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# ISOLATION AND CHARACTERIZATION OF PHOSPHOLIPIDS OF ERI-PUPAL OIL FROM PUPAE GROWN ON CASTOR AND TAPIOCA LEAVES

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## ABSTRACT

**Objective:** The aim of the present study was isolation and characterization of phospholipids from eri pupal oils.

**Methods:** Silkworm lipids were extracted from pupae fed on castor and tapioca leaves and further separated in to individual class of lipids by column chromatography. Fatty acid composition of each lipid class was studied along with positional distribution of fatty acids in major phospholipids by enzymatic hydrolysis.

**Results and Conclusion:** The eri pupal oils were found to contain 96.72-97.33% of neutral lipids, 0.51-0.21% of glycolipids and 2.65-2.39% phospholipids. Phosphatidylethanolamine (PE) was the predominant classes of phospholipid present (65.65 and 64.29%) in tapioca and castor fed pupal oils respectively. Phosphatidylcholine (PC) was observed in 19.50 and 19.22% respectively in tapioca and castor fed pupal oils. Cardiolipin (CL) and phosphatidylinositol (PI) are observed in minor levels. Positional distribution of fatty acids showed that, α-linolenic acid (ALA) was found to be more in *sn-2* position of PE. Thus pupal oil offers a good natural source of PE with enriched ALA.

**Key Words:** Eri silkworm pupae, Lipid classes, Phospholipids, Positional distribution, Fatty acid composition

## INTRODUCTION

Eri silk is obtained from non-mulberry silkworms and is known for its elegance and is mostly produced in the North-eastern parts of India. Silkworms are reported to show association with the host plants on which they feed for the necessary nutrients required for their metabolic activities and also for silk production[1]. The host plants reported for growing of eri silkworms are mainly castor (*Ricinus communis Linn.*) and tapioca (*Manihot utilissima Phol.*) followed by some other species also[2]. Besides production of silk, sericulture also produces large quantities of silkworm pupae which are thrown as sericulture waste or used as fertilizer and as a constituent of chick and fish feeds[3-4]. The desilked silkworm pupae are reported to have high nutritional value because of the presence of high protein and fat and these are considered as a source of nutritive components and protein supplements[5-7]. The silkworm pupae have been reported to be a good source of alpha linolenic acid (ALA) and the oil is reported to contain high amount of phosphorous[3]. The study on nutritional and toxicological evaluation of silkworm oil revealed that eri pupal oil is safe and nutritionally equivalent to common vegetable oils, which increase its potential as source of ALA[8].

The neutral lipids of eri pupae were isolated and characterized previously and the phosphorous content was reported to be 3-3.5%[9]. In mulberry silkworm oil, phospholipid content of 10% was reported where the major PLs identified were PE and PC[10]. Lecithins from vegetable oils like rice bran[11], corn[12], rapeseed[13], sunflower[14], cottonseed and peanut[15] are known to be good sources for phospholipids such as PC, PE and PI. In milk phospholipids, PE is the major component where as PC, sphingomyelin are almost in equal portions[16].

Studies on isolation and characterization of phospholipids from vegetable oils are abundant, whereas PLs from other

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sources is very rare. Literature reports are also available on fatty acid composition of total lipids of silkworm pupae *Bombyx mori* L [17]. However, there is no report on the detailed study on phospholipids obtained from eri silkworm oil. Therefore, the present investigation was aimed at the determination of phospholipid composition in lipids extracted from pupae fed on both castor and tapioca leaves.

## MATERIALS AND METHODS

Pupae fed on castor and tapioca leaves collected from Shadnagar, Ranga Reddy district, Telangana and Rampachodavaram, East Godavari district, Andhra Pradesh, India were supplied by Central Silk Board, Bengaluru, India. Fatty acid mixtures, references PE, PC, PI, CL and snake venom (Naja naja Atra) phospholipase $A_2$ source were purchased from M/s Sigma Chemicals, St Louis, USA. Silica gel (60-120 mesh) for column chromatography was purchased from Acme Synthetic Chemicals, Mumbai, India. Pre-coated thin layer chromatography (TLC) plates (silica gel $60F_{254}$) were procured from Merck, Darmstadt, Germany. HPLC grade solvents procured from M/s Merck, Mumbai, India. All other analytical reagent-grade chemicals and solvents were purchased from M/s SD Fine Chemicals, Mumbai, India.

### Total Lipid Extraction

The matured pupae grown on castor and tapioca leaves were dried at 80-90°C for 6-8 hours using vacuum oven, followed by the oil extraction using Soxhlet extractor with hexane as reported earlier[9]. The crude total lipids of castor and tapioca pupal oils were dried under reduced pressure, stored for further use.

### Separation of Lipid classes

Total lipids (TL) of eri silkworm oils were separated in to neutral lipids (NL), glycolipids (GL) and phospholipids (PL) using column chromatography[18] by the elution with chloroform, acetone and methanol respectively. GLs and PLs were qualitatively identified by TLC using chloroform/methanol/water (65:25:4, v/v/v) with suitable spray reagents[19-20]. The phospholipid mixtures obtained from column chromatography of castor (0.619 g) and tapioca (0.687 g) fed pupal lipid extracts were further individually re-chromatographed to separate individual phospholipids. The column was eluted with a gradient of 5-10, 20, 40 and 50% of methanol in chloroform to obtain CL, PE, PC and PI respectively[21]. The separated phospholipids were identified by comparison with individual standards and confirmed by spray reagents[20]. The isolated yields of phospholipids were found to be in the following amounts. For castor based PLs: CL (0.058g, 9.37%), PE (0.398g, 64.29%), PC (0.119g, 19.22%), PI (0.042g, 6.78%) and for tapioca based PLs: CL (0.060g, 8.73%), PE (0.451g, 65.65%), PC (0.134g, 19.50%), PI (0.041g, 5.96%).

### Positional Distribution of Fatty Acids

Positional distribution of fatty acids in individual phospholipids of both was carried by phospholipase $A_2$ mediated regiospecific hydrolysis as described by Christie[22]. Briefly, PE (50 mg) was dissolved in 4 ml of diethyl ether and to this 300 µL of snake venom solution (6 mg of snake venom in 1 ml of borate buffer; pH 7.0) was added and shaken vigorously for 1 hour. After complete hydrolysis, the ether solution was evaporated under nitrogen. The mixture containing liberated free fatty acids (FFA) and lysophospholipids were separated by column chromatography with the elution of gradient methanol in chloroform[23]. PC, PI were also hydrolyzed in a similar manner and the fatty acid composition was determined[22-23].

### Fatty Acid Methyl Esters (FAME)

All the lipid fractions isolated were converted to FAME using 2% sulfuric acid in methanol[24] and the FFA was treated with diazomethane. All the FAMEs were analyzed by GC-FID for fatty acid composition.

### Gas Chromatography (GC)

GC was performed on Agilent 6890N series Gas Chromatograph equipped with a flame ionization detector (FID) on a split injector. A fused silica capillary column (DB-225MS, 30 m x 0.25 mm i.d. J&W Scientific, USA) was used for separation. The oven temperature was programmed at 160°C for 2 min, increased to 230°C at 5°C/min and hold for 20 min at 230°C. The injector and detector temperatures were maintained at 230 and 250°C respectively. Nitrogen used as carrier gas with a flow rate of 1 mL/min. Identification of fatty acids was carried out by comparing with the retention time of respective commercial standards.

### HPLC analysis of Phospholipids

The phospholipid mixtures separated by column chromatography were qualitatively analyzed by Agilent HPLC chromatograph equipped with a quaternary pump and an evaporative light scattering detector (ELSD2000, Alltech, Deerfield, IL, USA). The drift tube temperature was set at 50°C and the nitrogen gas flow set at 1.5 L/min. The castor and tapioca phospholipids of 1 mg/mL concentration was separated on a SunFire™ Prep Silica column (5 µm, 4.6 x 250 mm; Sunfire columns, Waters, Ireland) at a mobile phase flow 0.5 mL/min. A binary gradient mobile phase composed of eluent A [chloroform/ methanol/ammonium hydroxide (80:19.5:0.5, v/v/v)] and eluent B [chloroform/methanol/ammonium hydroxide/water (60:34:0.5:5.5, v/v/v/v)] was used for elution as follows[25]: 0-10 min, 95% B; 10-15 min, 100% B and 15-20 min, 95% B. Identification of phospholipids was carried out

by comparing the retention times of the respective commercial standards. All the analysis was carried out in duplicate.

## RESULTS AND DISCUSSION

The present study describes the separation and characterization of lipid classes from eri pupae (*Samia Cynthia ricini*) grown on castor and tapioca leaves. Total lipids were extracted from pupae and were found to be in the range of 18-20 % as reported earlier[26]. The extracted total lipids were separated into 3 lipid classes by column chromatography and the data is shown in Table 1.

**Table 1: Composition of lipid classes (wt %) in pupal oil by column chromatography.**

| Lipid class | Castor leaf fed | Tapioca leaf fed |
|---|---|---|
| Neutral lipids | 97.33±0.12 | 96.72±0.13 |
| Phospholipids | 2.39±0.09 | 2.65±0.14 |
| Glycolipids | 0.21±0.01 | 0.51±0.03 |

The data revealed that the NLs were the major constituents followed by PLs and GLs in minor amounts in both the silkworm oils. The castor leaf fed pupal lipids showed a slight higher content of NLs than the tapioca leaf fed pupal lipids, where as the polar lipids were found to be higher in tapioca fed pupal lipids compared to castor fed lipids.

**Table 2: Fatty acid composition (wt %) of classes of lipids in castor leaf fed pupal oil.**

| Fatty acid | Total lipids | Neutral lipids | Glycolipids | Phospholipids |
|---|---|---|---|---|
| 14:0 | 0.43±0.01 | 0.44±0.0 | 1.58±0.05 | 0.31±0.0 |
| 16:0 | 28.81±0.08 | 28.84±0.06 | 30.27±0.21 | 17.76±0.05 |
| 16:1 | 1.89±0.06 | 1.91±0.01 | 1.01±0.52 | 0.61±0.02 |
| 18:0 | 3.82±0.01 | 3.69±0.05 | 15.25±0.06 | 15.26±0.06 |
| 18:1 | 19.11±0.06 | 19.07±0.06 | 21.0±0.10 | 20.67±0.06 |
| 18:2 | 5.68±0.05 | 5.61±0.01 | 7.42±0.26 | 12.19±0.05 |
| 18:3 | 40.04±0.06 | 40.25±0.05 | 19.92±0.11 | 30.51±0.17 |
| 20:0 | 0.15±0.06 | 0.14±0.0 | 1.81±0.15 | 1.59±0.05 |
| 22:0 | 0.06±0.01 | 0.04±0.01 | 1.72±0.0 | 1.07±0.04 |

**Table 3: Fatty acid composition (wt %) of classes of lipids in tapioca leaf fed pupal oil.**

| Fatty acid | Total lipids | Neutral lipids | Glycolipids | Phospholipids |
|---|---|---|---|---|
| 14:0 | 0.30±0.0 | 0.30±0.01 | 0.47±0.21 | 0.29±0.0 |
| 16:0 | 24.89±0.05 | 24.91±0.06 | 24.43±0.20 | 18.09±0.05 |
| 16:1 | 1.12±0.01 | 1.12±0.0 | 0.86±0.11 | 0.61±0.01 |
| 18:0 | 4.64±0.06 | 4.48±0.05 | 18.63±0.23 | 15.32±0.06 |
| 18:1 | 13.29±0.12 | 13.19±0.06 | 19.32±0.36 | 15.76±0.23 |
| 18:2 | 4.33±0.01 | 4.28±0.05 | 5.83±0.10 | 8.40±0.06 |
| 18:3 | 51.13±0.11 | 51.46±0.05 | 27.04±0.36 | 38.59±0.21 |
| 20:0 | 0.19±0.0 | 0.16±0.06 | 1.84±0.06 | 1.69±0.05 |
| 22:0 | 0.10±0.01 | 0.08±0.0 | 1.57±0.05 | 1.22±0.01 |

The fatty acid composition of total, neutral, glyco and phospholipids of both varieties were determined by GC and are given in Tables 2&3. It was observed that, ALA was the major fatty acid followed by palmitic (16:0) and oleic (18:1) in all tapioca lipids compared to castor fed lipids which could be due to the influence of host plants as reported earlier[9]. The ALA content in castor and tapioca fed PLs was observed 30.51% and 38.59% respectively. Previous studies on phospholipids from mulberry silkworm oil reported 40% of ALA[10]. Among other fatty acids, stearic (18:0), oleic and linolenic (18:2) acids were observed in greater amounts in both PLs and GLs of both varieties compared with neutral

lipids. However, ALA content was observed to be low in PLs and GLs compared with NLs in both pupal oils. Long chain saturated fatty acids like arachidic (20:0) and behenic (22:0) acids were found slightly higher amounts in polar lipids compared with NLs of both pupal lipids.

The phospholipids from both silkworm oils were identified by HPLC and quantified by column chromatography into CL, PE, PC and PI. The results showed that the castor and tapioca phospholipids are major source for PE (64-65%). Such a high PE content was earlier observed in the phospholipids of an obligate intracellular parasitic bacterium, *Rickettsia prowazeki,* where the PE reported 60-70%[27]. It was reported that mammalian and plant tissues have lesser occurrence of PE than PC, where as in bacteria PE is the principal phospholipid present[28]. In vegetable oil phospholipids, only castor seed oil was reported to contain high amounts of PE[29].

The fatty acid compositions of individual phospholipid classes and their hydrolysis products were determined by GC and are given in Tables 4&5. In cardiolipin isolated from castor and tapioca PLs, palmitic acid was the major fatty acid followed by oleic, stearic, ALA and other fatty acids. ALA content was more in PE of both pupal oils, followed by stearic, oleic, palmitic and other fatty acids compared to other PLs of both varieties.

**Table 4: Fatty acid distribution in individual phospholipid classes of castor leaf fed.**

| PL | Positional distribution | Fatty acid composition (wt %) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 14:0 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:0 | 22:0 |
| CL | Total | 1.56±0.15 | 28.01±1.25 | 0.36±0.05 | 20.17±0.15 | 22.89±0.15 | 6.66±0.21 | 17.39±0.72 | 0.27±0.06 | 2.66±0.25 |
| PE | Total | 0.30±0.01 | 14.60±0.26 | 0.32±0.01 | 17.52±0.01 | 28.72±0.10 | 9.51±0.10 | 28.04±0.12 | 0.60±0.01 | 0.39±0.06 |
| | *Sn-1* | 0.27±0.05 | 20.82±0.12 | 0.38±0.06 | 25.69±0.21 | 17.33±0.15 | 9.07±0.06 | 18.31±0.43 | 4.42±0.05 | 3.71±0.06 |
| | *Sn-2* | - | 3.72±0.06 | 0.41±0.0 | 1.46±0.05 | 30.59±0.29 | 18.24±0.067 | 45.56±0.38 | - | - |
| PC | Total | 0.43±0.20 | 25.08±1.65 | 0.47±0.23 | 14.55±0.29 | 23.48±0.25 | 8.94±0.23 | 23.28±0.76 | 1.89±0.21 | 1.87±0.38 |
| | *Sn-1* | 0.08±0.001 | 16.85±0.05 | 0.27±0.05 | 30.57±0.05 | 19.45±0.11 | 7.67±0.06 | 19.39±0.19 | 3.27±0.05 | 2.45±0.06 |
| | *Sn-2* | - | 13.18±0.36 | 0.35±0.05 | 6.15±0.15 | 24.96±0.15 | 16.11±0.05 | 39.25±0.36 | - | - |
| PI | Total | 0.12±0.01 | 19.30±1.06 | 0.46±0.06 | 30.10±0.10 | 26.68±0.29 | 7.16±0.39 | 10.16±0.05 | 3.35±0.11 | 2.66±0.11 |
| | *Sn-1* | 0.41±0.01 | 18.35±0.11 | 0.21±0.10 | 34.24±0.11 | 13.96±0.23 | 8.27±0.11 | 13.59±0.10 | 5.31±0.09 | 5.63±0.11 |
| | *Sn-2* | - | 19.55±0.15 | 0.45±0.15 | 23.40±0.25 | 21.86±0.11 | 11.22±0.01 | 23.51±0.15 | - | - |

**Table 5: Fatty acid distribution in individual phospholipid classes of tapioca leaf fed.**

| PL | Positional distribution | Fatty acid composition (wt %) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 14:0 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:0 | 22:0 |
| CL | Total | 1.04±0.11 | 26.36±0.63 | 0.81±0.06 | 23.26±0.38 | 19.28±0.10 | 6.47±0.21 | 19.10±0.36 | 1.40±0.52 | 2.27±0.06 |
| PE | Total | 0.12±0.01 | 11.71±0.06 | 0.28±0.01 | 27.37±0.05 | 19.05±0.11 | 7.40±0.06 | 29.77±0.21 | 2.35±0.05 | 1.93±0.07 |
| | *Sn-1* | 0.46±0.05 | 18.15±0.06 | 0.16±0.05 | 33.98±0.06 | 13.65±0.27 | 8.01±0.10 | 13.45±0.28 | 6.22±0.01 | 5.92±0.06 |
| | *Sn-2* | - | 4.72±0.01 | 0.35±0.01 | 3.76±0.06 | 26.13±0.15 | 13.17±0.05 | 51.74±0.20 | 0.13±0.11 | - |
| PC | Total | 0.09±0.01 | 24.50±1.58 | 0.37±0.21 | 22.06±1.98 | 15.70±0.62 | 16.48±0.49 | 19.20±0.52 | 1.10±0.10 | 0.49±0.43 |
| | *Sn-1* | 1.81±0.19 | 34.21±0.77 | 0.13±0.05 | 19.59±0.11 | 20.09±0.81 | 8.67±0.06 | 9.88±0.05 | 1.86±0.38 | 3.69±0.15 |
| | *Sn-2* | - | 28.99±0.46 | 0.26±0.05 | 16.23±0.15 | 16.24±0.45 | 15.96±0.24 | 22.31±0.36 | - | - |
| PI | Total | 0.10±0.01 | 31.30±2.01 | 1.40±0.47 | 17.55±2.65 | 20.90±0.81 | 15.17±0.93 | 11.50±1.06 | 1.15±0.32 | 0.90±0.80 |
| | *Sn-1* | 1.78±0.05 | 34.14±0.15 | 0.12±0.01 | 19.64±0.06 | 24.23±0.26 | 9.71±0.11 | 6.91±0.15 | 2.02±0.06 | 1.43±0.21 |
| | *Sn-2* | - | 32.19±0.34 | 0.52±0.05 | 13.77±0.21 | 16.19±0.17 | 9.47±0.06 | 27.84±0.15 | - | - |

The results (Table 4&5) indicate that, ALA was present in higher amounts at *sn-2* position of tapioca PE (51.74%) compared to castor PE (45.56%). Saturated fatty acids were majorly located at *sn-1* and unsaturated fatty acids were predominantly located at *sn-2* position for all the phospholipid classes. A higher content of ALA and their predominance at *sn-2* position increases the nutritional importance of the lipid[9]. Hence, the phospholipids of castor and tapioca fed oils are rich source of ALA with predominant distribution at *sn-2* position. In addition, phospholipids are a major component of cell membranes and required for signal transduction, metabolic regulation and maintenance of living cells[30]. The higher content of PE with ALA can be helpful for formation of fluid membrane structures which can have potential applications in signal transduction and other biological applications.

## CONCLUSIONS

In this study, eri pupal oil is shown as a novel source for phosphatidylethanolamine containing ALA. The extracted oil from eri pupae was separated into different lipid classes and further characterization of phospholipids is reported for first time. With a few exceptions, castor and tapioca leaf fed eri silkworm pupal oils showed similar characteristics in lipid classes, fatty acid composition and positional distribution of fatty acids in phospholipids. The phospholipid fraction of eri pupal lipids with a high amount of PE could be a useful product for pharma and food applications.

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## REFERENCES

1. Unni BG, Kakoty AC, Khanikor D, Bhattacharya PR, Pathak MG, et al. Lipid and fatty acid composition of muga silkworm, *Antheraea assama*, host plants in relation to silkworm growth. J Lipid Mediat Cell Signal 1996; 13: 295-300.
2. Suryanarayna N, Das PK, Sahu AK, Sarmah MC, Phukan JD. Recent advances in eri culture. Indian Silk 2003; 41: 5-12.
3. Udayasekhara Rao P. Chemical composition and nutritional evaluation of spent silkworm pupae. J Agric Food Chem 1994; 42: 2201-2203.
4. Ichhponani JS, Malik NS. Evaluation of deoiled silkworm pupae meal and corn steep liquor as protein sources in chick rations. **Br Poult Sci** 1971; 12: 31-234.
5. Sing KC, Suryanarayan N. Eri pupae: A popular cuisine too. Indian Silk 2003; 41: 57-58.
6. Wang J, Wu Fu-An, Liang Y, Wang M. Process optimization for the enrichment of α-linolenic acid from silkworm oil using response surface methodology. Afr J Biotechnol 2010; 9: 2956-2964.
7. Mishra N, Hazarika NC, Narain K, Mahanta J. Nutritive value of non-mulberry and mulberry silkworm pupae and consumption pattern in Assam, India. Nutrition Research 2003; 23: 1303-1311.
8. Longvah T, Manghtya K, Qadri SSYH. Eri silkworm: a source of edible oil with a high content of α-linolenic acid and of significant nutritional value. J Sci Food Agr 2012; 92: 1988-1993.
9. Kaki SS, Shireesha K, Kanjilal S, Kumar SVLN, Prasad RBN, et al. Isolation and characterization of neutral lipids of desilked eri silkworm pupae grown on castor and tapioca leaves. J Agric Food Chem 2006; 54: 3305-3309.
10. Kotake NE, Yamamoto K, Nozawa M, Miyashita K, Murakami T. Lipid profiles and oxidative stability of silkworm pupal oil. J Oleo Sci 2002; 51: 681-690.
11. Adhikari S, Adhikari DJ. Indian rice bran lecithin. J Am Oil Chem Soc 1986; 63: 1367-1369.
12. Schneider M. *In lecithins: Sources, Manufacture and Uses.* American Oil Chemical Society, Champaign-III; 1989. P. 109-130.
13. Sosulki P, Zadernowski R, Babuchowsk K. Composition of polar lipids in rapeseed. J Am Oil Chem Soc 1981; 58: 561-564.
14. Morrison WH. Sunflower lecithin. J Amer Oil Chem Soc 1981; 58: 902-903.
15. Vijayalaxmi B, Rao SV, Achaya KT. The nature of cottonseed phospholipids. Fette Seifen Anstrichm 1969; 1: 757-761.
16. Morrison WR, Jack EL, Smith LM. Fatty acids of Bovine milk Glycolipids and phospholipids and their specific distribution in diacylglycerophospholipids. J Am Oil Chem Soc 1965; 42: 1142-1147.
17. Sreekantaswamy HS, Siddalingaiah KS. Sterols and fatty acids from the neutral lipids of desilked silkworm pupae. Fette Seifen Anstrichm 1981; 83: 97-99.
18. Rouser G, Kritchevsky G, Yamamata A. Column chromatographic and associated procedures for separation and determination of phosphatides and glycolipids. In: Marinetti GV. Lipid chromatographic analysis, Marcel Dekker; 1967. P. 99-161.
19. Jacin H, Mishkin AR. Separation of carbohydrates on borate-impregnated silica gel G plates. J Chromatogr A 1965; 18: 170-173.
20. Mangold HK. Thin layer chromatography of lipids. J Am Oil Chem Soc 1961; 38: 708-727.
21. Christie WW. Lipid analysis; isolation, separation, identification and structural analysis of lipids. 2nd ed, Pergamon, Oxford; 1982. P. 109-111.
22. Christie WW. Structural analysis of lipids by means of enzymatic hydrolysis. *Lipid Analysis.* (2nd ed), Pergamon Press, Oxford, UK; 1982. P. 155-166.
23. Hanahan DJ, Brockerhoff H, Barron EJ. The site of attack of phospholipase A on lecithin. J Biol Chem 1960; 235: 1917-1923.
24. Christie WW. The preparation of derivatives of lipids. *Lipid Analysis*. (2nd ed.). Oxford, UK, Pergamon Press; 1982. P. 51-61.
25. Avalli A, Contarini G. Determination of phospholipids in dairy products by SPE/HPLC/ELSD. J Chromatogr A 2005; 1071: 185-190.
26. Ravinder T, Kaki SS, Prabhakar INSS, Rao BVSK, Swain SK, Prasad RBN. Effect of Natural and Synthetic Antioxidants on Oxidation of Eri silkworm oils. IAJPR 2015; 5: 3666-3675.
27. Winkler HH, Miller ET. Phospholipid composition of *Rickettsia prowazeki* grown in chicken embryo yolk sacs. J Bacteriol 1978; 136: 175-178.

28. Vance JE. Biochemistry of Lipids, Lipoproteins and Membranes. (4th ed) Elsevier, Amsterdam; 2002.
29. Paulose MM, Venkob Rao, Achaya KT. Nature of castor seed phospholipids. Indian J Chem 1966; 4: 529-532.
30. Yanagita T. Nutritional functions of dietary phosphatidyl inositol. Inform 2003; 14: 64-66.