Proceedings of the 1st International Conference on Natural Resources Engineering & Technology 2006
24-25th July 2006; Putrajaya, Malaysia, 233-236

Neutral Lipid Profile of Sago Worm, the Larva of Weevil *Rynchophorus Ferrugineus*

Ozair Mohd Ali, K.L. Chu, and C.W. Wang*

Department of Molecular Medicine,Faculty of Medicine,University of Malaya

Abstract

The larva of the weevil *Rynchophorus ferrugineus* is popularly known as sago worm. The sago worms were purchased from the wet market in Kuching, Sarawak. The fat tissues were dissected separately from the viscera and the cuticle. The neutral lipid content of the fat tissue was extracted with 150 ml of dichloromethane by using a Soxhlet apparatus. The neutral lipid content per gm fat tissue (fresh weight) from the viscera was found to be 59.8% whereas that from the cuticle was 11.7%. The profile of triacylglycerols was analyzed by using high performance liquid chromatography (Waters 501 HPLC pump) equipped with evaporative light scattering detector (ELSD, VAREX MKIII). The HPLC column used was Supelcosil LC-18 column and the solvent system was acetonitrile /dichloromethane (68:32,v/v). The identification of triacylglycerols was carried out using purified olive and linseed oils and commercially available lipid standards. From the HPLC-ELSD chromatograms, two (2) major triacylglycerols, namely triolein (OOO) and l-palmitoyl-2-oleoyl-3-oleoyl-rac-glycerol (POO) and at least ten (10) other minor triacylglycerols were identified. The percentage (w/w) of triolein (OOO) in neutral lipid of the fat tissues from both viscera and cuticle was found to be 50% whereas that of l-palmitoyl-2-oleoyl-3-oleoyl-rac-glycerol (POO) was 41.7%. Other minor triacylglycerols identified in the neutral lipid of fat tissue from viscera were l-palmitoyl-2-oleoyl-3-linolenoyl-rac-glycerol, POLn (0.6%), 1-palmitoyl-2-linoleoyl-3-linoleoyl-rac-glycerol, PLL (0.1%), l-palmitoyl-2-palmitoyl-3-linoleoyl-rac-glycerol, PPL(1.1%) and l-palmitoyl-2-stearoyl-3-linoleoyl-rac-glycerol, PSO (2.9%) whereas that of cuticle were POLn (0.2%), PLL (1.0%), PPL(1.1%) and PSO (2.9%).

Key Words: sago worm, Rynchophorus ferrugineus, weevil, triacylglycerol analysis, HPLC- ELSD.

1.0 Materials and Methods

1.1 Fat tissues of sago worm

The sago worms or larvae of the weevil *Rynchophorus ferrugineus*, were freshly bought from the wet market in Kuching, Sarawak (Fig. 1a). There were well-packed and air-transported to Kuala Lumpur within 24 hrs. They were then kept alive at 2-4°C for 2 days. The fat tissues were dissected from the visceral and the cuticle and blotted dried with Whatman filter paper No. 1. The fat tissues are white or yellow loose ribbon-shaped fat cells lying over the haemocoel (Figure 1b).

* *Corresponding author: Tel. No. 03-79675745 Fax No. 03-79674957E-mail: wangcw@um.edu.my*

Figure 1 (a) Fresh lava of *Rhynchophorus ferrungineus* (sago worm) (b) White or yellow loose ribbon-shaped fat tissues

1.2 Lipid extraction

The total lipid content of 800 mg fat tissues (wet weight) was extracted with 150 ml dichloromethane (CH_2Cl_2) by using a Soxhlet apparatus. 8 mg of eicosane ($C_{20}H_{42}$) was also added as the internal standard. The dichloromethane-extracted lipid content is expressed as the dry weight of extractable materials per mg fat tissue.

1.3 Detection and Identification of triacylglycerols

The profile of triacylglycerols was analyzed by using high performance liquid chromatography (Waters 501 HPLC pump) equipped with an evaporative light scattering detector (ELSD, VAREX MKIII) according to the modified method of Héron and Tchapla [1]. The HPLC column used was Supelcosil LC-18 column and the solvent system used was acetonitrile /dichloromethane (MeCN:CH_2Cl_2, 68:32,v/v). The column was maintained at 30±1°C and the drift-tube temperature of the ELSD detector was maintained at 90±1°C. The exhaust temperature was found to be at 54±1°C. Oxygen-free nitrogen was used as the carrier gas at a flow rate of 2.00±0.05 SLPM and the gas pressure was maintained at 50.0±2.0 psig. The solvent flow rate and pressure were maintained constantly at 0.8 ml/min and 16±1 psig, respectively. The injected volume of neutral lipid sample was 20 µl. The identification of triacylglycerols was carried out using purified olive and linseed oils as well as commercially available triacylglyerol standards. A HPLC-ELSD chromatogram of triacylglycerols of cuticle's fat bodies was shown in Figure 3.

2.0 Results and Discussion

The neutral lipid content per gm fat tissue (fresh weight) from the viscera was found to be 59.8% whereas that from the cuticle was 11.7%. The neutral insect lipids function mainly as an important source of metabolic energy for cell maintenance, flight, reproduction, embryogenesis and metamorphosis. The fat body lipids were first quantitatively analyzed by Chino and Gilbert [2]. Triacylglycerols are the major lipid store in fat body, accounting for 78% or more of the total lipids. Most of the neutral lipid content isolated from the cuticle may be used up during the process of metamorphosis.

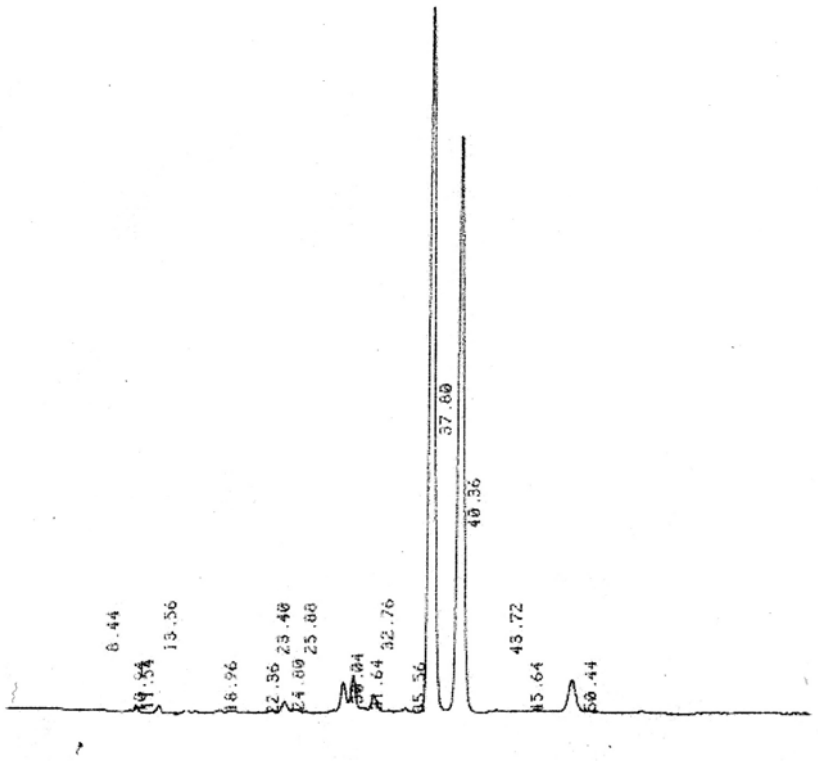

Figure 3 HPlC-ELSD chromatogram of triacylglycerols from cuticle's fat bodies

Table 1 shows the identification of triacylglycerols from fat tissues isolated from visceral and cuticle. Two major triacylglycerols, namely triolein, OOO and l-palmitoyl-2-oleoyl-3-oleoyl-rac-glycerol, POO as well as at least ten (10) other minor triacylglycerols were identified.

The percentage (w/w) of triolein (OOO) in neutral lipid of the fat tissues from both viscera and cuticle was found to be about 50% whereas that of l-palmitoyl-2-oleoyl-3-oleoyl-rac-glycerol (POO) was 41.7%. Other minor triacylglycerols identified in the neutral lipid of fat tissue from viscera were l-palmitoyl-2-oleoyl-3-linolenoyl-rac-glycerol, POLn (0.6%), 1-palmitoyl-2-linoleoyl-3-linoleoyl-rac-glycerol, PLL (0.1%), l-palmitoyl-2-palmitoyl-3-linoleoyl-rac-glycerol, PPL(1.1%) and l-palmitoyl-2-stearoyl-3-linoleoyl-rac-glycerol, PSO (2.9%) whereas that of cuticle were POLn (0.2%), PLL (1.0%), PPL(1.1%) and PSO (2.9%). Although there was no previous report on triacylglycerol analysis of insect lipids, the fatty acid composition of *Rhynchophorus phoenicis* larva oil has been analyzed recently [3]. Palmitic (32.4%), oleic (40.1%) and linoleic (13.0%) acids are the major fatty acids. The high triolein content may offer a nutritive advantage when the larvae are consumed as a delicacy in Sarawak.

Furthermore, six unidentified triacylglycerol peaks were separated from visceral fat. More purified unidentified samples are required for triacylglycerol identification using FAME methodology. The position of fatty acids in a triacylglycerol can be confirmed by using 1,3-specific lipase [4].

Table 1: Triacylglycerols of Visceral Fat And Fat Bodies On The Cuticle

No. of Peaks	Retention time (Mins)	Percentage of triacylglycerols (w/w)* *Visceral Fat*	Percentage of triacylglycerols (w/w)* *Cuticle's fat bodies*	Identification of Triacylglycerols
1	8.44	0.08	0.08	Unidentified
2	10.94	0.02	0.05	LnLnLn
3	13.56	0.14	0.33	Unidentified
4	18.96	-	0.15	PLLn
5	22.36	-	0.05	OOLn
6	23.40	0.63	0.20	POLn
7	24.80	0.10	0.95	PLL
8	25.88	0.08	0.48	OOL
9	28.60	0.13	-	POL
10	30.04	2.30	2.16	Unidentified
11	31.64	0.20	0.21	Unidentified
12	32.76	1.10	1.08	PPL
13	33.52	0.03	-	Unidentified
14	35.56	0.21	0.25	Unidentified
15	37.80	50.17	48.97	OOO
16	40.36	41.74	41.71	POO
17	43.72	0.05	0.22	PPO
18	45.64	0.11	0.18	SOO
19	50.44	2.89	2.93	PSO

References

[1] Héron, S. and A. Tchapla. 1995. *Finger prints of triacylglycerols from oils and fats by HPLC isocratic elution and evaporative light scattering detection ELSD Sedex 45*, Sedere, France.
[2] Chino, H. and L I. Gilbert. 1965. Lipid release and transport in insects. *Biochim. Biophys. Acta* 98:94-110.
[3] Ekpo, K.E. and A.O. Onigbinde. 2005. Nutritional potentials of the larva of *Rhynchophorus phoenicis* (F). *Pakistan J. Nutrition* 4:287-290.
[4] Goh, S.H., S.K. Yeong, and C.W. Wang. 1993. Transesterification of cocoa butter by fungal lipases: Effect of solvent on 1,3-specificity. *J. American Oil Chemists' Society* 70: 567-570.