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Comprehensive Identification of Principal Lipid Classes and Tocochromanols in Silkworm (*Antheraea pernyi* and *Bombyx mori*) Pupae Oils

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A comprehensive identification of lipid compositions and tocochromanols in *Antheraea pernyi* (*A. pernyi*) and *Bombyx mori* (*B. mori*) pupae oil is reported in the present study. Fatty acid profiling shows that both oils contain high levels (79.67% vs 71.11%) of unsaturated fatty acids, especially linoleic acid and linolenic acid. Moreover, linolenic acid is preferentially enriched at the *sn*-2 positions of triacylglycerols (TAGs). Liquid chromatograph-mass spectrometry (LC-MS) analysis demonstrates that POO (TAG with one palmitoyl and two oleoyls) is the primary TAG form with percentages of 20.18% in *A. pernyi* and 15.00% in *B. mori*. The dominating phospholipid species are phosphatidylcholine (PC, 30.40% vs 54.61%) and phosphatidylethanolamine (PE, 34.82% vs 20.39%). Four sterol constituents with total contents of 382.56 $\pm$ 3.12 and 371.65 $\pm$ 2.98 μg g^{-1} are identified and analyzed quantitatively. Additionally, the levels of tocochromanols (20.15 $\pm$ 0.89 vs 17.15 $\pm$ 0.71 mg g^{-1}) are quantified in both silkworm pupae oils. Overall, silkworm oil acts as an enriched source of functional lipids and tocochromanols.

Practical Applications: **A systematic investigation on the principal lipid classes and tocochromanols of *Antheraea pernyi* pupae and *Bombyx mori* pupae oil is reported in this study. The informative data provide supporting evidence for comprehensive utilization of silkworm oil for production of nutritional and healthy products.**

1. Introduction

The sericulture development paved the way for China's engagement with global trade on Silk Road 2000 years ago. Silkworms still act as an efficient large-scale producer of silk thread nowadays. Most of the commercially available silkworms, including mulberry *Bombyx mori* (*B. mori*), non-mulberry *Antheraea pernyi* (*A. pernyi*), and *Samia cynthia ricina* (*S. cynthia ricina*) are harvested in China, India, and Thailand. In recent 5 years, the average annual production of silkworm pupae has reached up to almost 450 000 tons in China.[1] However, tons of silkworm pupae were only used as fertilizers, fodder, and even disposed as industrial wastes after the extraction of silk threads.[2] The potential functions and nutritive value of silkworm pupae have not been fully recognized.[3] Recent publications demonstrate that silkworm pupae are characterized by high levels of protein, lipids, and biologically active substances such as vitamin.[2,4–6] It is documented that the *A. pernyi* pupae powders contain 71.9% of crude protein, compared with 48–60% in *B. mori* pupae, with all essential amino acids included.[7] The Ministry of Health and State Food and Drug Administration PR China has approved the use of several new functional foods and medicines derived from silkworm pupae.[8] Moreover, the contents of total lipids in *A. pernyi* pupae and *B. mori* pupae reach 28–35% and 25–31%, respectively. Specifically, unsaturated fatty acids, especially oleic acid, linoleic acid, and α-linolenic acid (ALA), are highly enriched in both resources.[2,9] Admittedly, dietary ALA intake relates to lower risk of cardiovascular diseases, coronary heart disease, myocardial infarction, coronary artery disease, and carotid atherosclerosis.[10] ALA also serves as a natural precursor of eicosapentanoic acid (EPA) and docosahexanoic acid (DHA), both of which associate with an array of health benefits including anti-inflammation, fetal development, and healthy aging.[11] Therefore, silkworm pupae oil is considered a healthier edible oil than some common commercialized plant oils.

It is generally acknowledged that both lipid compositions and bioactive components (e.g., tocochromanols) in edible oil play crucial roles in the oxidation stability and health benefits.[12] However, to the best of our knowledge, with total fatty acid compositions being extensively investigated, a detailed analysis of the lipids in *A. pernyi* and *B. mori* pupae oil can be hardly found. The bioactive compounds have not been fully elucidated yet, either. Therefore, this research focuses on the characterization of the lipid compositions and the bioactive compounds in *A. pernyi* and *B. mori* pupae oil. The characterization includes new data of

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neutral, polar lipid classes and identification of fatty acid compositions and positional distribution. Additionally, tocochromanols were analyzed qualitatively and quantitatively.

2. Experimental Section

2.1. Materials

Fresh *A. pernyi* Guérin-Méneville and *B. mori* Linnaeus were obtained from The Sericulture Farm & Farm Produce Processing Research Institute of Guangdong Academy of Agricultural Sciences (Guangdong, China). (−)-α-Tocotrienol, σ-tocopherol, (±)-α-tocopherol, plant sterol mixtures, and cholesterol were purchased from Shanghai ZZBIO Co., Ltd (Shanghai, China). Mixtures of trioleoyl glycerol, dioleoyl glycerol (15% of *sn*-1,2 dioleoyl glycerol and 85% of *sn*-1,3 dioleoyl glycerol), oleic acid, and 37 fatty acid methyl esters were purchased from Sigma-Aldrich (Shanghai, China). Isopropanol, *n*-heptane, *n*-hexane, formic acid, and tetrahydrofuran were of liquid chromatography-mass spectrometry (LC-MS) grade. Other organic reagents were of analytical grade.

2.2. Methods

2.2.1. Lipid Extraction

The silkworm pupae samples were dried in vacuum at 60 °C for 24 h till the moisture content was less than 5%, which was determined according to the Chinese standard method (GB 5009.3-2016, National Standard of China). The dried samples were slightly grinded and sieved by a 60-mesh sieve. Subsequently, lipid extraction was conducted according to the conventional method of Folch and Sloane.[13] Briefly, the silkworm pupae powder (10 g) was mixed with 140 mL of chloroform/methanol (2:1, v/v). The lipid extractions were performed in a water bath thermostat at 50 °C for 2 h and filtered. Saturated NaCl solution (30 mL) was then added into the filtrate. The lower organic layer was transferred to another clean flask and evaporated with a rotary evaporator at 45 °C. The total lipids were stored at 4 °C for subsequent analysis after the yield being calculated.

2.2.2. Analysis of the Lipid Composition by HPLC

The acylglycerol compositions were analyzed by a normal-phase high performance liquid chromatography (NP-HPLC, refractive index detector) equipped with a Phenomenex Luna silica column (250 mm × 4.6 mm i.d., 5 µm particle size) with the column temperature of 30 °C. The mobile phase was a mixture of *n*-hexane, 2-propanol, and formic acid (21:1:0.003, v/v/v) with a flow rate of 1 mL min^{-1}.

2.2.3. Lipidomic Analysis by LC-MS

Five microliters of each sample was injected by the autosampler and eluted using a Waters CORTECS C18 column (150 mm × 2.1 mm i.d., 2.7 µm particle size) with a gradient mobile phase system consisting of solvents (A): H_2O:acetonitrile = 40:60 (v/v) containing 0.1% formic acid and 10 mM ammonium formate, and (B) 2-propanol:acetonitrile = 90:10 (v/v) containing 0.1% formic acid and 10 mM ammonium formate at a flow rate of 0.4 mL min^{-1}. Samples were applied to the column at 80% A for 0.2 min, and eluted with linear decrease in A (80–40% from 0.2 to 2 min), then reached 0% at 9 min, and maintained at 0% for 1 min. Finally, A was linearly increased to 80% from 10 to 10.5 min followed by equilibration at 80% for 3.5 min.

The following key MS parameters were used: electrospray voltage was 3.5 kV (positive) and −3.0 kV (negative), with the following ion-source properties: sheath gas 45, auxiliary gas 12, sweep gas 1, ion transfer tube temperature 320 °C, vaporizer temperature 350 °C. All MS data were acquired using the following conditions: scan range, m/z 200–1200; intensity threshold, 5.0×10^4; detector type, Orbitrap; RF lens, 60%; automated gain control (AGC) target, 5.0×10^5; maximum injection time, 100 ms; microscans, 1; data type, profile; and polarity, positive. The orbitrap resolution was set at 120 000. All ddMS2 data were acquired using the following conditions: isolation mode, quadrupole; activation type, high energy collision induced dissociation (HCD); detector type, Orbitrap; Orbitrap resolution, 30 000; first mass, m/z 50; AGC target, 5.0×10^4; maximum injection time, 100 ms; microscans, 1; and data type, profile. The HCD collision energy was set to 25%.

2.2.4. Analysis of the Fatty Acid Compositions by GC-FID

All lipids were methylated to fatty acid methyl esters according to the standard method of ISO 5509:2000, and then analyzed by gas chromatography-flame ionization detector (GC-FID) equipped with an Agilent HP-88 column (100 m × 0.25 mm, 0.2 µm film thickness). With respect to fatty acyls at *sn*-2 positions of triacylglycerol (TAG), analysis was carried out according to the method of ISO 6800: 1997. Measurements were performed with nitrogen as carrier gas with a flow rate of 25 mL min^{-1}, hydrogen flow rate of 45 mL min^{-1}, and air flow rate of 450 mL min^{-1}. Injections (1 µL) in split mode (100:1) were carried out at 250 °C, with the detector temperature of 260 °C. The GC oven program started at 140 °C for 5 min. Then, the temperature ramped to 210 °C at 5 °C min^{-1} and was held for 15 min. The final temperature of 230 °C was reached with a ramp of 3 min $°C^{-1}$ and held for 20 min. Fatty acid methyl esters were identified by comparison of retention times of standards.

2.2.5. Analysis of Tocochromanols by HPLC

The detection of tocochromanols was carried out by HPLC equipped with a Silica column (4.6 mm × 250 mm i.d., 5 µm particle size) with a flow rate of 1 mL min^{-1} according to the method of ISO 9936: 2006. The temperature of column oven was 30 °C. The mobile phase was *n*-heptane containing 3.85% tetrahydrofuran.

2.2.6. Analysis of Sterols by GC-MS

After the silkworm pupae oil being saponated, the remaining unsaponifiables dissolved in ethanol were analyzed by gas

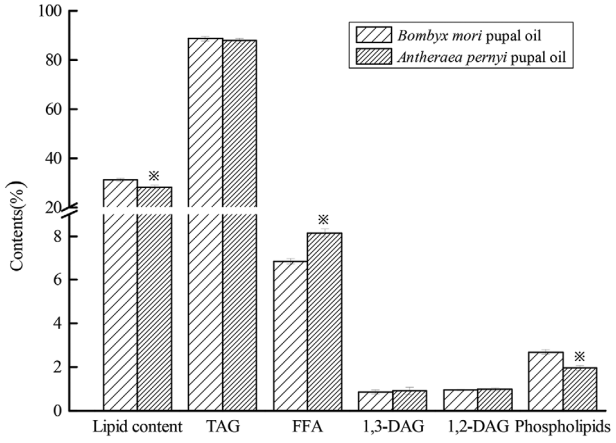

Figure 1. Lipid classes in *A. pernyi* pupae oil and *B. mori* pupae oil.

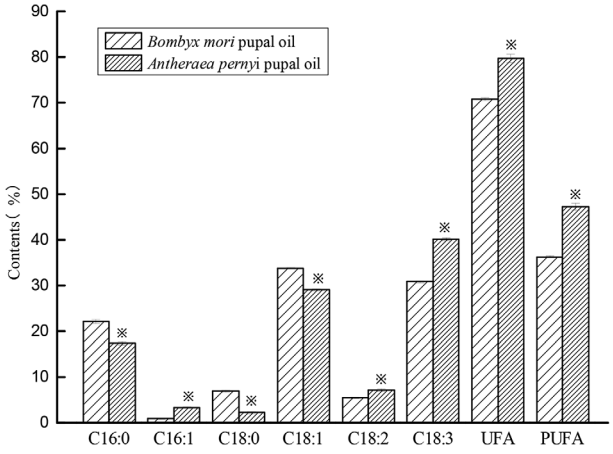

Figure 2. Fatty acid compositions of TAG in *A. pernyi* pupal oil and *B. mori* pupal oil.

chromatography-mass spectrometry (GC-MS) equipped with an Agilent DB-5MS UI column (30 m × 0.25 mm, 0.25 μm film thickness). All measurements in the full-scan mode (m/z 30–550) were conducted with the following oven program: the temperature raised to 280 °C with a ramp of 15 °C min^{-1} from initial 180 °C and was held for 25 min. The injector temperature was 280 °C; the carrier gas was helium gas with a flow rate of 25 mL min^{-1}. Injections (1 μL) in split mode (10:1) were performed with an autosampler.

2.3. Statistical Analysis

All experiments were carried out in triplicate and the data were expressed as the mean ± standard deviation. The statistical significance of differences between the groups was measured by a one-way analysis of variance (ANOVA). The significance of differences was conducted by the *t*-test ($p < 0.05$) of statistical product and service solutions (SPSS) software.

3. Results and Discussion

3.1. Lipid Classes

The lipid compositions of *A. pernyi* and *B. mori* pupae oil are illustrated in **Figure 1**, which clearly displays a remarkable similarity of lipid classes between the above two oil samples. However, a significant difference ($p < 0.05$) was observed with respect to the total lipid levels, which accounted for 31.31% and 28.29% of dry weight in *A. pernyi* pupae and *B. mori* pupae, respectively. The primary lipids are triglycerides in both *A. pernyi* pupae and *B. mori* pupae with concentrations of more than 87%. In addition, HPLC analysis also demonstrated that the silkworm pupae oil contained a relatively high content of free fatty acids (FFAs, 5–10%), a few of *sn*-1,3-DAG, *sn*-1,2-DAG (≈1%), and phospholipids (2–4%). In detail, the *B. mori* pupae oil had a slightly higher level of FFA (≈8%), compared with ≈7% in *A. pernyi* pupae oil. Yet any significant differences with regard to the content of *sn*-1,3-DAG and *sn*-1,2-DAG were not observed between *A. pernyi* pupae oil and *B. mori* pupae oil ($p > 0.05$). *B. mori* pupae oil (2.69%) was 1.37 times the phospholipid content of *A. pernyi* pupae oil (1.96%).

Table 1. Comparison of fatty acid compositions (wt% of total fatty acids) in different animal oils.

Oil	Saturated fatty acid	Unsaturated fatty acid	Linolenic acid	Ref
Beef tallow	57.00	57.33	0.63	[16]
Chicken fat	39.60	39.58	1.80	[16]
Pork lard	39.70	39.74	3.38	[16]
Mutton tallow	40.00	47.60	2.50	[17]
Fish oil	≥31.00	≤63.70	≤3.60	[18]
Silkworm pupae oil	≤28.88	≥71.11	≥30.81	This paper

3.2. Fatty Acid Profiles

The fatty acid profiles of *A. pernyi* pupae oil and *B. mori* pupae oil are illustrated in **Figure 2**. The same fatty acid compositions could be observed between these two oil samples, both of which contained high levels of unsaturated fatty acids. The contents of unsaturated fatty acids showed a significant difference ($p < 0.05$), with 79.67% in *A. pernyi* pupae oil and 71.11% in *B. mori* pupae oil. Specifically, the unsaturated fatty acids were mainly composed of oleic acid, linoleic acid, and linolenic acid, with linoleic acid and linolenic acid as main polyunsaturated fatty acids. The total level of polyunsaturated fatty acids in *A. pernyi* pupae oil (47.28%) was significantly higher than that in *B. mori* pupae oil (36.28%). The content of linoleic acid and linolenic acid in *A. pernyi* pupae oil reached up to 40.12% compared with 30.88% in *B. mori* pupae oil. The concentrations of saturated fatty acids and unsaturated fatty acids in common animal oils were compared with the special focus on linolenic acid, the results of which are demonstrated in **Table 1**. It can be observed that the percentage of unsaturated fatty acids in silkworm pupae oil was higher than that in all the other animal oils. Remarkably, linolenic acid in silkworm pupae oil was more than ten times the levels in other animal oils. It has been generally acknowledged that linoleic acid and linolenic acid are essential fatty acids in human bodies.

Table 2. Fatty acid compositions (wt% of total fatty acids) and position distribution of total fatty acids of TAG isolated from silkworm pupae oil.

Fatty acid	*A. pernyi* pupae oil		*B. mori* pupae oil	
	Total	*sn*-2 position	Total	*sn*-2 position
Palmitic acid C16:0	17.25 ± 0.32	8.61 ± 0.12	22.04 ± 0.49	2.47 ± 0.09
Palmitoleic acid C16:1	3.42 ± 0.10	1.14 ± 0.08	0.92 ± 0.01	0.55 ± 0.05
Stearic acid C18:0	2.23 ± 0.12	ND	6.84 ± 0.23	1.42 ± 0.11
Oleic acid C18:1	29.15 ± 0.26	29.67 ± 0.57	33.91 ± 0.08	39.22 ± 0.76
Linoleic acid C18:2	7.14 ± 0.12	7.29 ± 0.14	5.48 ± 0.14	5.70 ± 0.12
Linolenic acid C18:3	40.28 ± 0.35	53.29 ± 0.43	30.81 ± 0.21	51.24 ± 0.37
Unsaturated fatty acid	79.67 ± 0.52	91.39 ± 0.65	71.11 ± 0.31	96.71 ± 0.45
Polyunsaturated fatty acid	47.28 ± 0.48	60.58 ± 0.43	36.29 ± 0.26	56.94 ± 0.43

ND: not detected.

Additionally, linolenic acid acts as an important precursor for the synthesis of long-chain polyunsaturated fatty acids such as EPA and DHA, which have functions of anti-atherosclerosis and protect neurons.[14,15] Therefore, silkworm pupae oil as a new insect oil resource rich in unsaturated fatty acid, especially linolenic acid, will serve as a promising functional edible oil in oil industry.

3.3. Fatty Acid Distribution of TAG

It is documented that the functions and health benefits of TAG are greatly affected by not only the total fatty acid compositions, but also fatty acids at the *sn*-2 position of the glycerol backbone.[19] Therefore, fatty acid profiles were investigated by GC-FID with special emphasis on the fatty acids at the *sn*-2 positions of TAG after the crude silkworm pupae oil was deacidified and degummed. A very low acid value (0.18 mg KOH per g) was determined. The results are shown in **Table 2**. As can be seen in Figure 1, TAG is the dominating lipid class in the silkworm pupae oil. It was demonstrated that TAG had similar fatty acid species with the total lipids. However, the proportion of unsaturated fatty acids at the *sn*-2 position was significantly higher than the total levels in TAG. This is consistent with the composition of the neutral lipids in the *S. cynthia ricina* pupae oil.[20] More obviously, this characteristic was also observed in *B. mori* pupae oil. Compared with total contents of unsaturated fatty acids in neutral lipids, the levels at the *sn*-2 position increased by 11.72% in *A. pernyi* pupae oil, with an increment of 25.60% in *B. mori* pupae oil. Moreover, in *B. mori* pupae oil, palmitic acid (22.04%) and stearic acid (6.84%) in neutral lipids were higher than those (palmitic acid of 2.47% and stearic acid of 1.42%) at the *sn*-2 position, while oleic acid (33.91%) and linolenic acid (30.81%) were significantly lower than those (oleic acid of 39.22% and linolenic acid of 51.24%) at the *sn*-2 position. In *A. pernyi* pupae oil, palmitic acid (17.25%) and palmitoleic acid (3.42%) in neutral lipids were higher than palmitic acid (8.61%) and palmitoleic acid (1.14%) at the *sn*-2 position, whereas linolenic acid (40.28%) was significantly lower than that (53.29%) at the *sn*-2 position.

Table 3. TAG profiles of *A. pernyi* pupae oil and *B. mori* pupae oil.

Triacylglycerols	Abbreviation	Molecular formula	*A. pernyi* pupae oil [wt%]	*B. mori* pupae oil [wt%]
TAG (16:0/16:0/18:1)	PPO	$C_{53}H_{100}O_6$	ND	5.58 ± 0.08
TAG (16:0/16:0/18:3)	PPLn	$C_{53}H_{96}O_6$	0.82 ± 0.05	4.55 ± 0.11
TAG (16:0/18:0/18:1)	PSO	$C_{55}H_{104}O_6$	ND	3.78 ± 0.10
TAG (16:0/16:1/18:1)	PPaO	$C_{53}H_{98}O_6$	3.83 ± 0.13	0.88 ± 0.07
TAG (16:0/16:1/18:3)	PPaLn	$C_{53}H_{94}O_6$	2.55 ± 0.11	0.51 ± 0.06
TAG (16:0/18:1/18:1)	POO	$C_{55}H_{102}O_6$	20.18 ± 0.75	15.00 ± 0.16
TAG (16:0/18:1/18:2)	POL	$C_{55}H_{100}O_6$	ND	3.99 ± 0.10
TAG (16:0/18:1/18:3)	POLn	$C_{55}H_{98}O_6$	2.68 ± 0.09	13.40 ± 0.18
TAG (16:0/18:2/18:3)	PLLn	$C_{55}H_{96}O_6$	4.02 ± 0.17	2.49 ± 0.09
TAG (16:0/18:3/18:3)	PLnLn	$C_{55}H_{94}O_6$	14.22 ± 0.30	10.18 ± 0.19
TAG (16:1/18:3/18:3)	PaLnLn	$C_{55}H_{92}O_6$	4.48 ± 0.12	ND
TAG (18:0/18:1/18:1)	SOO	$C_{55}H_{106}O_6$	4.09 ± 0.17	2.57 ± 0.07
TAG (18:0/18:1/18:3)	SOLn	$C_{55}H_{102}O_6$	5.58 ± 0.21	ND
TAG (18:1/18:1/18:1)	OOO	$C_{55}H_{104}O_6$	4.72 ± 0.13	2.65 ± 0.05
TAG (18:1/18:1/18:2)	OOL	$C_{55}H_{102}O_6$	ND	3.14 ± 0.07
TAG (18:1/18:1/18:3)	OOLn	$C_{55}H_{100}O_6$	0.78 ± 0.04	2.68 ± 0.06
TAG (18:1/18:2/18:3)	OLLn	$C_{55}H_{98}O_6$	8.80 ± 0.14	3.81 ± 0.11
TAG (18:1/18:3/18:3)	OLnLn	$C_{55}H_{96}O_6$	1.83 ± 0.22	11.70 ± 0.20
TAG (18:3/18:2/18:3)	LnLLn	$C_{55}H_{94}O_6$	10.54 ± 0.15	2.96 ± 0.07
TAG (18:3/18:3/18:3)	LnLnLn	$C_{55}H_{92}O_6$	8.71 ± 0.09	7.46 ± 0.14
Others	—	—	2.17 ± 0.05	2.67 ± 0.09

P, palmitic acid; S, stearic acid; Pa, palmitoleic acid; O, oleic acid; L, linoleic acid; Ln, linolenic acid; ND, not detected.

3.4. Triacylglycerol Profiles

The quality and nutritional value of lipids are related to not only fatty acid compositions, but also the chemical structure of glycerides.[21] Currently, the development of the state-of-the-art shotgun lipidomics and lipidomics based on LC-MS and LC-MS/MS enables the systematic analysis of lipid classes and species. The LC-MS analysis showed that *B. mori* pupae oil was mainly composed of 18 kinds of glycerides compared with 16 in *A. pernyi* pupae oil (**Table 3**). The TAG in *B. mori* pupae oil mainly consisted of POO (15.00%), POLn (13.40%), PLnLn (10.18%), and OLnLn (11.70%), while the main components in *A. pernyi* pupae oil were POO (20.18%), PLnLn (14.22%), and LnLLn (10.54%). In addition, *B. mori* pupae oil and *A. pernyi* pupae oil had comparable levels of LnLnLn (TAG with three linolenic acyls) with 7.46% in the former and 8.71% in the latter.

3.5. Phospholipid Profiles

As mentioned above, the phospholipid species and fatty acid compositions in *A. pernyi* pupae oil and *B. mori* pupae oil were also analyzed by LC-MS, the results of which are demonstrated in **Table 4**. Phosphatidylcholine (PC) (54.61%) acted as the primary phospholipid species in *B. mori* pupae

Table 4. Phospholipid profiles of *A. pernyi* pupae oil and *B. mori* pupae oil.

Phospholipids	Composition	*A. pernyi* pupae oil [wt%]	Total [wt%]	*B. mori* pupae oil [wt%]	Total [wt%]
LPC	LPC (15:0)	ND	20.99 ± 0.33	12.49 ± 0.16	14.67 ± 0.12
	LPC (18:0)	3.74 ± 0.10		ND	
	LPC (18:1)	8.45 ± 0.13		1.16 ± 0.12	
	LPC (18:2)	2.71 ± 0.14		ND	
	LPC (18:3)	6.09 ± 0.27		1.02 ± 0.09	
LPE	LPE (15:0)	3.64 ± 0.13	12.49 ± 0.16	0.89 ± 0.07	4.62 ± 0.22
	LPE (16:0)	ND		0.69 ± 0.05	
	LPE (18:0)	ND		0.85 ± 0.06	
	LPE (18:1)	5.12 ± 0.19		2.19 ± 0.16	
	LPE (18:2)	2.40 ± 0.08		ND	
	LPE (18:3)	1.33 ± 0.07		ND	
PC	PC (16:0/18:0)	1.48 ± 0.12	30.41 ± 0.08 (ALA-PC, 8.59 ± 0.45)	ND	54.61 ± 0.78 (ALA-PC, 35.63 ± 0.49)
	PC (16:0/18:1)	6.87 ± 0.21		6.17 ± 0.15	
	PC (16:0/18:2)	2.20 ± 0.15		2.62 ± 0.10	
	PC (16:0/18:3)	5.32 ± 0.21		7.38 ± 0.32	
	PC (18:0/18:1)	2.92 ± 0.09		3.62 ± 0.11	
	PC (18:0/18:2)	ND		6.57 ± 0.20	
	PC (18:1/18:1)	8.35 ± 0.26		ND	
	PC (18:0/18:3)	0.87 ± 0.09		16.21 ± 0.26	
	PC (18:1/18:3)	0.92 ± 0.06		5.21 ± 0.21	
	PC (18:2/18:3)	0.66 ± 0.08		2.65 ± 0.12	
	PC (18:3/18:3)	0.82 ± 0.06		4.18 ± 0.15	
PE	PE (16:0/18:1)	ND	34.82 ± 0.64 (ALA-PE, 25.97 ± 0.86%)	0.99 ± 0.07	20.37 ± 0.96 (ALA-PE, 13.59 ± 0.57)
	PE (16:0/18:2)	ND		0.82 ± 0.07	
	PE (16:0/18:3)	6.64 ± 0.22		3.86 ± 0.13	
	PE (18:0/18:1)	1.31 ± 0.04		1.36 ± 0.09	
	PE (18:0/18:2)	2.18 ± 0.12		3.61 ± 0.17	
	PE (18:0/18:3)	16.84 ± 0.73		7.44 ± 0.19	
	PE (18:1/18:2)	5.36 ± 0.14		ND	
	PE (18:1/18:3)	2.49 ± 0.05		0.48 ± 0.13	
	PE (18:1/18:3)	ND		0.62 ± 0.09	
	PE (18:1/18:3)	ND		1.19 ± 0.06	
PI	PI (18:0-18:3)	ND	ND	4.64 ± 0.19	4.64 ± 0.19
PS	PS (18:0/18:2)	1.29 ± 0.11	1.29 ± 0.11	ND	1.07 ± 0.12
	PS (18:1/18:1)	ND		1.07 ± 0.12	

PS, phosphatidylserine; PI, phosphatidylinositol; ND, not detected.

oil, while the most abundant species in the other sample was phosphatidylethanolamine (PE, 34.82%). In addition, higher levels of lyso-phosphatidylcholine (LPC) than lyso-phosphatidylethanolamine (LPE) could be observed in both pupae oil samples. Unexpectedly, pentadecanoic acid (15:0) was detected in phospholipids of *B. mori* pupae oil, yet it was not found in total fatty acids. This might be due to the fact that pentadecanoic acid was only present in phospholipids, the content of which was far less than neutral lipids. Therefore, the abundance was less than the limit-of-detection (LOD) of GC-FID.

3.6. The Composition and Concentration of Tocochromanols

As an important natural lipophilic antioxidant in edible oil, tocochromanols has been receiving growing attention because of the anticarcinogenic, anticancer, and anti-atherosclerosis activities.[22–24] Therefore, it is necessary to analyze the composition and concentration of tocochromanols in silkworm pupae oil. However, a systematic and comprehensive analysis of tocochromanols in *A. pernyi* pupae oil and *B. mori* pupae oil can be rarely found. Herein, HPLC analysis of tocochromanols in the above two samples is shown in **Table 5**. Obviously, the similar

Table 5. The content of tocochromanols (μg g^{-1}) in *A. pernyi* pupae oil and *B. mori* pupae oil.

Silkworm pupae oil	α-Tocopherol	β-Tocopherol	γ-Tocopherol	γ-Tocotrienol	σ-Tocopherol	Total
A. pernyi	255.00 ± 2.63^{a}	22.73 ± 0.61^{a}	29.68 ± 0.90^{a}	75.15 ± 0.79^{a}	ND	382.56 ± 3.12^{a}
B. mori	205.38 ± 1.75^{b}	38.37 ± 0.68^{b}	37.05 ± 1.12^{b}	55.34 ± 0.98^{b}	35.24 ± 1.25	371.65 ± 2.98^{b}

Different superscripts (^{a,b}) donate significant differences ($p < 0.05$); ND, not detected.

Table 6. The content of sterols (mg g^{-1}) in *A. pernyi* pupae oil and *B. mori* pupae oil.

Silkworm pupae oil	Campesterol	Cholesterol	β-Sitosterol	Stigmasterol	Total
A. pernyi	0.18 ± 0.02^{a}	16.46 ± 0.84^{a}	3.36 ± 0.19	0.15 ± 0.01^{a}	20.15 ± 0.89^{a}
B. mori	0.76 ± 0.05^{b}	12.69 ± 0.64^{b}	3.34 ± 0.16	0.33 ± 0.02^{b}	17.15 ± 0.71^{b}

Different superscripts (^{a,b}) donate significant differences ($p < 0.05$).

compositions could be observed between *A. pernyi* pupae oil and *B. mori* pupae oil. More importantly, the contents (382.56 ± 3.12 μg g^{-1} in *A. pernyi* pupae oil and 371.65 ± 2.98 μg g^{-1} in *B. mori* pupae oil) were significantly higher than those in some common edible oil resources such as olive oil (127 μg g^{-1}), peanut oil (281 μg g^{-1}), and sunflower seed oil (201 μg g^{-1}).[25] Specifically, a comparably higher concentration (255.00 ± 2.63 μg g^{-1} in *A. pernyi* pupae oil vs 205.38 ± 1.75 μg g^{-1} in *B. mori* pupae oil) of α-tocopherol can be observed, which is different from most vegetable oils, including soybean oil (80–110 μg g^{-1}) and peanut oil (151 μg g^{-1}).[26–28] Admittedly, α-tocopherol has better physiological activities and antioxidant activities than any other analogues.[29] Moreover, β-tocopherol, γ-tocopherol, and γ-tocotrienol were all found in both *A. pernyi* pupae oil and *B. mori* pupae oil, whereas σ-tocopherol was only identified in the latter.

3.7. The Composition and Concentration of Sterols

The compositions and concentrations of sterols in *A. pernyi* and *B. mori* pupae oil are displayed in **Table 6**. It was observed that the former oil had a higher level of total sterols (20.15 ± 0.89 mg g^{-1}) than the latter one (17.15 ± 0.71 mg g^{-1}). As can be seen in Table 6, cholesterol and β-sterol are the main compositions, which is in parallel with previous findings.[30] Additionally, a few of campesterols and stigmasterols were identified. Significant differences ($p \leq 0.05$) in levels of cholesterol, campesterol, and stigmasterol were noticed, while the content of β-sitosterol did not show any significant differences between the two oil samples. Obviously, the concentrations of β-sitosterol in *A. pernyi* pupae oil (3.36 ± 0.19 mg g^{-1}) and *B. mori* pupae oil (3.34 ± 0.16 mg g^{-1}) were higher than those in peanut oil (1.61–1.72 mg g^{-1}) and soybean oil (1.55–1.75 mg g^{-1}).[31,32] It is documented that β-sitosterol contributes to lowering of the levels of serum total cholesterol and low-density lipoprotein cholesterol.[33] Therefore, silkworm pupae oil acts as a potential functional oil due to the cholesterol-lowering effects.

4. Conclusion

This research comprehensively analyzed the principal classes of lipids (fatty acid profiles, fatty acid distribution in TAGs, phospholipid profiles, sterols) and tocochromanols (tocopherols and tocotrienols) in two silkworm pupae oils obtained by extraction from mulberry *A. pernyi* silkworm pupae and non-mulberry *B. mori* silkworm pupae. The findings demonstrated that the lipid classes and fatty acid compositions were similar in the above two silkworm oil samples, in which the unsaturated fatty acids are highly enriched. Most importantly, the paper showed that silkworm oil is the most abundant source of linolenic acid in commercial animal oils. Additionally, attentions were focused on tocochromanols with high antioxidant activities. The results showed that silkworm oil is an important source of ALA, much richer than commercial animal fats. Furthermore, both investigated silkworm pupae oils have been found to contain significant levels of tocochromanols (tocopherols and tocotrienols) with high antioxidant activity as well as biological activity. These observations provide us an insight into the health benefits and promising prospects of natural silkworm oil resources in edible oil.

Abbreviations

ALA, α-linolenic acid; DAGs, diacylglycerols; DHA, docosahexanoic acid; EPA, eicosapentanoic acid; FFAs, free fatty acids; LOD, limit-of-detection; LPC, lyso-phosphatidylcholine; LPE, lyso-phosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; TAGs, triacylglycerols

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

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