ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info/jasr

ISSN
0976-9595
Research Article

Variation in Lipid Classes and Fatty Acid Content During Ovarian Maturation of *Albunea Symmysta*

Srinivasan P[a*], Rameshthangam P[b], Prabhu NM[c], Arulvasu C[d]

[a]*Department of Bioinformatics,* [b]*Biotechnology,* [c]*Animal Health and Management, Alagappa University, Karaikudi – 630 003, Tamil Nadu, India*

[d]*Department of Zoology, University of Madras, Chennai – 600 025, Tamil Nadu, India*

**Corresponding author: sri.bioinformatics@gmail.com*

ABSTRACT

Variation in lipid classes and fatty acid profiles were studied during different developmental stages of ovary of *Albunea symmysta*. The values of neutral, phospho and glycolipid were recorded high (10.0±0.001, 14.7±0.002, 21.0±0.002) at ripen stage and declined (3.2±0.001, 5.4±0.002, 10.5±0.001) at spent stage. The concentration of lauric, myristic, palmitic and lignoceric acid reached high at ripen stage (stage IV) (0.144, 0.816, 15.92 and 1.514 %) and those acid concentration were lowered (0.118, 0. 380, 2.568, and 0.872 %) at spent stage (stage V). The concentration of stearic, arachidic and behanic acid were fluctuated in the stage III and IV (4.027- 0.683, 1.872-1.832 and 3.815-3.869 %) and lowered (0.925, 0.961, 1.905 %) The palmitolic acid values were fluctuated (0.673-0.626 %) in the stage III and IV and its concentration was lowered (0.225 %) at spent stage. The oleic acid concentration was recorded higher than that of all other fatty acids and its values increased (42.27 %) at ripen stage and declined (18.52 %) at spent stage. All the polyunsaturated fatty acids were fluctuated in the ripening stage (especially stage III & IV) and those acids value reached maximum at the stage III and lowered at spent stage.

Keywords: *Albunea symmysta*, glycolipid, neutral lipid, ovarian maturation, phospholipid.

1. INTRODUCTION

Albunea symmysta, an anomuran crab with the burrowing mode of life, was chosen for the present investigation. They belong to the family albuneidae of the reptantian decapods. They inhabit the sandy beaches, mostly along the tropical seacoast. *Albunea symmysta* also known as sand crab, is found in the lower level of inter tidal regions. The peculiar feature of the animal is that they possess a pair of respiratory siphon at its anterior region. The third pair of walking legs is highly developed with claws for the purpose of digging into the sand. The female crabs were found to be bigger than the male crabs. Crustaceans lay large number of yolk – laden eggs at each spawning. Female of the decapods extrude their eggs and carry them under the abdomen until hatching. The eggs are rich in yolk substances and other chemical constituents .The yolk substances closely correlate with embryonic development [1, 2]. Lipid content, which is relatively higher in decapods eggs, is one of the main energy sources that the female stored. During embryonic development stages, the lipid is not only a kind energy source but also a component of biological membrane and pigment of compound eyes [1]. Lipid play a central role in embryonic metabolism as they represent the most important energy source and form at least 60 % of the total energy expenditure of the developing embryo [3]. In this present investigation, an attempt has been made to analyse major lipid classes content and fatty acid profiles of ovary of different developmental stages of the anomuran crab, *Albunea symmysta* which form an important substitute in supplementing the nutritional requirement as well as the livelihood of local fishing communities of the madras coastal region.

2. MATERIAL AND METHODS

Specimen of the female sand crabs *Albunea symmysta* in ranging size from 15 to 30 mm carapace length were collected from the intertidal region of Elliot's beach at Besant Nagar, Chennai, India. The carapace length was measured from posterior margin along the mid dorsal line to the tip of the rostrum. Mostly the specimen was available in the morning and in late evening hours. More than fifty crabs were handpicked at each collection and brought to the laboratory with least disturbance in polythene bags containing wet sand. They were kept in two plastic tanks with sufficient aeration with clean sand spread in slanting position. Seawater was changed every day and the sand was changed once in a week. The female crab was distinguished from the male by presence of three pairs of pleopods on ventral side of the abdomen. The female crabs were dissected out to observe the colour of ovary. The ovarian stages were identified based on the colour changes from whitish to orange as outlined by Kerr [4].

Total lipid was extracted by the following method of Folch et al. [5]. One gram of sample was homogenized in 5mL of chloroform/methanol (2:1 v/v) and shaken for 20 min in an Erlenmeyer flask with a magnetic stirrer. After filtering, the liquid phases were mixed and washed with 0.5ml of 0.9% sodium chloride solution. The lower phase was collected after phase separation, and the solvent was evaporated. The residue was transferred to a 10ml glass vial and maintained at -20°C until analysis was performed. The total lipid content was determined according to Barnes and Blackstock [6]. Briefly, an aliquot of 0.5ml of lipid sample was taken and allowed to evaporate under nitrogen. The individual dry samples were digested with 0.5ml of concentrated sulphuric acid in a boiling water bath for 15 min. A known quantity (0.2ml) of acid digest was taken, to which 5ml of phosphovanillin reagent was added. The mixture was allowed to stand for 30 min and the colour intensity was determined by spectrophotometry at 520 nm. Chloroform and cholesterol was used as the blank and standard, respectively. The eparation of phospholipid, glycolipid and neutral lipid were extracted from total lipid by silicic acid column chromatography as outlined by Rouser et al. [7].

2.1. Activation of silicic acid

Fifteen gram of silicic acid was taken and mixed with 50 ml of 3N HCl and kept it oven at 100ºC over night or 6-8 hrs and then cool it to room temperature 10- 15 mins.

2.2. Column preparation

Silicic acid (activated) powder was taken in 50ml of chloroform and poured into a column until a height of 15-20cm. The column bed was washed with chloroform (250ml) for proper packing and fraction size was 2ml/min.

2.3. Application and elution of the sample

Two hundred milligram of ovary was taken for total lipid extraction and dissolved in 3ml of chloroform and methanol with 1:1 ratio and then add the sample down side of the column.

2.4. Elution of neutral lipid

Add gradually 10 times column volume of chloroform (500ml) without disturbing the sample at the flow rate of 2ml / mins and the elution was collected in a known weighed beaker.

2.5. Elution of glycolipid

Acetone (100ml) was added to the same column without disturbing the sample and 3-5ml of elution was discarded and the fraction was collected in a known weighed beaker at a flow rate of 2ml/min.

2.6. Elution of phospholipids

Methanol (500ml) was added to same column without disturbing the sample and 2-3ml of elution was discarded and the fraction was collected in a known weighed beaker at the flow rate of 2ml/min.

2.7. Quantification of neutral, glyco and phospholipids

The collected three lipid fractions were dried under nitrogen gas. The dried beaker was weighed and lipid fractions were determined, from these values the amount of neutral, glyco and phospholipids were estimated in the total lipid. The same procedure was followed for all the ovarian stages.

2.8. Fatty acids analysis

Fatty acid composition from the total lipids were analysed as fatty acid methyl esters derivates (FAME) according to the method of Morrison and Smith [8]. Briefly, 100mg of lipid was saponified with 2ml of sodium methoxide in methanol (0.5N) and incubated 10 min in a boiling water bath. The solution was then cooled at room temperature, and to this was added 2ml of boron trifluoride/methanol complex (14%). The solution was then heated for 20 min in boiling water bath (80°C). After cooling, 1ml of hexane was added, and the mixture was heated for another 2 min and cooled at room temperature. To this was added 1.25ml of saturated sodium chloride. The mixture was shaken vigorously and after phase separation, the organic layer was suctioned with a Pasteur pipette and transferred to a 2ml vial containing 1mg of anhydrous sodium sulfate. The fatty acid methyl esters were analysed using a Chemito 8610 gas chromatography, equipped with capillary column. Nitrogen was used carrier gas at a flow rate of 1.3ml/min^{-1}. The injector and detector (Flame Ionization Detector) temperature was adjusted to 200°C and 230°C respectively. The oven temperature was programmed from an initial temperature and time of 160°C for 10 min to 180°C at 15°C min^{-1} respectively and maintained for 5 min. The integrated peak areas of the fatty acid methyl esters were identified by comparison with known standards.

3. RESULTS

The first two stages of ovary are not visible as they are completely embedded among the hepatopancreatic mass. However the ovarian stages III and IV can be identifiable externally. The colour of the ovary was orange. In the fifth stage, the egg mass were also seen in the pleopods.

Total lipid content was increased with ovarian maturation process (stage I - 33.5±0.005; stage II - 40.0±0.006, stage III - 45.2±0.005, stage IV - 45.5±0.002) and suddenly decreased in the spent stage (stage V - 35.0± 0.005). Similarly, the

neutral, phospho and glycolipid were also increased from immature stage to mature stage and abruptly decreased at the spent stage neutral lipid content was 4.0± 0.001 in stage I, 4.3±0.005 in stage II, 8.0±0.002 in stage III, 10.0±0.001 in stage IV and 3.2±0.001 in stage V. The phospholipid content was 6.4±0.001 in stage I, 6.7±0.005 in stage II, 10.5±0.005 in stage III, 14.7±0.002 in stage IV and 5.4±0.002 in stage V. Glycolipid content was 7.0±0.004 in stage I, 7.4±0.001 in stage II, 20.0±0.001 in stage III, 21.0±0.002 in stage IV and 10.5±0.001 in stage V (Table-1).

Table 1: Major lipid classes content in ovary at different development stages of Albunea symmysta. (Values are expressed in mg/g)

Stages	Total Lipid	Neutral Lipid	Phospholipid	Glycolipid
I	35.5 ± 0.005	4.0± 0.001	6.4± 0.001	7.0± 0.004
II	40.0± 0.006	4.3± 0.005	6.7± 0.005	7.4± 0.001
III	45.2± 0.005	8.0± 0.002	10.5± 0.005	20.0± 0.002
IV	45.5± 0.002	10.0± 0.001	14.7±0.002	21.0± 0.002
V	35.0± 0.005	3.2± 0.001	5.4± 0.001	10.5± 0.001

Table 2: Major fatty acids content in ovaries at different maturity stages of Albunea symmysta (Values are expressed in %)

Fatty acid	Stage I	Stage II	Stage III	Stage IV	Stage V
Saturated					
Lauric acid	0.105 ± 0.01	0.108 ± 0.06	0.113 ± 0.04	0.144 ± 0.03	0.118 ± 0.01
Myristic acid	0.380 ± 0.05	0.392 ± 0.06	0.460 ± 0.04	0.816 ± 0.03	0.380 ± 0.02
Palmitic	5.092 ± 1.93	9.682 ± 1.02	14.86 ± 1.02	15.92 ± 1.96	2.568 ± 0.93
Stearic acid	1.250 ± 0.28	2.925 ± 1.32	4.027 ± 0.60	0.683 ± 0.76	0.925 ± 0.01
Arachidic acid	0.960 ± 0.03	0.968 ± 0.01	1.874 ± 0.09	1.832 ± 0.87	0.961 ± 0.06
Behanic acid	1.762 ± 0.09	1.875 ± 0.09	3.815 ± 0.03	3.689 ± 0.07	1.905 ± 0.05
Lignoceric acid	0.465 ± 0.03	0.948 ± 0.07	1.303 ± 0.02	1.514 ± 0.09	0.872 ± 0.03
Monounsaturated					
Palmitolic acid	0.253 ± 0.01	0.468 ± 0.03	0.673 ± 0.02	0.626 ± 0.03	0.225 ± 0.06
Oleic acid	20.49 ± 1.02	25.96 ± 1.34	39.39 ± 1.65	42.27 ± 1.86	18.52 ± 0.92
Polyunsaturated					
Linoleic acid	18.52 ± 0.93	22.90 ± 1.23	30.96 ± 1.48	28.85 ± 1.02	15.68 ± 0.86
Linolenic acid	0.117 ± 0.02	0.155 ± 0.01	0.169 ± 0.01	0.168 ± 0.02	0.108 ± 0.03
Eicosapentaenoic acid	0.285 ± 0.01	0.489 ± 0.03	0.665 ± 0.02	0.114 ± 0.03	0.104 ± 0.06
Docosahexaenoic acid	0.528 ± 0.02	0.625 ± 0.05	0.759 ± 0.03	0.180 ± 0.06	0.525 ± 0.03
Σ SFA	10.014 ± 2.42	16.898 ± 2.63	26.452±1.84	24.598 ± 3.81	7.729 ± 1.11
Σ MUFA	20.743 ± 1.03	26.428 ± 1.37	40.063±1.67	42.896 ± 1.89	18.745 ± 0.98
Σ PUFA	19.450 ± 0.98	24.169 ± 1.32	32.552±1.54	29.312 ± 1.13	16.417 ± 0.98

3.1. Fatty acids content in the ovarian maturation of *Albunea symmysta*

3.1.1. Saturated fatty acid

The fatty acids with high concentration were found in ovary during different maturity stages. Lauric, myristic, palmitic and lignoceric acid were increased from stage I-IV. Lauric acid content was 0.105, 0.108, 0.113, 0.144 and decreased 0.118 in the stage V. Myristic acid was 0.380, 0.392, 0.460, 0.816 and declined 0.380 in the spent stage V. Palmitic acid content was 5.092, 9.682, 14.86, 15.92 and decreased 2.568 in the stage V. Lignoceric acid content was 0.465, 0.948, 1.303, 1.514 and fell down 0.872 in the stage V. The recorded values of stearic, arachidic and behanic acid were fluctuated in relation to different maturity stages (especially in the stage III- IV) and those acid values were 0.925, 0.961 and 1.905 lowered in the stage V.

3.1.2. Monounsaturated fatty acid

The concentration of palmitolic acid was fluctuated in the stage III & IV (0.673-0.626) and abruptly decreased in the stage V (0.225), while the oleic acid concentration was steadily increased from the stage I-IV (20.49, 25.96, 39.39, 42.27) and suddenly fell in the stage V (18.52).

3.1.3. Polyunsaturated fatty acid

The concentrations of linoleic, linolenic, ecosapetaenoic and docosahexaenoic acid were fluctuated from the stage III -IV (30.96-28.85, 0.169-0.168, 0.665 -0.114, - 0.759 - 0.180) and those acid concentrations were lowered in the spent stage (15.68, 0.108, 0.104 and 0.525).

4. DISCUSSION

In the present study, the total lipid and major lipid classes (i.e.) neutral, phospho and glycolipid isolated and quantified from the ovary to investigate the variations in their fatty acid profiles in detail. As a result, the total lipid, neutral, phospho and glycolipid of the ovary were found to have high during maturation, while those acid depositions were lowered after the completion of ovarian development (spent stage) as evidenced by Tirumalai and Subramoniam [9] in *Emerita asiatica*.

Hence, lipids are thought to play an important role in reproductive process in *Albunea symmysta*. The similar result has also been reported in some other shrimps *Penaeus japonicus*, *Penaeus duorarum*, *Pleoticus mulleri* and other decapod crustacean [10-13]. The origin of lipids reaching the ovary is not fully understood but lipid stored in hepatopancreas have been shown to be transported the ovary during vitellogenesis. Result shows that lipids are needed as a source of energy and to maintain the structure and functions of cell membrane. They also play an important role in buoyancy control in some fishes [14, 15]. Apart from this each lipid has specific function (i.e.) phospholipid essential for growth because crustacean have limited ability to synthesis this lipid *de novo* [16]. As a consequence some species require dietary phospholipids for normal growth and survival of larvae. It also plays an important role in the transport of cholesterol [17, 18] and composition of gametes [19] because this is involved in synthesis of vitellus, which is phospholipoprotein [20]. Accumulation of neutral lipid in the ovary of *Albunea symmysta* due to containing predominant fatty acids which include palmitolic, palmitic, oleic, myristic acid and hence neutral lipid is essential for synthesis of other fatty acids and transport them [1, 13]. Glycolipid is essential for alternation of pleopods, growth and stress resistance [21].

In *Albunea symmysta* ovary, the concentration of lauric, myristic, palmitic and lignoceric acid are increased with maturity especially in the stage III and IV, while those acid values are lowered in the stage V (spent stage). The concentration of stearic, arachidic, and behanic acid are fluctuated in the ripening stage (stage III & IV), while those acids concentration are lowered in the spent stage (stage V). Similar results have been reported in some prawn such as *Penaeus japonicus* [13], *Penaeus duorarum* [10] and also some fishes [22]. These acids are essential for over all body maintenance [23]. Among those fatty acids palmitic, stearic, and arachidic acid value were recorded high, which were responsible for carry out some specific functions, essential for *de novo* synthesis in marine crustacean [24]. Stearic acid plays an important role in embryonic development [25-27]. Arachidic acid is essential for production of phospholipids including phosphoidylinositol as indicated by Gunasekara et al. [28] and Gallgher et al. [22].

The concentration of Palmitolic acid fluctuated in the ripening stage (stage III and IV) and fell quietly in the stage V, while the concentration of oleic acid constantly increased with maturity and decreased with spent stage. The increasing concentrations of monounsaturated fatty acid in the ovary indicate that these compounds are considered to be major energy source during embryonic and larval development [29, 30]. Monounsaturated fatty acid content was higher than that of polyunsaturated fatty acid. These similar results have been reported in *Macrobrachium rosenbergii* [31].

The concentrations of polyunsaturated fatty acid (linoleic, linolenic, eicosapetaenoic and docosahexaenoic acid) are fluctuated in the ripening stage (stage III and IV) and fell quite in the stage V (spent stage). The concentration of linoleic acid was recorded high in stage III compared to other fatty acids. This investigation clearly indicated that polyunsaturated fatty acids are not only necessary for successful ovarian maturation and also need (especially eicosapentaenoic, docosahexaenoic acid) as a nutrient for developing embryo. Similar result has been reported in *Macrobrachium nipponense* [24] and *Nephrops norvigicus* [26] and in milk fish [32]. The importance of the compounds have been invented and indicated in some marine crustaceans [33-46].

5. CONCLUSION

Since, ecdysteroid is a vital compound for performing growth and reproduction, the increase in ovarian lipid classes and fatty acid with maturation in *Albunea symmysta* is related to conversion of cholesterol as precursor for ecdysteroid. The present observation represented here will provide a base line data towards the lipid metabolism in relation to ovarian maturation of *Albunea symmysta*.

5. REFERENCES

1. Holland DL. Lipid reserves and energy metabolism in the larvae of benthic marine invertebrates. In: Malins DC and Sargent JR (Eds.) Biochemical and biophysicsical perspectives in marine biology, 1978 pp85-123.Luo W, Zhou
2. ZL, Zhao YC. *J East China Normal University*, 2004; **1**: 88-92.
3. Wehrtmann LS, Graeve M. *Comp Biochem Physiol*, 1998; **121B**: 457-463.
4. Kerr MS. *Dev Biol*, 1969; **20**: 1-17.
5. Folch J, Lees M, Stanely GHS. *J Biol Chem*, 1957; **266**: 497-509.
6. Barnes H, Blackstock J. *J Exp Mar Biol Ecol*, 1973; **12**: 103-118.

7. Rouser G, Kritchlvsky G Yamamoto A. *Lipid Chromatographic analysis.* Dekker, New York, 1967; pp99-162.
8. Morrison WR, Smith LM. *J Lipid Res*, 1964; **5**: 600-608.
9. Tirumalai R, Subramoniam T. *Mol Reprod Develop*, 1992; **33**: 16-26.
10. Gehring WR. *Comp Biochem Physiol*, 1974; **49A**: 511-524.
11. Clark A. *J Exp Mar Biol Ecol*, 1979; **38**: 1-17.
12. Teshima S, Kanzawa A. *Bull Jap Soc Sci Fish*, 1983; **49**: 957-962.
13. Teshima S, Kanazawa A, Koshio A, Hrinouchi K. *Comp Biochem Physiol*, 1989; **92B**: 45-49.
14. Cavalli RO, Menschaert G, Lavens P, Sorgeloos P. *Aquaculture*, 2000; **8**: 41-58.
15. Castille FC, Lawrence AC. *J Crust Biol*, 1989; **9**: 202-211.
16. Ackman RG, Eaton CA. *J Fish Res Board of Canada*, 1970; **27**: 1669-1683.
17. Shieh HS. *Comp Biochem Physiol*, 1969; **30**: 679-684.
18. Teshima S, Kanazawa A, Koshio S, Horinouchi K. *Nipp Sui Gakk*, 1988; **54**: 1123-1129.
19. Calli RO, Lavens P, Sorgeloos P. *Aquaculture*, 1999; **179**: 387-402.
20. Pollero RJ, Iribarne DO. *Comp Biochem Physiol*, 1988; **90B**: 317-320.
21. Fujii T. *Acta Embryo Morphol Exp*, 1960; **3**: 260-285.
22. Kontara KK, Coutteau M, Sorgeloos P. *Aquaculture*, 1997; **158**: 305-320.
23. Gallagher ML, Paramori L, Alves D, Rulifsion R. *J Fish Biol*, 1998; **52**: 1218-1228.
24. Barclay MC, Dall W, Smith DM. *J Exp Mar Biol Ecol*, 1983; **68**: 229-244.
25. Kayama M, Hirata M, Kanazawa A, Tokiwa S, Saito M. *Bull Jap Soc Sci Fish*, 1980; **46**: 483-488.
26. Li H, Zhao Y, Wang Q. *J Fish China*, 2003; **27(6)**; 545-549.
27. Rosa R, Morais S, Cslado R. *Aquaculture*, 2003; **221**: 507-522.
28. Rosa R, Costa P, Banarra R, Nunes MC. *Biol Bull*, 2005; **208**: 100-113.
29. Gunasekara RM, De Selva SS. *Aquaculture*, 2000; **134**: 169-183.
30. Clarke A, Brown JH, Holmes LJ. *Comp Biochem Physiol*, 1990; **96B**: 505-511.
31. Roustaian P, Kamarudin MS, Saad CR, Ahamad MH. *Aquacult Res*, 1999; **30**: 815-824.
32. Ogata HY, Chavez DR, Garibay ES, Furuita H, Suloma A. *Jap Aqua Res Quat* 2006; **40(4)**: 393-402.
33. Guary JC, Kayama M, Murakami Y. *Bull Jap Soc Sci Fish*, 1974; **40(10)**: 1027-1032.
34. Brown A, McVey JP, Middleditch BS, Lawrence AC. *Proc Worl Maricult Soc*, 1979; **10**: 435-444.
35. Brown A, McVey JP, Scott BM, William TD, Middleditch BS, Lawrence AL. *Proc Worl Maricult Soc*. 1980; **11**: 488-499.
36. Middleditch BS, Missler SR, Ward DG, McVey JP, Brown A, Lawrence AL. *Proc Worl Maricult Soc*, 1979; **10**: 472- 476.
37. Middleditch BS, Missler SR, Hines HB, McVey JP, Brown A. *J Chromatography*, 1980; **195**: 359-368.
38. Lawrence AL, Akamine Y, Middleditch B, Champerlain S, Hutchins GD. *Proc World Maricult Soc*, 1980; **11**: 481-487.
39. Lilly ML, Bottino NR. *Lipid*, 1981; **16**: 871-875.
40. Sargent JR, McVoy LA, Estevez A. *Aquaculture*, 1999; **79**: 217-229.
41. Navarro J, Villanueva C. *Aquaculture*, 2000; **183**: 161-177.
42. Navarro JC, Villanueva R. *Aquaculture*, 2003; **219**: 613-631.
43. Cuvalli RO, Tamtin M, Lavens S, Sorgeloos P. *Aquaculture*, 2001; **193**: 311-324.
44. Kanazawa A, Teshima S, Tokiwa S. *Bull Jap Soc Sci Fish*, 1977; **43**: 849-856.
45. Kanazawa A, Teshima S, Endo M. *Mem Fac Fish Kagoshima Univ*, 1979a; **28**: 27-33.
46. Kanazawa A, Teshima S, Ono K. *Comp Biochem Physiol*, 1979b; **63(b)**: 295-298.