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EXTRACTION AND CHARACTERIZATION OF CHITIN FROM MARINE BYCATCH CRUSTACEANS EMPLOYING CHEMICAL METHOD

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Abstract

Chitin is a versatile compound and most abundant biopolymer next to cellulose. It has plenty of applications for its resourceful potentials especially as artificial skin, anti-cancer drug, waste water treatment agent, etc. In the present study, an attempt has been made to extract chitin from trash crabs *Calappa lophos, Dromia dehaani, Dorippe facchino* and stomatopod *Squilla* spp. The chitin extracted from exoskeleton shells by demineralisation with acid treatment and deproteinisation with strong alkali treatment. The chitin yield was 37.1%, 27.7%, 8.97% and 24.18% from *C. lophos, D. dehaani, D. facchino* and *Squilla* spp. respectively. The extracted chitin was confirmed by FT-IR analysis compared with standard grade chitin. The quality examined by analysis of moisture, ash, protein and lipid contents. Surface of the chitin was examined under Scanning Electron Microscope (SEM).

Key words: Chitin, Demineralization, *Calappa lophos,* FT-IR, Scanning Electron Microscope, Deproteinization and Bycatch

1.Introduction

The ever increasing demand for fish and fishery products from a burgeoning human population contribute to an alarmingly increase in global fishing effort. Trawl fishery reached in its zenith in the last three decades that paved way to indiscriminate and irrational over exploitation. Demersal trawl aiming at shrimp catch is the most destructive forms of fishing that reduces the structural heterogeneity of benthic habitats of a range of fish and invertebrates (Frid and Hall, 1999; Haywood *et al.*, 2005; Jennings *et al.*, 2005). Along with the target fishes, this type of fishing will indiscriminately catch quite a large number and biomass of non-target species (bycatch) which was estimated to be between 6.8 and 20 million tons per annum globally (FAO, 1999; Kelleher, 2005). It has been estimated that 23% of global fisheries, that amounts to nearly 20 million tons are discarded every year (FAO, 2004).

Discarded bycatch organisms landed in fish landing centres brought back to sea create many problems as majority of them die after capture or in such a moribund state that they will not survive. Moreover, discarded bycatch species has adverse impacts either biologically, ecologically or economically (Raffi, 2006). The by-catch issue is also one of waste; the millions of tons of protein dumped in the ocean and the waste of animal lives was often condemned on moral grounds (Hall *et al.*, 2000).

Understanding this distressing state, FAO Code of Conduct for Responsible Fisheries emphasized that "maritime states should improve the use of bycatch to the extent that this is

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consistent with responsible fisheries management practices" (clause 8.4.5) (Clucas, 1997).The Code of Conduct for Responsible Fisheries also emphasize the maritime states to " encourage those involved in fish processing, distribution and marketing to improve the use of bycatch to the extent that is consistent with responsible fisheries management practices" (FAO, 1995). Recently, the global trend has been focusing on the better utilization of bycatch and to reduce the quantity of bycatch landed (Zynudheen *et al.*, 2004). Trash crustacean exoskeleton shells are rich in the chitin content (Arbia *et al.*, 2012; Palpandi *et al.*, 2009; Das and Ganesh, 2010).

Chitin, a naturally abundant mucopolysaccharide and distributed in the shells of crustaceans, in the cuticle of insects and in some cell wall of fungus (Muzzarelli, 1994). Like cellulose, it functions as structural polysaccharides and serving as a supportive and protective material in crustacean, molluscs, insects, bacterial cell wall and fungi (Sugimoto *et al.*, 1998). This biopolymer consists of 2-acetamido 2-deoxy-β-D-glucose through a β (1-4) linkage and that has intra and inter-molecular hydrogen bonds (Yusof *et al.*, 2004). Chitin has vast applications such as: waste water treatment, industrial effluent treatment by heavy metal adsorption, haemostatic, fungistatic, spermicidal, antitumour, anticholestremic activity, accelerates bone formation etc., (Dutta *et al.*, 2004). Annual production of chitin is estimated that nearly 10^{10}-10^{11} tons by global (Nair and Dufresne, 2003). Japan and USA are the main countries in the production of chitin but Indian production is very less. In India, this polymer found to be a good growth promoter of broiler chicks. In countries such as Brazil, Cuba, Ireland, Norway, Uruguay and Russia, production of this polymer is under consideration (Thirunavukkarasu *et al.*, 2011). Based on this evidence the present study focused on the production of chitin.

2. Materials and Methods

Raw material collection

The specimens *C. lophos, D. dehaani, D. facchino* and *Squilla* spp. were collected from Mudasalodai (Lat. $11^0$29'N; Long. $79^0$46'E) fish landing centre, Tamilnadu, India. Samples were washed thoroughly with tap water to remove the sand and other dirty particles. The exoskeleton separated and dried in hot air oven at 60^0 C for 48 hours.

2.2.Chitin Production

The powdered exoskeleton subjected to extraction of chitin by following the methodology of Takaguchi (1991).The chitin yield was estimated after the demineralisation and deproteinisation steps. To get average yield the experiment was repeated for three times.

2.3. FT-IR spectral analysis

IR characterization of chitin was performed with Perkin Elmer Spectrum RX1 type FT-IR instrument at Central Instrumentation Laboratory, Annamalai University. The standard grade chitin (Marine Chemicals, Cochin, India) was compared with that of the chitin obtained from all the species by following the methodology of Kumirska *et al.* (2010). The spectrum of chitin was obtained with a frequency range of 4000 - 400 cm^{-1}.

2.4.Scanning Electron Microscope Analysis

The physical structure and nature of chitin was obtained with the help of Scanning Electron Microscope (SEM) (Model: JEOL.JSM 5160 with INCAEDS, version 1.1, Japan) at Central Instrumentation Laboratory, Annamalai University, Tamilnadu. Powdered chitin was well dried in hot air oven at 60°C for 6 hours. Prior to analysis the chitin samples were sprinkled onto carbon tapes which are adhesive and supported on metallic disks and coated with Au (silver). Images of the sample surfaces were recorded at different areas and magnifications. SEM images of chitin produced only from *C. lophos* were compared with that of standard chitin. Since, it was fetched higher yield.

2.4.Quality analysis

The extracted chitin from various species were subjected to moisture and ash content analysis by followed the methodology of AOAC (1995). Besides above protein (Raymont *et al.*, 1964) and lipid (Folch *et al.*, 1957) were estimated by adopting standard methods.

3.Results and Discussion

3.1. Chitin Production

The average yield of chitin from 100 g of raw exoskeleton of *C. lophos, D. dehaani, D. facchino* and *Squilla* spp. are 37.1 g, 27.7 g, 8.97 g and 24.18 g respectively (Table - 1). Among four species used in the present study, chitin obtained from *C. lophos* showed higher yields of 37.1%; which was followed by *D. dehaani*, *D. facchino* and *Squilla* spp. The acid was added to the crustacean shell powder, demineralisation could be achieved at a faster rate, with minimal decrease in residual ash content (Zakaria *et al.*, 1998). This process was depending on the concentration of acid in such a way that higher the acid concentration, the more efficient was the solubilisation of calcium carbonate ($CaCO_3$), with minimal residual ash content. The HCl (2N) caused a complete decrease of the minerals from the shells by reducing the retention time from 12 to 24 hours (Rodde *et al.*, 2008). The deproteinisation process conducted with a strong alkali in the pH range of 14 and with high temperature ranged between 75 to 80ºC; destroys the protein molecules from the crustacean shells. Alkaline solutions with higher concentration of chitin (5%) resemble semitransparent, opalescent, viscous mass.

Earlier works done on yield analysis proved that content of chitin in shells varied depending on species and also with different body parts within the species. Nair and Madhavan (1989) extracted chitin from *Scylla serrata* reported that the chitin content in carapace was 20.05%, which was more than that of claw (10.0%) and leg (14.0%). Contradictory to this, Das *et al.* (1996) observed that chitin content were 16.7%, 11.67% and 10.42% in *S. serrata* whereas in *Portunus pelagicus* it was 20.19%, 13.51% and 11.67% in leg, carapace and claw respectively.

Green and Mattick (1979) postulated that the proportions of chitin and the non-chitinous fractions vary with species and with season. Subasinghe (1999) observed higher chitin yield in snow crab legs (32%). Tseng *et al.* (1999) evidenced 21% of chitin extraction from shrimps and 15 % from crab shell waste. Varghese (2002) observed an average of 2-3.9 g (12-19%) of chitin from the exoskeleton of stomatopod *Harpiosquilla melanoura.* Tirunavukkarasu (2005) reported an average yield of 10.74% from the carapace of *S. tranquebarica;* whereas Odote *et al.* (2005) reported that the percentage wise yield of chitin was 23.0% for *S. serrata* (brachyuran crabs), 15.7% for *Panulirus ornatus* (lobster) and 28.0% from *Penaeus indicus* (shrimps). Abdou *et al.* (2008) reported about 21.53% and 23.72% percentage of chitin from shrimps *P. aztecus* and *P. durarum* respectively, 16.73% from crab shells and 20.60% from crayfish *Procambarus clarkia* shells, 5.40% from cuttlefish pens and 49% from squid pens. Kanagaraj (2007) extracted chitin from brachyuran crabs *Doclea ovis, Podopthalmus vigil, Charybdis natator, C. miles* and *C. lophos,* which were to the tune of 18.95%, 12.38%, 10.84%, 7.41% and 21.45% respectively.

Table - 1: Table showing the average yield of chitin produced from marine trash crustaceans

Source	A		B		C		Average yield of the chitin (g)
	Raw material (g)	Chitin (g)	Raw material (g)	Chitin (g)	Raw material (g)	Chitin (g)	
C. lophos	100	36.8	100	37	100	37.5	37.1
D. dehaani	100	26.67	100	28	100	26.51	27.7
D. facchino	100	8.9	100	9.01	100	9	8.97
***Squilla* spp.**	100	23.8	100	24.56	100	24.18	24.18

3.2. FT-IR Analysis

The results of FT-IR spectral peaks assignment of *C. lophos, D. dehaani, D. facchino* and *Squilla* spp. chitin and standard chitin were represented in Fig – 1 to 5. The FT-IR investigation proved the existence of helical arrangement of chitin in standard chitin. The amide-A band of standard chitin was recorded at 3434 cm^{-1}, that showed that there were OH groups involved in free hydroxyl bonds. The amide-B band of chitin was found at 2960 cm^{-1} which was related to asymmetric and symmetric stretching H-C-H, were as amide-I, amide-II and amide-III bands were observed at 1425 cm^{-1}, 1418 cm^{-1} and 1261 cm^{-1} respectively. In the case of *C. lophos, D. dehaani, D. facchino* and *Squilla* spp. chitin, amide-I were showed at 1654 cm^{-1}, 1641 cm^{-1} and 1654 cm^{-1}respectively against those of standard chitin which is 1653 cm^{-1}. Amide-II was represented at 1425 cm^{-1}, 1413 cm^{-1}, 1379 cm^{-1} and 1411 cm^{-1} for *C. lophos, D. dehaani, D. facchino* and *Squilla* spp. respectively against at 1418 cm^{-1} for standard chitin representing CH_2 bend and CH_3 deformation. The amide-III band position of standard chitin was in the range of 1262 cm^{-1}, 1261 cm^{-1}, 1262 cm^{-1} and 1258 cm^{-1} respectively. From the results of the FT-IR spectral analysis, it was evident that, the chitin produced from the *C. lophos, D. dehaani, D. facchino* and *Squilla* spp. showed helical arrangement and their functional properties more or less matching with that of standard grade chitin. The stretching bands of the OH groups involved in hydrogen bonds O-3-H. O-5 occurs at 3440cm^{-1}(Pearson *et al.*, 1960 and Focher *et al.*, 1992). The C=O stretching region of the amide moiety between 1600 and 1500 cm^{-1} for chitin, the Amide-I band is split at 1656 and 1621cm^{-1} for β – chitin (Focher *et al.*, 1992).

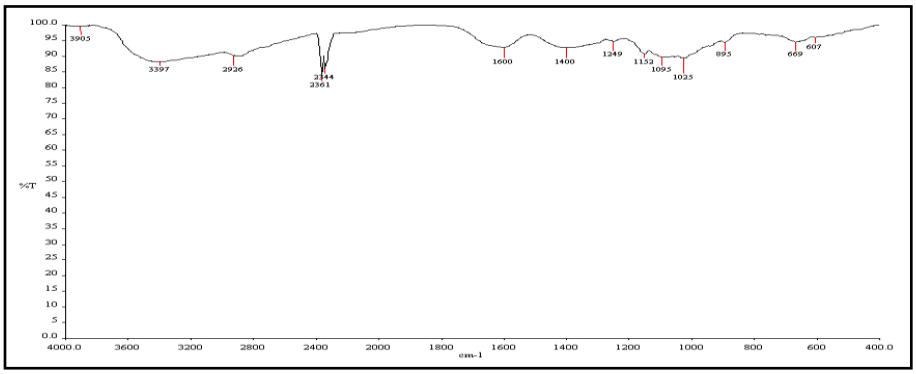

Fig - 1: FT-IR spectral analysis of standard grade chitin

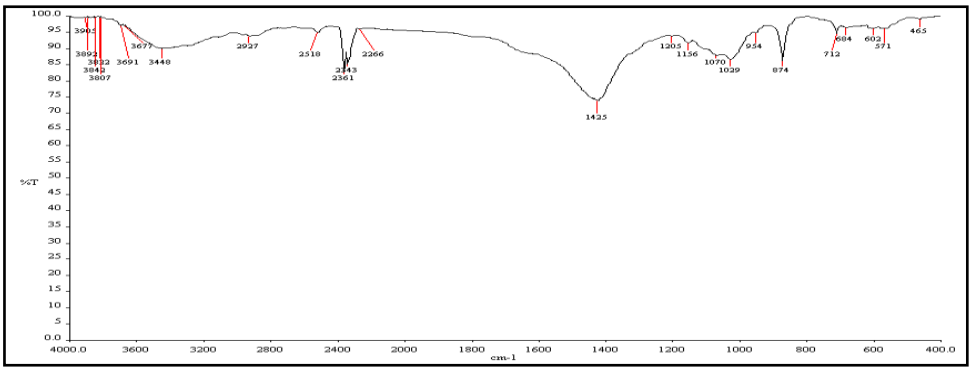

Fig - 2: FT-IR spectral analysis of chitin produced from *C. lophos*

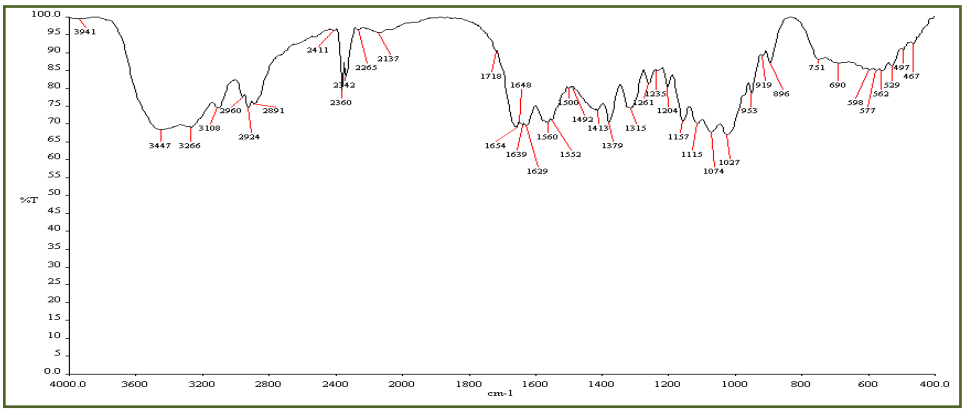

Fig.3: FT-IR spectral analysis of chitin produced from *D. dehaani*

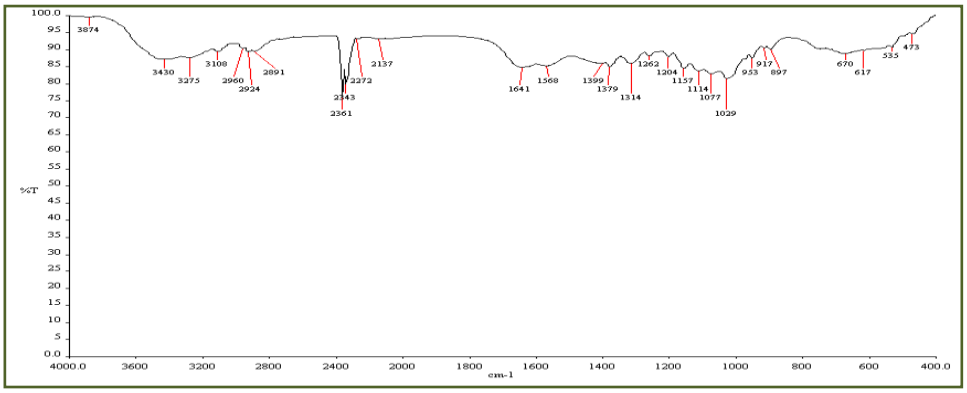

Fig.4: FT-IR spectral analysis of chitin produced from *D. facchino*

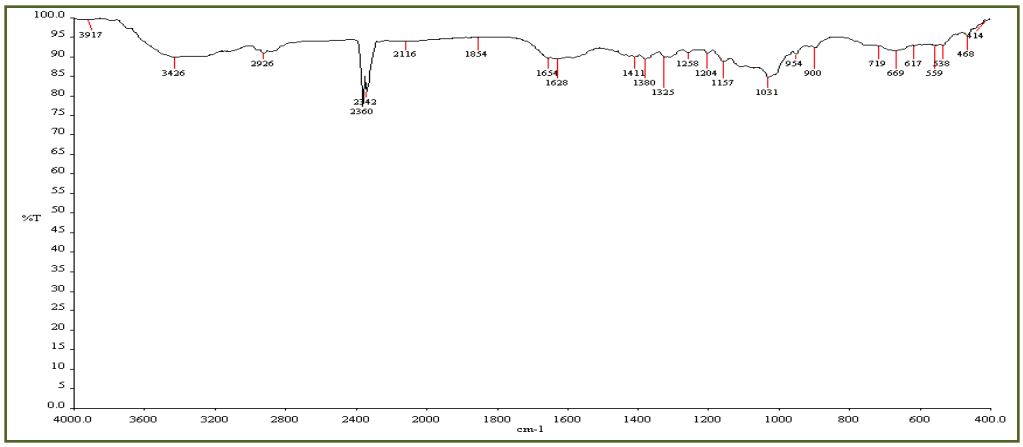

Fig.5: FT-IR spectral analysis of chitin produced from *Squilla* spp.

SEM Analysis

The SEM images with 1000X magnifications of chitin are shown in Fig - 6. In the present study, SEM images of chitin produced from *C. lophos* alone compared with that of standard chitin; since it fetched higher yield of the present study. It was observed that chitin biopolymer produced in the present study exhibits porous and fibril structures as that of standard chitin. Al-Sagheer *et al.* (2009) described the structure of chitin as crystalline and dense that was extracted from the exoskeleton of shrimps, *P. semisulcatus* (de Haan), *Metapenaeus affinis* (Milne-Edwards); from brachyuran crabs *P. pelagicus* (Linne), from lobster *Thenus orientalis* (Lund) and from cephalopod *Sepia* spp.

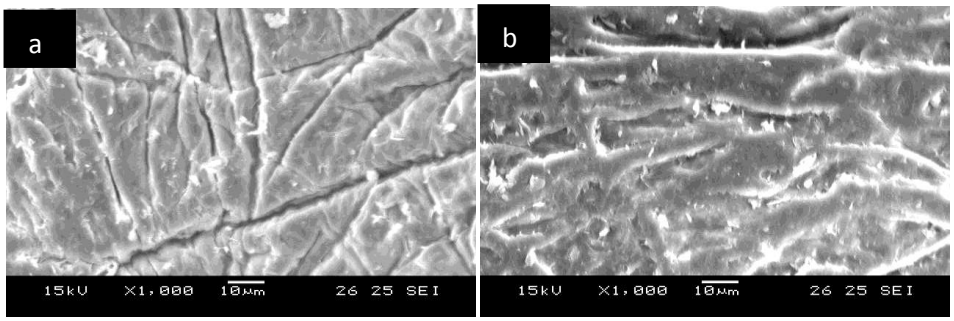

Fig - 6: SEM images of standard grade chitin (a) and chitin extracted from *C. lophos*(b) portrayed under 1000X magnification

3.4. Quality analysis

The chitin contains very little amount of moisture, lipid, protein, and ash content and was satisfactory in line with commercial international standards (Fig - 7). The extracted chitin contains very less protein that showed that deproteinisation was more effective. The proximate of chitin used to vary in its chitin and non–chitinous fractions which might be due to the variation in parent source (raw material). The quality of chitin is assessed based on its proximate composition, moisture content, ash content, DDA in such a fashion that lower the levels of protein, lipid, moisture and ash may results in better quality of chitin. Non – chitinous materials negatively affects the quality and property of chitin by interacting with it (Skaugrud and Sargent, 1990).

The proportion of chitin and the non– chitinous fractions varies with species (Green and Martrick, 1979). The chitin extracted by chemical method, contains very little amount of moisture content, lipid, protein, and ash content and is satisfactory in line with commercial international standard.

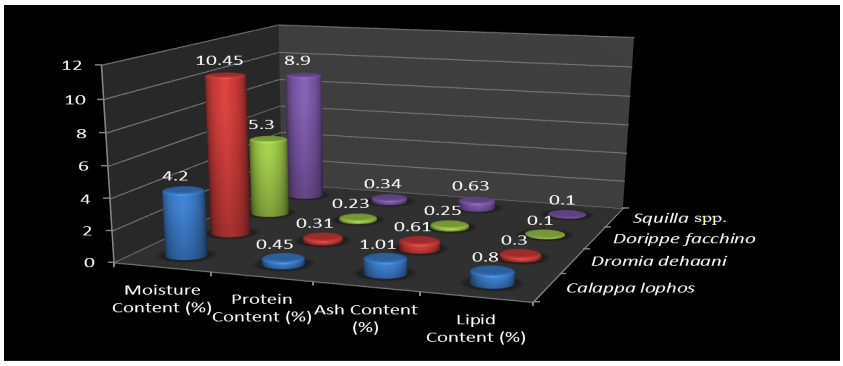

Fig - 7: Moisture content, Protein content, Ash content and Lipid content of chitin produced from *C. lophos, D. dehaani, D. facchino and Squilla* spp.

4. Conclusion

In the present study, the byproduct chitin was extracted from trash crustaceans. The chitin present in the trashes' shells proved that has high quality and quantity and nearly equal to commercial grade. This study directs to reduce the environmental pollution reduced by utilizing the trashes for the production of byproduct. These results supported that it is very meaningful for extraction of chitin from a very less expensive source since the most of the previous works on chitin extraction were done commercially valuable crabs and shrimps.

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5. Reference

1) Abdou, E.S., K.S.A. Nagy and M.Z. Elsabee, 2008. Extraction and characterization of chitin and chitosan from local sources. *Bioresource Technology*, 99: 1359-1367.
2) Al-Sagheer, F.A., M.A. Al-Sughayer, S. Muslim and M.Z. Elsabee, 2009. Extraction and characterization of chitin and chitosan from marine sources in Arabian Gulf. *Carbohydrate polymers*, 77(2): 410-419.
3) AOAC, 1995. Official methods of analysis (16thEds.). Association of official analytical chemists, Washington, DC.
4) Arbia, W., L. Arbia, L. Adour and A. Amrane, 2012. Chitin extraction from crustacean shells by biological methods – A review. Ecole Nationale Supérieure de Chimie de Rennes. Université européennede Bretagne, France.
5) Clucas, I., 1997. A study of the options for utilization of bycatch and discards from marine capture fisheries. FAO Fisheries Circular. No. 928. Rome, FAO.
6) Das, N.G., P.A. Khan and Z. Hossain, 1996. Chitin from the shell of two coastal Portunid crabs of Bangladesh. *Indian Journal of Fisheries*, 43(4): 413-415.
7) Das, S. and E.G.A. Ganesh, 2010. Extraction of chitin from trash crabs (*Podophthalmus vigil*) by an eccentric method. *Curr. Res. J. Biol. Sci*., 2: 72-75.
8) Dutta P.K., J. Dutta and V.S. Tripathi, 2004. Chitin and Chitosan : Chemistry, properties and application. *J.Sci. and Ind. Res.,* 63: 20-31.
9) FAO, 1995. Code of conduct for responsible fisheries. *Food and Agriculture Organization*, Rome, Italy.
10) FAO, 1999. The State of World Fisheries and Aquaculture (SOFIA) 1998. *Food and Agriculture Organization*, Rome, Italy.

11) FAO, 2004. Report of the expert consultation on fishing vessels operating under open registries and their impact on illegal, unreported and unregulated fishing, FAO Fisheries Report, p.722.
12) Focher, B., A. Naggi, G. Torro, A. Cosani and M. Terbojevich, 1992. Structural differences between chitin polymorphs and their precipitates from solutions – evidence from CP – MAS 13C – NMR, FT – IR and FT – Raman Spectroscopy. *Carbohydrate Polymer*, 17: 97-102.
13) Folch, J., M. Lees and G.H. Sloane-Stanle, 1957. A simple method for the isolation and purification of total lipids from animal tissue. *Journal of Biological Chemistry*, 226: 497 - 509.
14) Frid, C.L.J., and S.J. Hall, 1999. Inferring changes in North Sea benthos from fish stomach analysis. *Marine Ecological Progress Series*, 184: 183–188.
15) Green, J.H., and J.F. Matrick, 1979. Fishery waste management. *In*: Green, J.H., and A. Kremer (Eds.), *Food Processing waste management*, Westport, CJ, pp. 202-227.
16) Hall, M.A., D.L. Alverson and K.I. Metuzals, 2000. By-Catch: Problems and Solutions. *Marine Pollution Bulletin*, 41(1-6): 204-219.
17) Haywood, M.D.E., B.J. Hill, A.G. Donovan, W.A. Rochester, N. Ellis, A. Welna, S.R. Gordon, S.G. Cheers, K. Forcey, I.M. McLeod, Moeseneder, G.P. Smith, F.J. Manson, T.J. Wassenberg and S. Thomas, 2005. Quantifying the effects of trawling on seabed fauna in the Northern Prawn Fishery. Final Report on FRDC Project 2002/102, 1 921061 05 7CSIRO, Cleveland, Australia.
18) Jennings, S., S. Freeman, R. Parker, D.E. Duplisea and T.A. Dinmore, 2005. Ecosystem consequences of bottom fishing disturbance. *American Fisheries Society Symposium*, 41: 73–90.
19) Kanagaraj, R., 2007. Extraction of chitin and chitosan from marine trash crabs, *M.Sc. dissertation*, Annamalai University, Tamilnadu, India.
20) Kelleher, K., 2005. Discards in the world's marine fisheries. An update'. in *FAO Fisheries Techinical Paper* (No. 470), Rome, FAO, p.131.
21) Kumirska, J., M. Czerwicka, Z. Kaczyński, A. Bychowska, K. Brzozowski, J. Thöming and P. Stepnowski, 2010. Application of spectroscopic methods for structural analysis of chitin and chitosan. *Marine Drugs*, 8: 1567-1636.
22) Nair, K.G., and A. Dufresne, 2003. Crab shell chitin whisker reinforced natural rubber nanocomposites. 1. Processing and swelling behavior. *Biomacromolecules*, 4: 657-665.
23) Nair, K.G.R., and P. Madhavan, 1989. Advances in chitin research. *In:* K. Balachandran *et al.*, (Eds.): *Recent trends in processing low cost fisheries*, Society of Fisheries Technologies, India, p.174.
24) Odote, P.M.O., M.H. Struszczyk and M.G. Peter, 2005. Characterisation of chitosan from blowfly larvae characterisation of chitosan from blowfly larvae and some crustacean species from Kenyan marine waters prepared under different conditions. *Western Indian Ocean Journal of Marine Science*, 4(1): 99–107.
25) Palpandi, C., S. Vairamani and A. Shanmugam, 2009. Extraction of chitin and chitosan from shell and operculum of mangrove gastropod *Nerita* (*Dostia*) *crepidularia* Lamarck. *International Journal of Medicine and Medical Sciences*, 1(5): 198-205.
26) Pearson, F.G., R.H. Marchessault and C.Y. Liang, 1960. Infra red spectra of crystalline polysaccharides. V Chitin, *Journal Polymer Science*, 43(141): 101–116.
27) Raffi, S.M., 2006. Sustainable utilisation of bycatch resources. *In:* Kannaiyan, S., T. Balasubramanian, S. Ajmalkhan and K. Venkataraman (Eds.), *Biodiversity and Conservation of Marine Bioresources*., National Biodiversity Authority, pp.107-113.
28) Raymont, J.E.G., J. Austin and E. Lineford, 1964. Biochemical studies on zooplankton. 1. The biochemical composition of *Neomycis*

integer. *Journal of Cons. Int. Explor. Mer*., 28: 54-363.

29) Rodde, R.H., A. Einbu and K.M. Varum, 2008. A seasonal study of the chemical composition and chitin quality of shrimp shells obtained from northern shrimp (*Pandalus borealis*). *Carbohydrate Polymers,* 71(3): 388-393.

30) Skaugrud, O., and G. Sargent, 1990. Chitin and chitosan: Crustacean biopolymers with potential. *In: International* by products conference, Anchorage, Alaska, pp.61-72.

31) Subasinghe, S. 1999. Chitin from shellfish waste – health benefits over – shadowing industrial uses. *Info fish International,* 3(99): 58- 65.

32) Sugimoto, M., M. Morimoto, H. Sashiwa, H. Saimoto and Y. Shigemasa, 1998. Preparation and characterisation of water-soluble chitin and chitosan derivatives. *Carbohydr. Polym.*, 36: 49–59.

33) Takaguchi. Y., 1991. Physical properties of chitinous material. *In:* Chen, R.H., and H.C. Chen, (Eds.): *Proceedings from the Asia-Pacific Chitin,Chitosan- Jikken manual chapter1*. Advances in Chitin Science, Gihodou Shupan Kabushki Kaisha, Japan, pp.1-7.

34) Thirunavukarasu, N., 2005. Biology, nutritional evaluation and utilization of mud crab *Scylla tranquebarica* (Fabricius, 1798). *Ph.D Thesis*, Annamalai University, Tamilnadu.

35) Thirunavukkarasu, N., K. Dhinamala and M.R. Inbaraj, 2001. Production of chitin from two marine stomatopods *Oratosquilla* spp. (Crustacea). *Journal of Chemical and Pharmaceutical Research,* 3(1): 353-359.

36) Tseng, R.I., F.C. Wu and R.S. Juang, 1999. Pore structure and metal absorption ability of chitosan prepared from fishery wastes. *Journal of Environmental Science and Health. Part A: Toxic/hazardous Substances and Environmental Engineering,* 34(9): 1815-1828.

37) Vargese, K.J.J.P., 2002. Studies on biology of stomatopod *Harpiosquilla raphidea*. *Ph.D. thesis*, Annamalai University, Tamilnadu, India.

38) Yusof, N.L., L.Y. Lim and E. Khor, 2004. Flexible chitin films: structural studies. *Carbohydrate Research,* 339: 2701–2711.

39) Zakaria, Z., G.M. Hall and G. Shama, 1998. Lactic acid fermentation of scampi waste in a rotating horizontal bioreactor for chitin recovery. *Process Biochemistry*, 33: 1-6.