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Investigations on the non-conventional crab fishery resource, *Charybdis lucifera* (Fabricius, 1798) from Karnataka coast (India) and the need to commercialize the resource

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Original Article

Abstract

Charybdis lucifera, a seafood delicacy in many parts of the world, are discarded from commercial fishery operations of Karnataka coast (India), since it is not used for consumption in the region. Globally, in light of reports on overfishing and reduction in fishery, reducing the fishing pressure on many conventional species are becoming a priority. To meet the probable reduction in availability of seafood, due to the regulations on the fishery of conventional species, there is need to bring in more non-conventional species to commercial status. Information on nutritional quality of the non-conventional resources is the essential pre-requisite for effective use of these resources. This study, therefore, was focused to promote a non-conventional crab resource to commercial scale by unveiling information on their abundance, nutritional quality and economic importance. Our study revealed that *C. lucifera* is a regular component of trawl bycatch of Karnataka coast and annual average landing during 2015-2016 was estimated at 45 t. The species was found to have high meat content (27.2 ±5.9%) which is comparable or even better than most of the conventional edible crabs. Crude protein in male and female were 80.1 and 79.0% respectively, with high nutritive essential amino acid profile. Moreover, the species was found to possess rich PUFA, Eicosapentaenoic acid (13.5%) and Docosahexaenoic acid (16.8%) content, which qualifies it as a high quality seafood. At present these crabs are being disposed as "low value bycatch" for fertilizer and poultry feed after drying, with other trash fishes realizing an average price of Rs.5/kg which can be increased at least ten times, once it is popularized as a food crab. Moreover, its commercial use could eventually lead to increase in the landings of the species by discards. Even with the present annual catch data and with the projected value escalation, the revenue could be increased up to Rs.2.3 million (approx.30,000 $) every year, which definitely will help in improving the livelihood status of fishermen involved in coastal fishery. Its potential as a source of bio-active components like chitin, chitosan and astaxanthin was also projected in the study to supplement its economic significance.

Keywords: *Non-conventional resources, trawl discard, Chraybdis lucifera, nutritional quality, southwest coast of India, livelihood improvement*

Introduction

Globally, since 2013, one in eight people are deprived of enough food and most of them are in extreme hunger (FAO, IFAD and WFP, 2013). To feed a population, which was projected to reach nine billion by 2050 (Duarte *et al.*, 2009), the living resources from marine and coastal zones (comprising of 70% of the globe) are considered as potential source of healthy diet (Walford and Wilber, 1955; Duarte *et al*., 2009; Thilsted *et al*., 2014). Over the years due to adoption of technological advancements in fishing, fish production increased substantially. The production from the wild was 81.2 million t in 2015, wild catches were reported to be approaching its sustainable limit, as almost 90% of the global fish stocks are overfished (FAO, 2018). Conservation and management of the conventional resources and utilization of non-conventional fishery resources are considered viable options to meet the growing demand for fish from wild (Sajeevan and Nair, 2006). As far as exploitation and utilization of non-conventional marine resources are concerned, most of the earlier workers focused on exploitation and utilization of deep sea varieties from distant waters (Suda, 1973), however there were several difficulties in developing sustainable fishery for this resources, for example: technical difficulties, economic loss in deep sea fishing operation. Low resilience capacity of deep sea species, also made commercial deep-sea fisheries unsustainable (Elliott *et al*., 2012). Further it was estimated with "state of art" technological support, deep sea fishery could only contribute less than 1% of sea food. In this context, bringing more non-conventional fish protein to human diet is projected as one of the few alternatives to provide affordable protein supplement to growing population (Thilsted *et al*., 2014). Many non–conventional species with better preservation techniques and knowledge on the nutritional quality were brought out to provide human nutrition (Lai and Leung, 2003; Ilavarasan *et al*., 2015a, 2015b). Apart from utilizing for direct human consumption, guidelines are also available for preparation of food additives from conventional and non-conventional species (Mohanty and Roy, 1955; Olden, 1960). In addition to nutritional security, by using fishes from "discards" for human consumption, the economic status of the fishermen could be improved considerably with higher value realized for their catch (Thilsted *et al*., 2014).

In the present study attempts are made to explore the possibilities of using a species discarded during fishing as human nutritional supplement. While considering a non-conventional species for commercial exploitation, there are some prerequisites to be followed, to popularise its exploitation and consumption. Ascertaining the taxonomic status of the species, awareness of its commercial use elsewhere, ascertaining its availability in space and time for commercial exploitation are some of the prerequisites involved in the process. Even though availability of the species along Karnataka coast is known (Dineshbabu *et al*., 2011), to promote commercialisation of the species several aspects of biology, distribution and nutritional quality should be explored. Kumar *et al.* (2019) made a preliminary study on distribution and abundance of this species. Most importantly, while considering a species for popularisation, information on its meat content, its nutritional status and value addition are of prime importance. Preliminary studies on nutritional quality of *C. lucifera* was carried out on the east coast which suggests the possibility of acceptance of the species for human consumption (Ilavarasan *et al*., 2015a; Kumari *et al.,* 2015; Ramamoorthy *et al*., 2016). It was also suggested that there is regional variation in nutritional quality of this species, and in this context, the objective of the present study was to evaluate the distribution, abundance and nutritional quality of *C. lucifera* from west coast of India. Meat content, proximate composition, mineral content, amino acid profiles and fatty acid profiles were carried out in the present study, to explore its economic importance and also the chitin, chitosan and astoxanthin content available in the species were studied in detail.

Material and methods

Study area and sampling

Investigation was carried out from single day trawlers operating within 50 m depth along Karnataka coast (between 12.5^{0}N 75^{0}E and 15^{0}N and 74.5^{0}E) south west coast of India (Fig.1).

Study was carried out for two years from January 2016 to December 2017. A part of *C. lucifera* catch was kept in ice

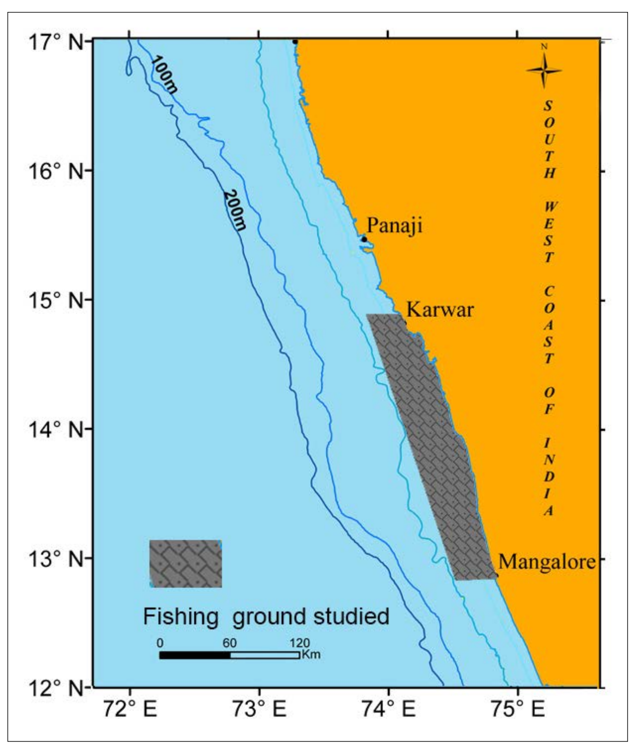

Fig. 1. Study area of *C. lucifera.*

packing and brought to the laboratory. In the laboratory, crabs were sex-wise segregated; measurements (carapace width to nearest mm and weight to nearest g) were noted. For Identification of *C. lucifera,* the keys provided by Mizzan and Vianello (2009) was followed.

Meat content

For extracting meat, carapace of individual crab was removed and meat content, including the meat from chelate legs, were removed. The meat was weighed and transferred to petri dish for further analysis. The male crabs used for the study ranged from 35 to 80 mm carapace width, weighing 18 to 108 g. In males 79 crabs having hard shells (inter-moult) were used for the study. In females from the inter-moult crabs 28 crabs without berried eggs were selected for the study. From the females 28 females 46 to 63 mm (CW) weighing 15 to 36 g. The overall meat percentage, meat percentage of males and females and meat percentage of the crabs in different size groups (carapace width) were derived (Balasubramanian and Suseelan, 2001).

Proximate analysis

Moisture, total protein, total lipids, ash and total carbohydrate of male and female crab were evaluated based on standard methods. Crude protein (N$\times$6.25) was evaluated by micro-Kjeldahl method (AOAC, 2012). The quantity of carbohydrate was determined by Phenol Sulphuric acid method (Dubois *et al.,* 1956). All determinations were done in triplicates. Mineral estimation was carried out by the method of inductively coupled plasma-optical emission spectrometric (ICP-OES) determination of elements using microwave-assisted digestion (Horwitz and Latimer, 2005).

The amino acid content of crab meat was determined based on the study of Hofmann *et al.* (2003) using isotope with gas chromatography–combustion–isotope ratio mass spectrometry (GCC-IRMS/MS) (GC: Hewlett-Packard 58590 series II, Germany; combustion series II-interface, IRMS MAT 252, Finnigan MAT, Germany; MS: GCQ, Finnigan MAT, Germany). The capillary column of dimension 50 m $\times$ 0.32 mm id. $\times$ 0.5 μm BPX5 (SGE) was connected to gas chromatography. The flow of carrier gas (helium) was maintained at 1.5 ml/min, with the head pressure 13 psi. The details of temperature program are given in Table 1. Classification of Nelson and Cox (2004) was adopted for classification of amino acids as nutritionally "essential" or "nonessential" or "conditionally essential".

Hot extracted crude fat from crab meat was used for determining the fatty acid methyl esters (FAMEs). The method suggested by Padua-Resurreccion and Banzon (1979) was used for this process. The analytical techniques such as FAMEs were quantified by gas chromatographer (GC2010, Shimadzu, Japan) equipped with fused silica column (BPX-70) and flame ionization detector (FID). The identification of peaks obtained from the lipid profiling was determined by comparing with National Institute of Standards and Technology Library (NIST 11 mass spectrometry library; NIST/EPA/NIH; version #2011). Further analysis was carried out following the methodology suggested by Nareshkumar (2007).

Chitin was extracted by demineralization and de-proteinization methods (Liu *et al.*, 2012) and for chemical composition followed (AOAC, 2012) methodology. Five gram of extracted Chitin was then subjected to deacetylation according to the method of Anand *et al.* (2014) and Chemical composition estimated by using standard method (AOAC, 2012). The surface morphological appearance of chitin and chitosan were examined with SEM (JSM 6380LA; JEOL, Tokyo, Japan). The presence of chitin and chitosan were confirmed by infrared spectrophotometer (Shimadzu FTIR-8700), characterized from 500 to 4,000 cm^{-1}.

Astaxanthin extraction was done by following the method of Dalei and Sahoo (2015). Quantification of astaxanthin was done by using the method of Kelley and Harmon (1972). The presence of astaxanthin was confirmed by infrared spectrophotometer (Shimadzu FTIR-8700), characterized from 500 to 4,000 cm^{-1}.

Results and discussion

Species description

Species identification was done using standard identification keys (Nguyen, 2002).

Classification

Phylum: Arthropoda
Subphylum: Crustacea
Order: Decapoda
Family: Portunidae
Genus: *Charybdis*

Species: *Charybdis (Charybdis) lucifera* (Fabricius, 1798) (Fig. 2)

Table 1. Temperature program for GC-C-IRMS/MS of Amino acid analysis

Time (min)	Temperature (°C)	Temperature/min
1	50	Start
10	50-100	10°C/min
10	100-175	3°C/min
10	175-200	3°C/min
10	250	stop

Fig. 2. *C. lucifera* (dorsal view)

C. lucifera is an edible variety which is commercially exploited and marketed along east coast of India (Ilavarasan *et al.*, 2015a). Along west coast of India this species is not considered for human consumption and often discarded or used in fish meal plants. Studies on the distribution of the species showed that they have been a part of the bycatch of single day trawl throughout the trawling season and is extensively distributed all along Southwest coast of India (Kumar *et al.*, 2019) and annual average landing in Karnataka coast during 2015-2016 was estimated at 45 t which is higher than the crab landings from east coast of India (Ilavarasan *et al.*, 2015a).

Meat content

Studies showed that overall meat content for the species was 27.2 ±5.90%. In males the average meat content was 27.7 ±5.71% and in females it was 22.1±5.78%. Size group-wise meat content in males and females are given in the Table 2.

Table 2. Meat content analysis of *C. lucifera* male and female. n=3, means ± SD

Classification for meat content	Average meat weight (percentage)
Overall meat content for the species	27.2±5.90
Meat content in males	27.7±5.71
Males, CW range 30-40mm	20.0±0.42
Males, CW range 40-50mm	26.5±3.88
Males, CW range 50-60mm	28.1±5.82
Males, CW range 60-70mm	27.2±5.98
Males, CW range 70-80mm	29.3±6.19
Meat content in females	22.1±5.78
Females, CW range 30-40mm	21.7±6.18
Females, CW range 40-50mm	26.3±0.97

Values are given as mean ± SD from triplicate determinations.

It is an established fact that male crabs yield more meat both from body and claws when compared to females (Sreelakshmi *et al.*, 2016). Even though the meat content in *C. lucifera* is not high as in *Scylla tranquebarica* (32.99%) reported by Sreelakshmi *et al.* (2016) and as in blue swimmer crab *Portunus pelagicus* (32%) (Wu *et al.*, 2010), but is comparable with those in *S. serrata* (30.44%) reported by Sreelakshmi *et al.* (2016) and was higher than in Chinese mitten crab *Eriocheir sinensis* (24.2%) as stated by Chen *et al.* (2007), the deep water crab *Charybdis smithii* (15.3%) (Balasubramanian and Suseelan, 2001), Atlantic spider crab *Maja brachydactyla* (17%) (Marques *et al.*, 2010) and brown crab *Cancer pagurus* (23%) (Barrento *et al.*, 2010). The present study throws light on the huge potential of crab meat availability if exploited commercially.

Biochemical compositions

Present study was conducted on dry weight basis. The moisture content in males and females were 89.27 and 84.31% respectively (Table 3), Crude protein formed 80.10 and 79.01% in males and females respectively. Apart from their delicacy, marine crustacean resources are well known for richness in nutrition (Heu *et al.*, 2003). Considering the health benefits of these sea food, there are growing number of research for promoting crustacean consumption (Rosa and Nunes, 2003; Chen *et al.*, 2007). Crabs specially crab muscle with their high protein and low fat and cholesterol content is getting acceptability as a healthy food globally (Barrento *et al.*, 2010). Studies conducted by Ramamoorthy *et al.* (2016) showed that protein content in *C. lucifera* was higher (22.57%) than conventional species *P. pelagicus* (20.15%). In the present study, carbohydrate in *C. lucifera* was 3.68% in males and 5.61% in females (Table 1). Ramamoorthy *et al.* (2015) found higher percentage of carbohydrate content in *C. lucifera* (1.17%) when compared to the conventional species *P. pelagicus* (0.54%), whereas the lipid content was comparatively lower than in *C. lucifera* compared to *P. pelagicus* (2.15 %). Since the present study was conducted on dry weight basis, direct comparison to the values observed by Ramamoorthy *et al.* (2016) was not possible. However comparison with studies conducted by Lyla *et al.* (2017) supports the nutritional superiority of *C. lucifera* over conventional species *P. pelagicus* and *P. sanguinolentus*

Table 3. Proximate composition of *C. lucifera* male and female (%, dry weight) n=3, means ± SD

Parameters (%)	*C.lucifera* male	*C.lucifera* female
Dry matter	10.73±0.22	15.69±0.41
Moisture	89.27±0.21	84.31±0.27
Crude Protein	80.10±0.09	79.01±0.07
Crude fat	3.00±0.00	4.92±0.18
Crude ash	13.22±0.02	10.46±0.21
Carbohydrate	3.68±0.04	5.61±0.08

Table 4. Proximate components of selected *C. lucifera* in comparison with conventional commercial species from the coast (%, dry weight of males) n=3, means ± SD

Parameters (%)	*C. lucifera*	*P. pelagicus*	*P. sanguinolentus*
Moisture	89.27±0.21	80.92±0.8	81.61±0.6
Protein	80.10±0.09	73.71±0.1	73.97±0.1
Lipid	3.00±0.00	5.68±0.01	6.96±0.1
Carbohydrate	3.68±0.04	1.39±0.8	1.40±0.5
Ash	13.02±0.02	12.06±0.6	12.66±0.0

Lyla *et al.* (2017)

(Table 4) as these two crabs were having 73.71 and 73.97% of protein. The protein content found in *C. lucifera* in the present study was higher than those reported in Chinese mitten crab (*E. sinensis*) (Chen *et al.*, 2007), *Podopthalmus vigil* (Sudhakar *et al.*, 2011) and *Portunus sanguinolentus* (Sudhakar *et al.*, 2009).

The richness of amino acids, which are the building blocks of proteins also determines the supremacy of seafood. In east coast of India Ilavarasan *et al.* (2015a) observed that the species to have vast potential in pharmaceutical formulation, with identification of 9 essential amino acids from the species, whereas present study showed that the species along west coast of India has 9 non-essential and 8 essential amino acids (Table 5) Ramamoorthy *et al.* (2016) while conducting a comparative study of the species recorded higher amount of amino acids in *C. lucifera* (3.92 g/100g) compared to *P. pelagicus* (2.79 g/100g). Richness of amino acids in *C. lucifera* from west coast of India is presented in Table 5. Total essential amino acid content of *C. lucifera* was above 42.30% which is considered as very good for human nutrition.

Table 5. Amino acid composition of *C. lucifera*, male and female (%, dry weight) n=3, means ± SD

Amino acid (g/100g)	*C.lucifera* male	*C.lucifera* female
Non essential		
Asp	4.79±0.09	4.26±0.09
Glu	9.05±0.09	8.85±0.02
Ser	2.34±0.01	2.42±0.01
Gly	14.64±0.05	13.46±0.12
Arg	8.02±0.06	8.91±0.07
Ala	8.70±0.02	8.47±0.03
Pro	5.79±0.09	7.28±0.09
Tyr	2.72±0.07	3.85±0.01
Cys	0.31±0.01	0.29±0.00
Essential		
Ile	4.81±0.07	4.86±0.11
Leu	7.54±0.05	7.46±0.01
Phe	3.69±0.09	3.77±0.03
Lys	13.31±0.19	12.53±0.10
His	2.04±0.01	1.64±0.03
Thr	3.15±0.02	3.03±0.08
Val	5.32±0.01	5.27±0.07
Met	2.44±0.03	2.37±0.07
TEAA	42.30±0.47	40.93±0.50
TAA	98.66±0.96	98.72±0.94

Classification of AAs as nutritionally "essential" or "nonessential" or "conditionally essential" is as per Nelson and Cox (2004).

Marine lipids with long-chain polyunsaturated fatty acids has proved to have cardio protective action (Kris-Etherton *et al.*, 2002; Ramamurthy *et al.*, 2015). In the present study, *C. lucifera* is found to have low fat content (3% in male and 4.92% in female), which was lower than those reported by Lyla *et al.* (2017) in *P. pelagicus* and *P. sanguinolentus*, which were 5.68 and 6.96% respectively (Table 3). The sea food with low fat and rich long chain polyunsaturated fatty is reported to have high dietary significance in human growth (Shahidi and Wanasundara, 1998, Dunstan *et al.*, 2007; Su *et al.*, 2008) and *C. lucifera* found to be rich in PUFA, Eicosapentaenoic acid and Docosahexaenoic acid content with 13.54 and 16.80% respectively (Table 6), similar findings of comparatively high percentage of PUFA in *C. lucifera* was reported in east coast also (Ramamoorthy *et al.*, 2015). Mineral analysis of crab meat showed 8 minerals, Sodium, Potassium, Magnesium, Calcium, Manganese, Iron, Zinc and Copper in the present study (Table 7) is one more from those reported from east coast (Kumari *et al.*, 2015) in which copper was absent.

Bioactive compounds

Crustaceans are considered as a good source of Chitin (Kaur and Dhillon, 2013) which is having many nutritional and medicinal utility. It was estimated that approximately half of weight of major crustaceans are formed of chitin (Islam *et al.*, 2004). *C. lucifera* is a rich source of chitin and the analysis of exoskeleton showed that it contained 16.72% protein, (Table 8). Percentage of extractable protein from the shell was 12.23% which is comparable with the chitin from commercial shrimps (12.46%) (Table 9). The presence of chitosan is confirmed by FTIR analysis compared with the study literature on crab by Quimque and Acas (2015). FTIR of extracted shell Chitosan of *C. lucifera* was found to be 3348 cm for hydroxyl group (-OH). Astaxanthin is a xanthophyll carotenoid which is found in various microorganisms and marine animals, which has

Table 6. Fatty acid methyl esters (g/100g lipid) of *C. lucifera* male and female (dry weight) n=3, means ± SD

Fatty acids (g/100g)		*Charybdis lucifera* (male)	*Charybdis lucifera* (female)
Saturated fatty acid			
Palmitic acid	C16:0	24.75±0.21	25.05±0.03
Heptadecanoic acid	C17:0	3.32±0.07	3.02±0.01
Stearic acid	C18:0	18.89±0.27	19.13±0.11
Hexanoic acid	C6:0	0.36±0.06	0.29±0.09
Octanoic acid	C8:0	0.65±0.12	0.99±0.02
Myristic acid	C14:0	1.00±0.01	1.43±0.12
Behenic acid	C22:0	0	0
Pentadecanoic acid	C15:0	0	0
tricosanoic acid	C23:0	0	0
Lignoceric acid	C24:0	0	0
Arachidic acid	C20:0	0	0
Monounsaturated fatty acid			
Palmitoleic acid	C16: 1 Cis	2.67±0.07	2.73±0.13
Oleic acid	C18 : 1 Cis	15.68±0.18	13.47±0.23
Eicosenoic acid	C20:1 Cis	0	0
Polyunsaturated fatty acid			
alfa Linolenic acid	C18: 3 Cis	0	0
Linoleic acid	C18:2 Cis	2.34±0.26	3.10±0.06
Eicosapentaenoic acid	C20 : 5 Cis	13.54±0.32	14.79±0.41
Docosahexaenoic acid	C22: 6	16.80±0.03	16.00±0.00
SFA		48.97±0.74	49.91±0.38
MUFA		18.35±0.25	16.20±0.36
PUFA		32.68±0.61	33.89±0.47
PUFA/SFA		0.66±0.82	0.67±1.23

Table 7. Mineral content of *C. lucifera* male and female (dry weight) n=3, means ± SD

Dry weight mg/100gm	*C. lucifera* male	*C. lucifera* female
Copper	0.95±0.36	0.65±0.36
Zinc	3.94±0.63	3.81±1.02
Manganese	0.07±0.03	0.07±0.05
Iron	2.48±0.45	1.80±0.27
Magnesium	69.40±0.32	48.80±0.28
Sodium	651.80±1.01	738.30±0.53
Potassium	251.80±0.51	254.20±0.42
Calcium	113.56±0.32	320.80±0.82

Table 8. Chemical composition of shell (exoskeleton) of crab *C. lucliera* (dry weight) n=3, means ± SD

%	*Charybdis lucifera*
Shell moisture	64.02± 0.30
Protein	16.72± 0.22
fat	0.72± 0.02
Ash	47.21± 0.15

Table 9. Chemical composition of Chitin from the shell of *C. lucliera* and commercial shrimp Chitin (dry weight) n=3, means ± SD

Chitin (%)	Commercial shrimp chitin	*Charybdis lucifera*
Yield	-	10.4± 0.09
Moisture	7.28 ± 0.16	8.12± 0.02
Protein	12.46 ± 0.33	12.23± 0.20
fat	0.54 ± 0.02	0.00
Ash	0.09 ± 00	0.0981± 0.01

great demand in food, feed, nutraceutical and pharmaceutical applications. Suganya and Asheeba (2015) and Sachindra *et al.* (2005) extracted astaxanthin from *Portunus sanguinolentus*, *Callinectes sapidus* and *Paralithodes brevipes, Charybdis feriata* and the present study showed that *C. lucifera* also can serve as a source for astaxanthin with extractable astaxanthin of 9.19 μg /g shell wet weight.

SEM pattern figure represents the SEM photographs of commercial shrimp chitin (Fig. 3) and the chitin obtained from the exoskeleton of *C. lucifera,* exhibited dense and firm surface morphology images under the electron microscopic examination at 100X to 2000X magnification (Fig. 4). The surface morphology of the chitin of *C. lucifera* at 100X and 1000X exhibited clear nanofibres. SEM photographs of commercial shrimp chitosan (Fig. 5) and the chitosan from *C. lucifera* exhibited dense and firm surface morphology images under the electron microscopic examination at 100X to 1000X magnification (Fig. 6). The surface morphology of chitosan from *C. lucifera* at 100X and 1000X exhibited clear nanofibres. The porous structure of chitin is used in metal ion absorption and tissue engineering and the fibrillary structure can be used in textiles (Zelencova *et al.*, 2015). There are several commercial brands already available in the global markets (Sastry *et al.*, 2015).

Present study showed that *C. lucifera* is rich in nutrients, is a good source of high quality chitin, chitosan and astaxanthin. At present the species is disposed as low value bycatch for fertilizer and poultry feed after drying, along with other trash fishes realizing an average price of Rs.5/kg. Often the species is discarded at sea, due to lack of facility for drying in most of

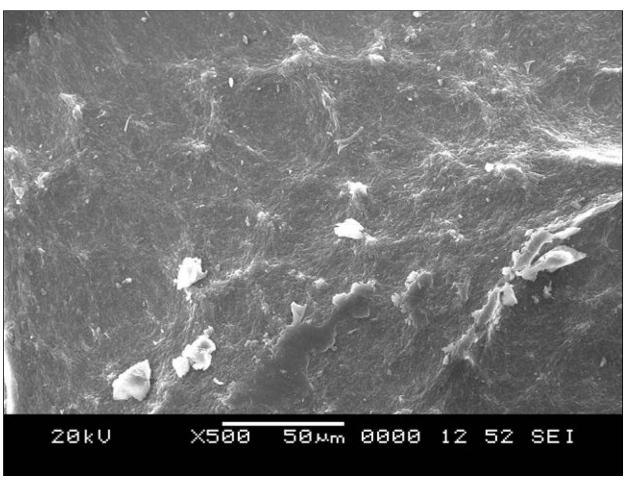

Fig. 3. SEM image of shell chitin of commercial shrimp

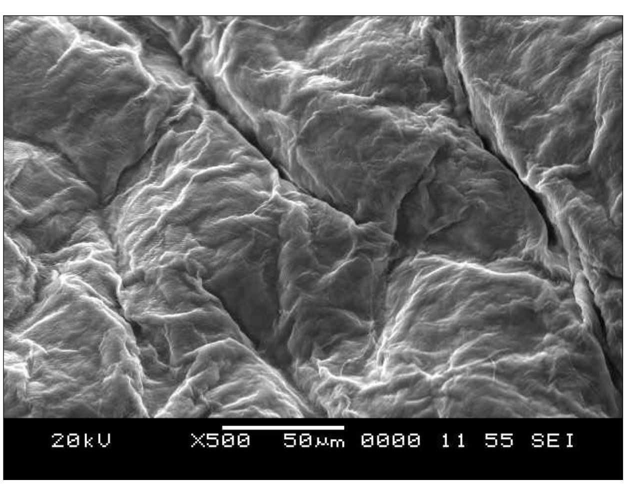

Fig. 4. SEM image of shell chitin of *C. lucifera*

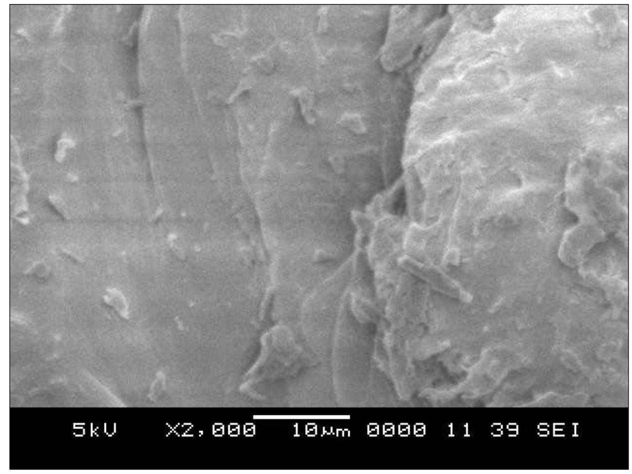

Fig.5. SEM image of shell chitosan of commercial shrimp

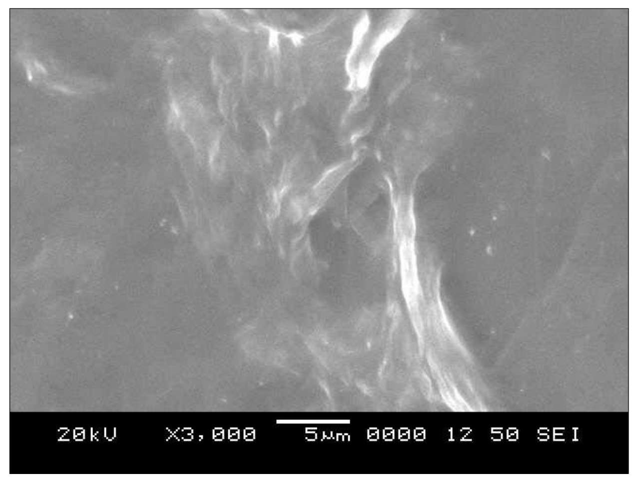

Fig.6. SEM image of shell chitosan of *C. lucifera*

the landing centres. The meat content in these crabs are as high as 27% which is comparable with most of the edible species in India, denoting the potential of these crabs for domestic consumption as well as export. Once it is popularized as human food with efficient processing and marketing methods, increased price can be obtained, which will be a great boon for the fishermen involved in the coastal fishery. If Rs. 50/kg is earned for the catch, with the annual landing reported. (45t in 2015-2016), the total value realized for the catch can escalate to Rs.2.3 million (approx.30,000 $), eventually improving the livelihood status of the fishermen.

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