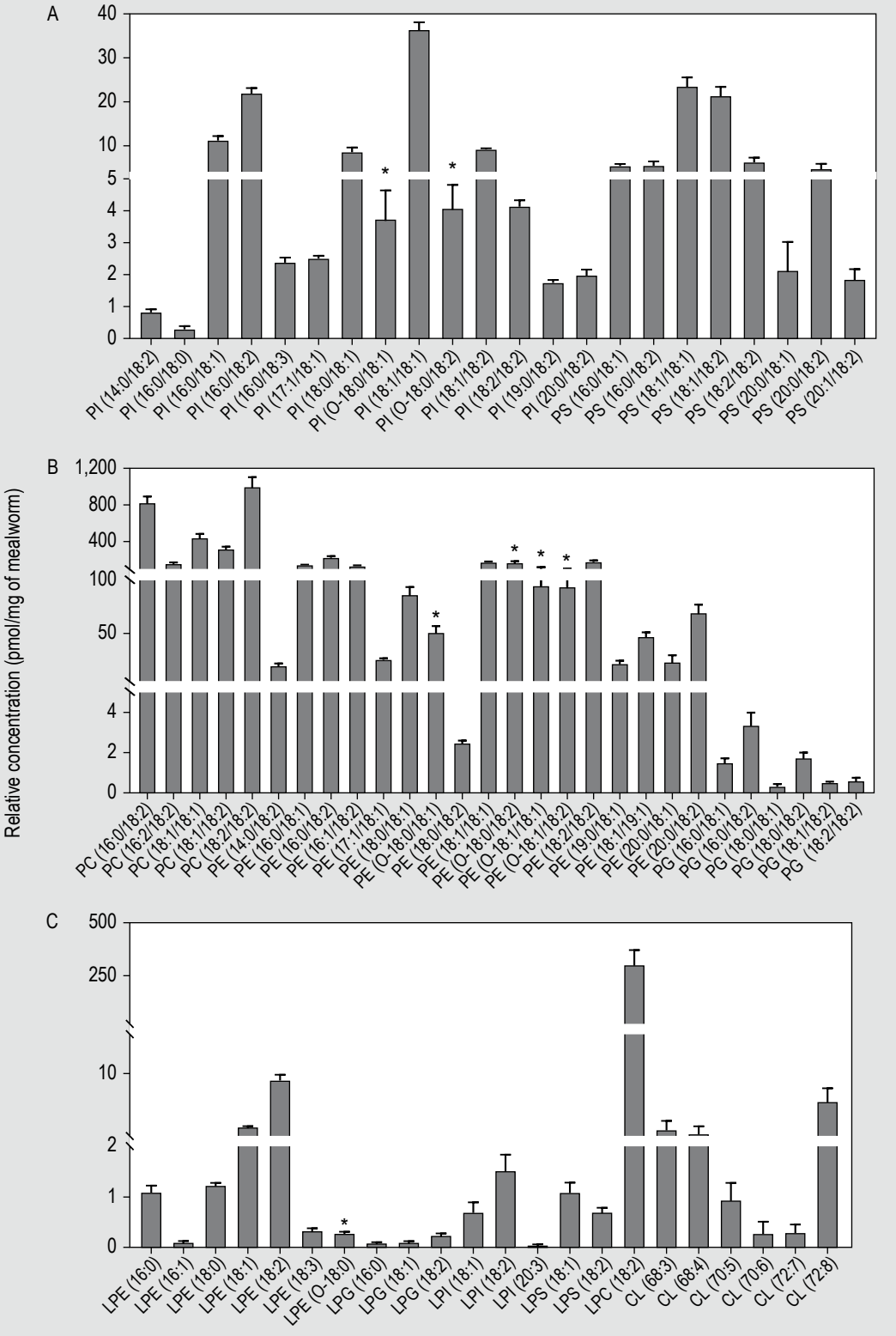

Figure 4. The concentrations of (A) phosphatidylinositol (PI) and phosphatidylserine (PS), (B) phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylglycerol (PG), and (C) lysophospholipids and cardiolipins (CL). [*denotes the O-alkyl chain containing lipids].

pmol. The feeding of lysophospholipids has been shown to exert a positive effect on the growth of animals (Huo *et al.*, 2019), suggesting the importance of *T. molitor* as a nutritive animal feed product. Further, more complex phospholipids such as cardiolipins were also characterised in yellow mealworms for the first time, with CL (72:8) being the most abundant species (Figure 4C). Moreover, cardiolipins characterised in yellow mealworms are highly unsaturated. The concentrations of cardiolipins per milligram of mealworm ranged from 0.3±0.1 to 6.4±0.8 pmol). Cardiolipins are known to be biologically important lipids for energy metabolism (Dolinsky *et al.*, 2016). The identification of these functional lipids will be extremely helpful in considering yellow mealworms as a functional food for animals and humans.

The concentrations of triacylglycerols, a major category of glycerolipids characterised from *T. molitor,* are shown in Figure 5A. The concentrations of triacylglycerols per milligram of mealworms ranged from 10.4±3.9 to 684.1±29.3 pmol. Triacylglycerols are the most abundant lipids relative to concentration among all the characterised lipids, and these results are consistent with a previous study which demonstrated the triacylglycerol profiles, determined by the equivalent carbon number method. This helped understand the size distribution of the triacylglycerol in lipids and their thin-layer chromatography profile in *T. molitor* (Tzompa-Sosa *et al.*, 2014). Interestingly, our LC/MS analysis results demonstrated that triacylglycerols with monounsaturated or polyunsaturated fatty acids are the major molecular species, with TAG 50:4 being the most abundant relative to their concentration. The high concentrations of polyunsaturated fatty acid derived-triacylglycerols again suggest that *T. molitor* is a potential source of high energy that can be useful for developing food and animal feed products.

The concentrations of free fatty acids ranged from 0.4±0.1 to 161.8±41.6 pmol and show the highest value for FA 18:1 and FA18:2 followed by FA 18:0 and FA 18:3 respectively (Figure 5B). PUFAs were the most abundant fatty acids (45%), followed by MUFAs (42%), and saturated fatty acids (13%). These results are consistent with previous reports that showed the abundance of PUFAs as major fatty acids in *T. molitor* (Stanley-Samuelson and Dadd, 1983; Tzompa-Sosa *et al.*, 2014) and MUFAs in their larvae of (Alves *et al.*, 2016). However, this composition could alter with the nature of the diet fed to mealworms (Van broekhoven *et al.*, 2015). Previous studies have demonstrated that diet has a direct influence on larval fat content and significantly changes the total fatty acid content (Alves *et al.*, 2016; Dreassi *et al.*, 2017; Fasel *et al.*, 2017; Tzompa-Sosa *et al.*, 2014). The comparison of proximate lipid content among various edible Thai terricolous insects also showed species-specific changes ranging from 5.4% in June beetle to 36.87% in queen caste (Raksakantong *et al.*, 2010). However, in this study, we did not explore any diet- or species-based lipid compositional changes, which should be focused on in the future.

The fatty acid FA 18:3 could be a bio-functional lipid as it generally refers to alpha-linolenic acid or gamma-linolenic acid and serves as a precursor for the biosynthesis of highly unsaturated PUFAs, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Although FA 18:3 is abundant in yellow mealworms, we did not detect any DHA or EPA, which are generally trace components in insects (Fontaneto *et al.*, 2011). Furthermore, the limitation of our study is that it is not an absolute lipid quantitation (generally carried out by constructing calibration curves in a targeted approach), and concentrations were expressed relative to the appropriate internal standard of the lipid subclass. Previously, odd-chain fatty acids such as FA 13:0, FA 15:0, FA 17:0, and FA 17:1 were identified in *T. molitor* pupae (Dreassi *et al.*, 2017), and in this study, in addition to these, we also detected other odd-chain fatty acids such as FA 19:0, and FA 19:1 respectively.

The concentrations of other lipid molecular species including sphingolipids and diacylglycerols, are shown in Figure 5C. In the sphingolipid class, sphingomyelins (298±58 to 4,944±761 pmol/mg of mealworm) are found to be the most abundant as compared to ceramides (0.21±0.07 to 0.92±0.11 pmol/mg of mealworm), with the highest concentration of SM (32:1;2O). Sphingomyelins are generally known to be involved in membrane function and structural organisation (Slotte and Ramstedt, 2007). To the best of our knowledge, this is the first study to characterise the sphingolipid molecular species in *T. molitor.* Dietary sphingolipids are often considered to have potential health benefits, including reduced chronic inflammation (Norris and Blesso, 2017). Furthermore, the concentrations of diacylglycerols ranged from 151±16 to 392±28 pmol/mg of mealworm. It has been known that diacylglycerols are the main form by which lipids are transported in insects (Horne *et al.*, 2009). Although diacylglycerols have been previously detected in *T. molitor* by thin-layer chromatographic analysis (Tzompa-Sosa *et al.*, 2014), this is the first study to report the molecular species with C_{34} and C_{36} total carbon chains by LC-MS analysis.

4. Conclusions

In conclusion, the untargeted total lipid profile of yellow mealworm larvae using UHPLC/LTQ Orbitrap MS led to the identification of several classes of functional lipids, including sphingomyelin, ceramide, cardiolipin, phosphatidylinositol, and phosphatidylethanolamines. The characterisation and distribution of functional lipids such as phospholipids, glycerolipids, free fatty acids, and sphingolipids suggest the potential use of *T. molitor* as a good nutritive source for developing food and animal

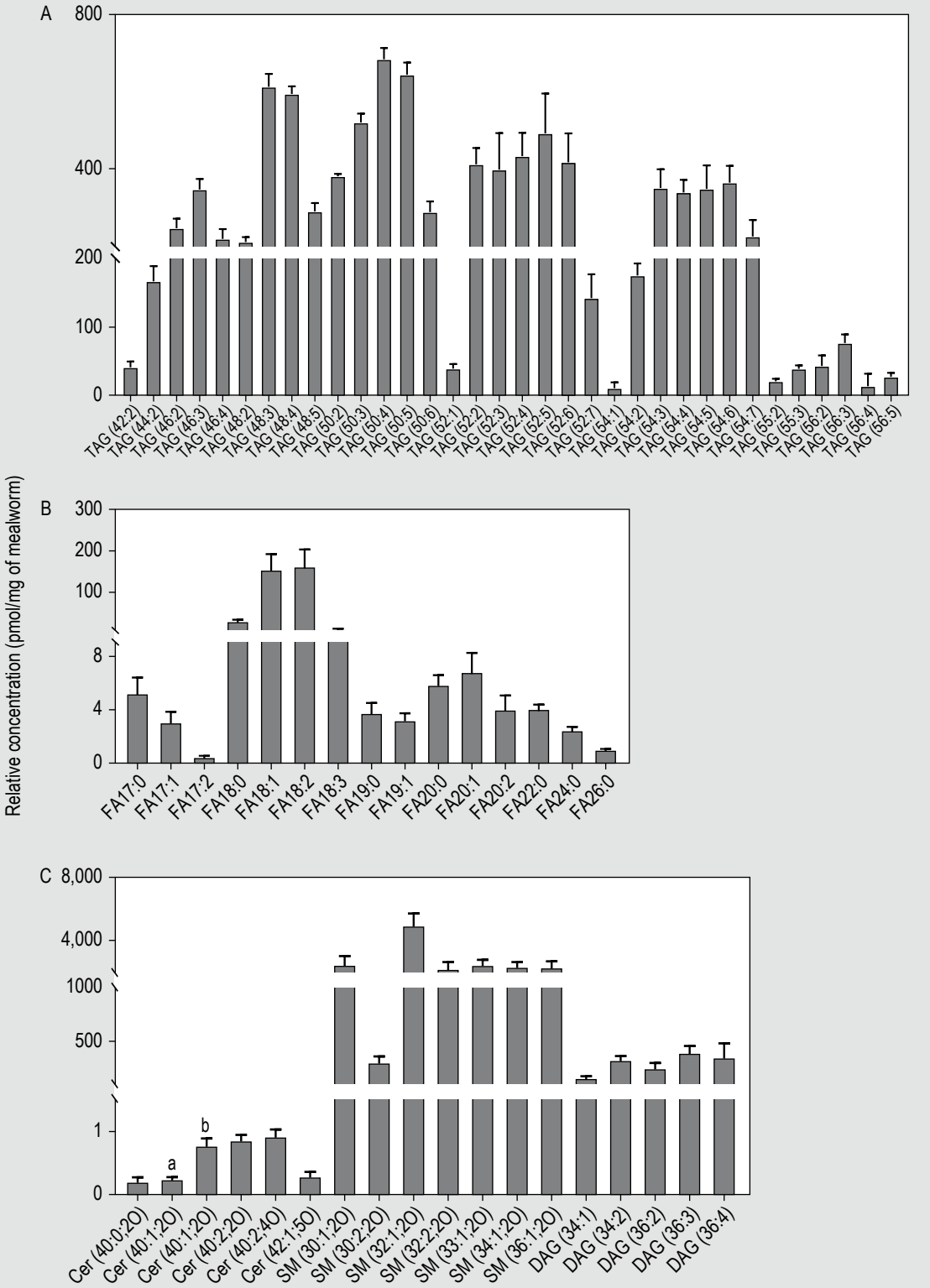

Figure 5. The concentrations of (A) triacylglycerols (TAG), (B) free fatty acids (FFA), (C) ceramides (Cer), sphingomyelin (SM), and diacylglycerols (DAG) in *T. molitor*. A and B are the $[M+CH3COO]^-$ and $[M-H]^-$ ions of Cer (40:1;2O).

feed products. This is the first study to elucidate the comprehensive lipid profiles of *T. molitor*, which may be highly beneficial for the insect industry to develop it as a functional food. Further investigations are needed on the biosynthesis of these lipids and their enrichment technologies.

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Conflicts of interest

The authors declare no conflict of interest.

Supplementary material

Supplementary material can be found online at https://doi.org/10.3920/JIFF2020.0119.

Table S1. List of lipid molecular species identified in mealworm.

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