Research Paper Chemistry

Chemical Analysis of The Edible Clam *Katelysia opima* From Mumbai, Maharashtra

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ABSTRACT

The clam *Katelysia opima* is an edible bivalve from Mumbai region. This investigation was undertaken to study the protein profile of this clam on a monthly basis. It was interesting to note that in all months, the protein content was considerable. However, especially during November and January protein contents reached their peaks. TLC chromatography of bivalve extract also showed presence of organic compounds. The pattern of organic compounds in the extract of clam, collected during different months was almost the same.

KEYWORDS marine clam, bivalve, chemical analysis, Seasonal study, nutrition, protein electrophoresis

INTRODUCTION

Shellfish fishery forms an important part of Indian economy as clams, oysters and mussels from marine waters are popular food items. It is already known that these bivalves are a good source of proteins and thus continue to serve the nutritional requirements of the people inhabiting coastal areas.

Katelysia opima is one of the common most edible bivalves in the North West Coast of India and is easily available in the local markets of Konkan region from Mumbai to Ratnagiri. A perusal of literature indicates that there are many reports available focused mainly on various biological and biochemical aspects such as the growth and breeding habits (Mane, 1974), reproduction (Nagabhushanam and Mane, 1975a), biochemical composition (Nagabhushanam and Mane, 1975b), edibility value (Mane, 1981) and respiration (Taware and Muley, 2013) of *Katelysia opima*.

The protein content is considered to be an important tool for the evaluation of physiological standards as it is essential for normal function, growth and maintenance of body tissues (Abdel-Salam, 2014). Though there are many studies undertaken which highlight the protein richness of the marine clams by simple biochemical analytical methods, the use of electrophoresis to separate and develop a protein profile of Indian clams is lacking. The purpose of this study was therefore, to assess the electrophoretic pattern of the *K. opima*. An attempt was also made to characterize the organic extract of this clam using thin layer chromatography.

MATERIALS AND METHODS

Collection of clams

K. opima (15 numbers) were collected every month during October, 2015 to March, 2016 from the local fish market of Mumbai. Clams were washed thoroughly with fresh water. The meat was separated from the shells and dried at 60°C for 48 h after which the sample was powdered and stored in a refrigerator until further used.

Protein extraction

Protein extraction from clam powder was carried out using tissue extraction reagent I which was obtained from Invitrogen (Cat. No. FNN0071). The clam powder (20 mg) was homogenized in 200 µl of Tissue extraction reagent I. The tissue was homogenized in a mortar and pestle placed in an ice bucket. The homogenate was then centrifuged at 10000 rpm for 5 minutes. The supernatant containing the proteins was pipetted out in a new tube and preserved at - 20°C until further used.

Protein quantification

Protein concentration was determined using Quant-iTTM Protein Assay Kit of invitrogen. The calibration was done using the three standards on Qubit fluorometer. Sample readings were taken and calculated for 2 µl. The results were obtained in µg/ml.

Polyacrylamide Gel Electrophoresis (PAGE)

SDS PAGE analysis was performed for the proteins extracted from clams collected during different months following the method of Saini and Sarin (2012). The gel was stained by silver staining technique (Chevallet et al., 2006).

Extraction of organic compounds

Clam powder (1 g) of different collection months was weighed and extracted separately with 10 ml methanol (cold extraction) for 24 h. The solvent was evaporated by using rotary evaporator. The crude dried extract was stored in a refrigerator.

Thin layer chromatography

The crude methanol extracts (total 6 extracts of 6 months) were used for Thin Layer chromatographic studies. The dried methanol extracts (2-3 µl) were applied on the TLC plate with the help of capillary tube. The plate was run by using methanol:chloroform (50:50 vol/vol) as a solvent system. After development, the solvent was evaporated and the dried plates were kept under UV to check UV visible compounds. TLC was developed by spraying 5% H2SO4 and ninhydrin for detection of various well separated bands. Rf values of the major spots were calculated.

RESULTS

The concentration of protein obtained from clams collected during different months is reported in the Table 1. The protein concentration was in the range of 1.1 mg/ml to 2.35 mg/ml. Least concentration was recorded in the month of October while the highest was in the month of November, 2015. It was observed that the number of protein bands were more in October 2015 and March 2016.

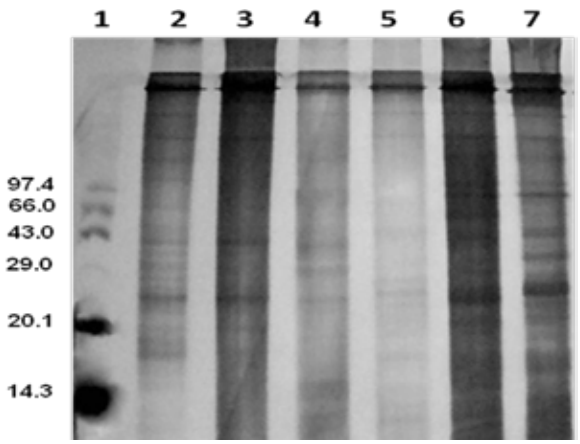
Table 1: Protein concentration in *K. opima* during October, 2015 to March, 2016

Month	Protein concentration in mg/ml	No. of bands
October, 2015	1.10	13
November, 2015	2.35	6
December, 2015	1.62	9

January, 2016	2.15	7
February, 2016	1.38	8
March, 2016	1.22	12

Protein pattern by PAGE
Electrophoretic protein profile is shown in the Fig. 1. Many proteins of different molecular weights were found to be present in the *K. opima.*

Figure 1: Protein profile of clam *K. opima* as generated by SDS – PAGE



Lane 1: Protein marker (ladder)
Lane 2: October; Lane 3: November sample
Lane 4: December; Lane 5: January sample
Lane 6: February; Lane 7: March sample

Thin layer chromatography (TLC)
TLC plate when developed and analyzed showed the presence of five major compounds in the clam extract (Fig. 2). The TLC pattern was found to be almost the same in all the months. Rf values of the major compounds observed on the TLC plate are given in the Table 2.

Figure 2: TLC plate showing compounds in the clam extract

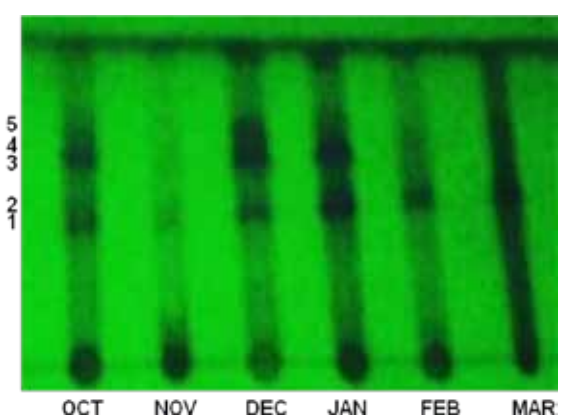


Table 2: Rf values of the compounds present in the calm extract separated by TLC

Sample code	Rf values of compounds on TLC
October 2015	0.45, 0.51, 0.68, 0.74, 0.82
November 2015	0.45, 0.51, 0.68 (two top spots are missing)
December 2015	0.45, 0.51, 0.68, 0.74, 0.82
January 2016	0.45, 0.51, 0.68, 0.74, 0.82
February 2016	0.45, 0.51, 0.68, 0.74, 0.82
March 2016	0.45, 0.51, 0.68, 0.74, 0.82

DISCUSSION
The results of the present study depict the protein profile of the clam *K. opima* collected during different months from Mumbai. The results indicated that there is no correlation between the protein concentration and number of total proteins in these clams. This is the first ever report of such kind and further studies are ongoing to study the electrophoretic patterns of other marine clams of Maharashtra state.

There are a few reports which highlight the usefulness of electrophoresis in studying protein profiles of bivalves.

Benzie and Williams (1998) used protein electrophoresis as a tool to analyze the phylogenetic relationships among giant clam species of the family Tridacnidae collected from Western Pacific region.

Gourdine and Smith-Ravin (2002) analyzed gill protein extracts of the clam Codakia orbicularis by SDS-PAGE and Western blotting and established its protein and glycoprotein profiles. Their results showed the presence of three major proteins whose molecular weights vary between 14,000 and 24,000 Daltons.

Upadhye and Jadhav (2010) reported a tissue-specific protein profiling of freshwater pearl producing mussel, Parreysia corrugata from Maharashtra, India using SDS-PAGE and showed that different tissues have different protein numbers and masses.

Abdel-Salam (2014) studied the protein content and the differences in the electrophoretic patterns of muscle proteins of commercially important species of crustaceans and molluscs collected from Egyptian and Saudi Arabian coasts. The author showed significant variation in the band numbers and molecular weight of the proteins in these animals and also reported a variation in the males and females of the same species. So such separate studies on male and female species of marine clams are warranted to investigate the variation and fluctuations in the protein profile.

CONCLUSION
The present investigation highlights the application of SDS-PAGE in characterizing the protein profile for a marine clam. To conclude, a seasonal variation in the protein concentration and number was observed in K. opima. Methanol is a recommended solvent to prepare the clam extract for separation of compounds by TLC.

REFERENCES
[1] Abdel-Salam, H. A. (2014), "Protein and Electrophoretic Analysis of Edible Muscle of Commercially Important Crustaceans and Mollusks Species from Egyptian and Saudi Arabia Costs". Animal and Veterinary Sciences, 2(4), 109-117.
[2] Benzie, J. A. and Williams, S. T. (1998), "Phylogenetic relationships among gi ant clam species (Mollusca: Tridacnidae) determined by protein electrophore sis". Marine Biology, 132(1), 123-133.
[3] Chevallet, M., Luche, S. and Rabilloud, T. (2006), "Silver staining of proteins in polyacrylamide gels". Nat Protoc., 1(4), 1852 -1858.
[4] Gourdine, J-P. and Smith-Ravin, E. (2002) "Electrophoretic separation of gill proteins of the clam Codakia orbicularis". Preparative Biochemistry and Bio technology, 32(4), 341-353.
[5] Mane, U. H., (1974), "The growth and breeding habits of the clam, Katelysia opima in Kalbadevi estuary at Ratnagiri". Indian Journal of Fisheries, 21, 386-398.
[6] Mane, U. H. (1981), "The edible clam, Katelysia opima from the Ratnagiri Coast". Mahasagar- Bulletin of N.I.O., 14(1), 91-94.
[7] Nagabhushanam, R. and Mane, U. H. (1975a), "Reproduction and breeding habits of the clam, Katelysia opima in Kalbadevi estuary at Ratnagiri". Indian Journal of Marine Sciences, 4, 86-92.
[8] Nagabhushanam, R. and Mane, U. H. (1975b), "Seasonal varioation in the biochemical com position of the clam, Katelysia opima". Rivista de Biologia, 68, 279-301.
[9] Saini, D. and Sarin, R. (2012), "SDS-PAGE Analysis of Leaf Galls of Alstonia

scholaris (L.) R. Br." J Plant Pathol Microb., 3, 121.
[10] Taware, S.S. and Muley, D. V. (2013), "Size-dependent seasonal variation in respiration of clam Katelysia opima (Gmelin) exposed to lower salinity ranges along Bhatye estuary, Ratnagiri, India". J. Mar. Biol. Ass. India, 55 (1), 42-49.
[11] Upadhye, M.V. and Jadhav, U. (2010), "Tissue specific protein profiling of freshwater pearly mussel Parreysia corrugata". Journal of Aquatic Biology, 25(1), 103-107.