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Extraction and Characterization of Chitosan from *Portunus sanguinolentus* and *Penaeus monodon*.

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ABSTRACT:
The present work aimed to compare the synthesis, characterization, physico- chemical and functional properties of Chitosan extracted from *Portunus sanguinolentus* and *Penaeus monodon*. Chitosan were synthesized from the shells of crab and prawn through the chemical process like Deproteinization, Demineralization and Deacetylation. The functional group of the chitosan was determined from its FTIR and UV Spectrum. The Physico-Chemical properties like moisture content and ash content of chitosan were determined. The Funtional properties like Fat Binding Capactity, Water Binding Capacity and Solubility of the Chitosan were also determined. The present study reveals that the yield of chitosan of *Portunus sanguinolentus* and *Penaeus monodon* was found to be 7.1% and 8.6% respectively. The moisture content of the chitosan of *Penaeus monodon* was noted as 40% when compared with *Portunus sanguinolentus* as 60%. The ash content of chitosan extracted from *Penaeus monodon* and *Portunus sanguinolentus* shells ranged from 1% and 17% respectively. The solubility of *Penaeus monodon* chitosan was recorded as 2% when compared to *Portunus sanguinolentus* as 10%. Among the functional properties analysed, water binding capacity was significantly different from *Penaeus monodon* chitosan as 294% and *Portunus sanguinolentus* 520% respectively. The results obtained for fat binding capacityof *Penaeus monodon* and *Portunus sanguinolentus* chitosan samples were different ranged from 78% and 70% respectively.

Key words: *Portunus sanguinolentus* , *Penaeus monodon*, Chitosan, Functional properties, Characterization.

1. INTRODUCTION

Chitosan is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D- glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit) (Dutta *et al.,* 2002). The actual difference between chitin and chitosan is the acetyl content of the polymer. Chitosan having a free amino group is the most useful derivative of chitin. Chitosan is a non- toxic, biodegradable polymer of high molecular weight and is very much similar to cellulose, a plant fiber. The only difference between chitosan and cellulose is the amine (-NH2) group in the position C-2 of chitosan instead of the hydroxyl (-OH) group found in cellulose. (Hudson and Smith, 1998).

Extraction of chitin requires the removal of proteins and a tiny amount of pigments and lipids by deproteinization and inorganic calcium carbonate by demineralization. In some cases, an additional step of decolourization is applied to remove the excess residual pigments (Younes and Rinaudo, 2015). Chitin and chitosan can exhibit a variety of chemical, physical and biological properties. According to elementary studies and analyses of different crustaceans (crab shrimp, lobster, and squid), there was great variability of this composition when chitin amounts were varied from species to species. Hence, there is a need to develop efficient demineralization and deproteinization processes to remove mineral content (20–30%) and protein content of approximately 40% in order to obtain chitin that is free of inorganic and protein content (Usman *et al.*, 2016).

Chitin and its derivative chitosan are of commercial interest due to their excellent biocompatibility, biodegradability, non-toxicity, chelating and adsorption power. With these characteristics especially chitosan has many attractive applications in biotechnology, food and pharmaceutical industry, in cosmetics, environmental engineering, in agriculture and aquaculture (Franco and Peter 2011).

Chitin and chitosan have attained increasing commercial interest as suitable resource materials due to their excellent properties including biocompatibility, biodegradability and adsorption ability to form films and to chelate metal ions. Chitosan has a number of commercial and possible biomedical uses (Freier *et al.*, 2005). It can be used in agriculture as a seed treatment and bio pesticide and helping plants to fight off fungal infections. In winemaking it can be used as a fining agent, also helping to prevent spoilage. In industry, it can be used in a self- healing polyurethane paint coating.

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2. MATERIALS AND METHODS

Collection of exoskeleton of *Portunus sanguinolentus* and *Penaeus monodon*

The marine crab (*Portunus sanguinolentus*) and marine shrimp (*Penaeus monodon*) exoskeletons were collected from thelocal fish market in Palayamkottai, Tirunelveli district, Tamil Nadu. The shrimp and crab shells were brought to the laboratory and shells were separated and washed thoroughly using running tap water and rinsed with double distilled water. The collected shrimp and crab shells were scraped free of loose tissues adhered and then processed for removing the water-soluble and insoluble impurities. Then the shrimp and crab exoskeleton shells were dried at room temperature for a week. The skeleton was placed in Ziploc cover and frozen overnight and then subsequently cut into smaller pieces using a meat tenderizer. The collected marine crab and shrimp shells were ground to get coarse powder thoroughly and kept for further studies. (Sakthidasan 2017).

Extraction of chitosan from shells of *Portunus sanguinolentus* and *Penaeus monodon*

Chitosan extraction from crab and shrimp shell involves three major steps such as deproteinization, demineralization and deacetylation according to Takiguchi, 1991 method was employed and extract chitosan from shell wastes.

i) Deproteinization

The sample was deproteinized with 300ml of 1N NaOH at 80°C for 24 hours with constant stirring. The NaOH was exchanged intermittently and the sample was washed with distilled water every time before adding fresh NaOH. After 24 hours, the sample was filtered. The sample filtrate was washed as before and dried and the obtained weight was noted.

ii) Demineralization

Deproteinized sample powder (20gm) was demineralised with 300ml of 2N HCl or 24 hours with constant stirring and thus filtered. The filtrate was again washed with distilled water and filtered till the liquid showed neutral pH. The filtrate was then dried in a vacuum dryer and weighed.

iii) Deacetylation

Chitin was deacetylated with 40% NaOH, heated for 6 hrs at 110°C in constant stirring then 10% acetic acid was added to the sample and stored for 12hrs at room temperature with constant stirring. The dissolved sample was re precipitated by adding 40% NaOH at pH 10. The sample was then dialyzed by deionized water to a pH of 6.5 and centrifuged at 10,000 rpm for 10 minutes and freeze dried (Sangeetha *et al.,* 2021).

iv) Determination of Percentage of chitosan Yield

Percentage of yield for chitosan was calculated from the weight of chitosan produced as a percentage of starting dry raw material (Zaku *et al.,* 2011).

$$\text{Chitosan \%} = \frac{\text{Weight of residue (g)}}{\text{Weight of initial dry raw material (g)}} \times 100$$

Characterization of chitosan of *Portunus sanguinolentus* and *Penaeus monodon*

i) Fourier transform infrared spectroscopy and Ultra violet-Visible spectroscopy

The spectra of the chitosan samples were measured in the spectral range from 400 cm-1 to 4000 cm-1 using Nicolet Nexus 470 spectrometer. Fourier Transform-Infrared spectrometer in transmittance mode with a resolution of 4 cm-1. FT- IR is based on the fact that almost all of the molecules can absorb infrared light in the region of the electromagnetic spectrum, and that gives FTIR the ability, as a sensitive advanced analytical technique, to identify and characterize most organic substances through the identification of the functional groups, side chains, and cross links in the organic molecular groups and compounds in the spectral ranges of 4000–400 cm−1. UV analysis of chitosan

Ultraviolet (UV) spectrums were recorded on Shimadzu UV-170 spectrophotometer. One milligram of the sample was dissolved in 10 ml of water and the spectra were recorded at 200– 400nm range. The spectrum was obtained using potassium bromide pellet technique. Potassium bromide was dried under vacuum at 100°C for 48 h and 100mg of KBr with 1mg of the sample was taken to prepare a KBr pellet. The spectrum was plotted as intensity versus wave number (Ashokkumar and Ramaswamy, 2014).

Physico – chemical properties of chitosan of *Portunus sanguinolentus* and *Penaeus monodon*

i) Moisture content of chitosan

Moisture content of all chitosan samples was determined by using the gravimetric method reported by (Black 1965). The samples were dried to constant weight in oven at 105 °C and moisture content was calculated as follows:

$$\text{Moisture content (\%)} = \frac{W1 - W2}{W2} \times 100$$

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Where, W1 = weight (g) of sample before drying.

W2 = weight (g) of sample after drying.

ii) Ash content of chitosan

The ash content was determined using laboratory muffle furnace as per AOAC method. In brief, 1 g of each sample was taken in pre weighed crucible with lid and placed in muffle furnace and was maintained at 575 ± 10 °C for 6 h. After cooling, the crucibles were removed from the furnace and were placed in the desiccators. The above process of heating and cooling was repeated until constant weight was obtained. The ash with crucible and lid was weighed when sample turns to grey. (Shilratan walke *et al.,* 2014). The percent ash was calculated as follows:

$$\text{Ash (\%)} = \frac{\text{Weight of Ash (g)}}{\text{Weight of sample (g)}} \times 100$$

2.1. Functional properties of chitosan of *Portunus sanguinolentus* and *Penaeus monodon*

i) Water binding capacity (WBC)

The chitosan samples were measured using a modified method of (Wang and Kinsella, 1976). Water absorption was initially carried out by weighing a centrifuge tube containing 0.5 g of sample each. 10 ml of water was added by mixing on a vortex mixture for 1 min to disperse the sample. The content was left at ambient temperature for 30 min with intermittent shaking every 10 min and was centrifuged at 2000 rpm for 25 min. The supernatant was decanted and the tube was weighed again. WBC was calculated as follows:

$$\text{Water binding capacity (\%)} = \frac{\text{Water bound (g)}}{\text{Sample weight (g)}} \times 100$$

ii) Fat Binding capacity (FBC)

The Chitosan samples were measured using a modified method of (Wang and Kinsella, 1976). Fat absorption was initially carried out by weighing a centrifuge tube containing 0.5 g of sample. To this 10 ml of oil was added by mixing on a vortex mixture for 1 min to disperse the sample. The content was left at ambient temperature for 30 min with intermittent shaking every10 min and was centrifuged at 2000 rpm for 30 min. After that supernatant was decanted the tubes were weighed again. FBC was calculated as follows:

$$\text{Fat Binding Capacity (\%)} = \frac{\text{Fat bound (g)}}{\text{Sample weight (g)}} \times 100$$

iii) Solubility of chitosan

The chitosan samples was dissolved in 1% acetic acid for 30 minutes and cooled at 30ºc. Then the mixed particles were read at 10,000rpm for 10min. The undissolved particles were separated and centrifuged at 10,000rpm and dried at 60ºc / 1 day. The residues were measured and calculated (Knorr, 1982).

$$\text{Solubility (\%)} = \frac{(\text{Initial tube weight + chitosan}) - (\text{Final tube weight + Chitosan})}{(\text{Initial tube weight + Chitosan}) - (\text{Initial tube weight})} \times 100$$

3. RESULTS AND DISCUSSION

In our present study, the results were observed for yield percentage, characterization, physicochemical properties and functional properties of chitosan from *Portunus sanguinolentus* and *Penaeus monodon*

Yield of chitin and chitosan from *Portunus sanguinolentus* and *Penaeus monodon*

The marine shrimp, *Penaeus monodon* and marine crab, *Portunus sanguinolentus* are one of the potential species of chitosan production. The result of present study reveals that 350g of shrimp sample produced 39g of chitosan and 350g of crab sample produced 25g of chitossan. It has been observed that the percentage of chitosan yield from shrimp waste collected from *Penaeus monodon* was found to be 8.6% and chitosan yield from biowaste of crab, *Portunus sanguinolentus* was found to be 7.1%. It was analysed that the amount of chitosan yield is proportional to the amount of chitin obtained from the biowaste of shrimp and crab species. The results are presented in Table 1.

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The yield of chitin was found more in the shell of *Portunus sanguinolentus* (23.8 g) than that of chitin yield present in *Scylla serrata* representing (22.3 g). The yield of chitosan was also found to be more in the shell of *Portunus sanguinolentus* than the *Scylla serrata*. The content of chitin and chitosan in crab shell varies depending on species as well as the physicochemical parameters similar results were reported by (Das *et al.,* 1996) in *Scylla serrata* and *Portunus sanguinolentus* , the biowaste of 580mg M.rosenbergii goes to 32.44gms chitosan, P.monodon produced 455 gms biowasteand this biowaste produced 26.64gms of chitosan (Panchakshari *et al.,* 2016).

Chitosan yield from biowaste of *Penaeus carinatus* and *Penaeus monodon* was found to be 34% (Yateendra *et al.,* 2012), 30% (Sibi *et al.,* 2013), 17% (Mohanasrinivasan, 2014). Chitosan yield from biowaste of *Penaeus monodon*, chitosan yield from the biowaste of *Penaeus monodon* was found to be 67.47% and 46% (Anshar patria, 2013; Divya *et al.,* 2014).It is obvious that the amount of chitosan yield is proportional to the amount chitin obtained from the bio-waste of shellfish, the amount of chitin yield inter depends on the amount of biowaste obtained from shellfish. The yield of extracted chitosanof crab shell and fungi was found to be 38% and 39.3% respectively (Abirami *et al.,* 2021)

Table.1 Yield of bio-waste from *Portunus sanguinolentus* and *Penaeus monodon*

Name of the Species	Dry Shell powder Weight (g)	Yield of Deproteinization (g)	Yield of Demineralization (g)	Yield of Deacetylation (Chitosan)(g)	Yield Of Chitosan (%)
Portunus sanguinolentus	350	290	31	25	7.1
Penaeus monodon	350	250	45	39	8.6

Characterization of chitosan of *P. monodon* and *P. sanguinolentus*

a) Fourier transforms infrared spectroscopy:

FTIR is one of the most important and widely used analytical techniques available to characterize the structure of chitosan. FTIR spectra of chitosan of *Penaeus monodon* shown in Figure 1. The presence of similar groups were alkyl halides, P-H phosphine, alkanes, aromatics and phenols in the position of a peak at $475^{cm^{-1}}$ to $3800^{cm^{-1}}$. The FTIR-spectra showed the peaks of chitosan at $3802^{cm^{-1}}$ (O-H) stretch, $3671cm^{-1}$ (O-H) alcohol, $3136cm^{-1}$ (O- H), $2924cm^{-1}$ $(-CH_3-CH_2)$,$2852cm^{-1}(CH_2)$,$1642cm^{-1}$(amide- 1) c=o, $1400cm^{-1}$ (amide) III- CN, $1164cm^{-1}$ (C-O), $890cm^{-1}$ (C-H), $669cm^{-1}$ (C-O-C) and $473cm^{-1}$ respectively.

The absorption efficiency of extracted chitosan was determined by FT-IR assay. Figure 2 shows the FT-IR spectrum of extracted chitosan of *Portunus sanguinolentus* for identification of functional groups, side chains and cross links in the organic molecular groups and compounds in spectral ranges of $460cm^{-1}$ to $3125cm^{-1}$. The spectrum of chitosan shows the usual characteristic stretching vibration bands at $3125cm^{-1}$ (O-H) alcohol, $2921cm^{-1}$ (alkanes), $2851cm^{-1}$ (CH_2), $2365cm^{-1}$ (O-H), $1670cm^{-1}$ (amide-1), $1160cm^{-1}$ (C-O) $710cm^{-1}$ (C=C) and $469cm^{-1}$ respectively. The results are presented in Figure1 and Figure 2.

FT-IR is an important technique for structural analysis of biomolecules and is especially used in determining active functional groups present in the selected from two marine crab samples such as *P.sanguinolentus* and *S.serrata.* The FT- IR spectrum was used to determine the active functional groups of compounds based on the peak value in the area of infrared radiation. In the present study crab *P. sanguinolentus, S. serrata* shell powder was conceded into the FT-IR as well as the functional groups. Groups of the components were separated based on the ratio are recorded and identified (Pillai and Nair 2014; Liu *et al.,* 2006). The presence of amide, alcohol, phenolic, carboxylic acids aldehydes, ketones, alkanes, primary amines, aromatics, esters, ethers, alkyl amides and aliphatic amine groups. The FT-IR spectrum of present study functional groups of active compounds were identified with standard peaks at 921.6cm-1, 4060 cm-1 as possible groups (Sangeetha *et al.,* 2021).

FT-IR peaks values of *P. sanguinolentus* crab shell powder showed 921.6, 1492,1153,1128 showed the presence of alkyl groups; 1641 confirms the presence of alkene, 2999 showed the presence of S1-0 which indicate the aliphatic alkane (CH2) n 4060cm-1(H-H) stretch, strong banded groups. FTIR spectrum of crab chitosan recorded major peaks lying between 500.05 [cm-1] and 3880.60 (cm-1). (Abirami *et al.,* 2021).

ii) UV spectrum analysis of chitosan

The UV-visible spectra for chitosan of *P. sanguinolentus* were range from 200-400 nm. In this study absorbance peak was observed in 270 nm, this peak indicates the presence of chitosan. The UV-visible spectra for chitosan of P. monodon were range from 200-900 nm. In this study absorbance peak was observed in 280 nm, this peak indicates the presence of chitosan. The results are presented in Figure3 and Figure 4.

UV-vis spectrum can be used for optical characterization of chitin and chitosan. Similar results were observed in chitin and chitosan isolated from *P. sanguinolentus, S. serrata* (Solani *et al.,* 2015).

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Figure 1: FTIR spectra of chitosan of *Penaeus monodon*

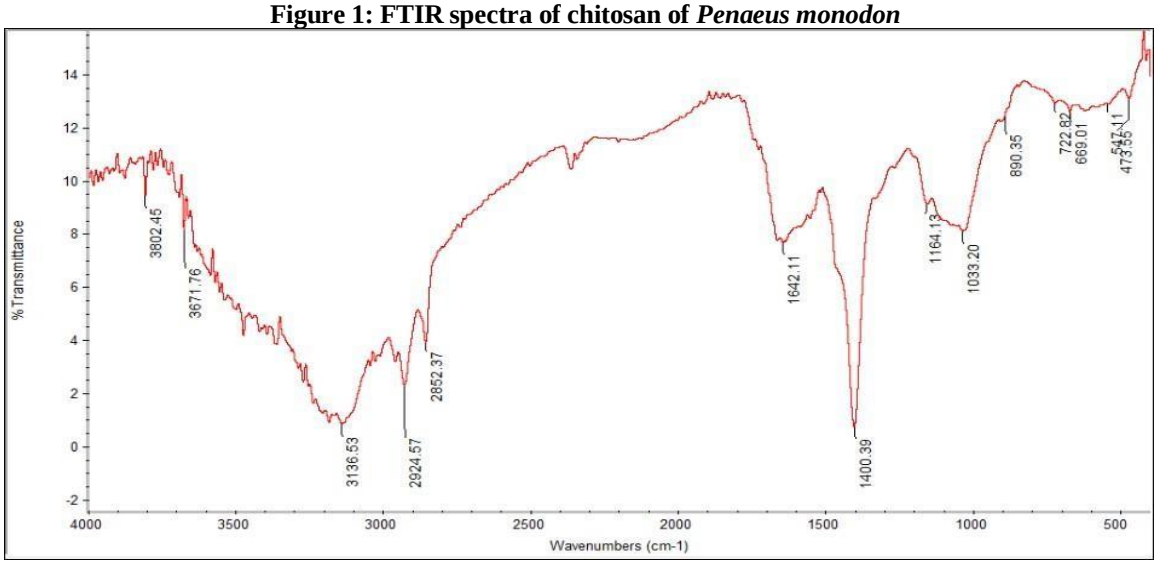

Figure 2: FTIR spectra of chitosan of *Portunus sanguinolentus*

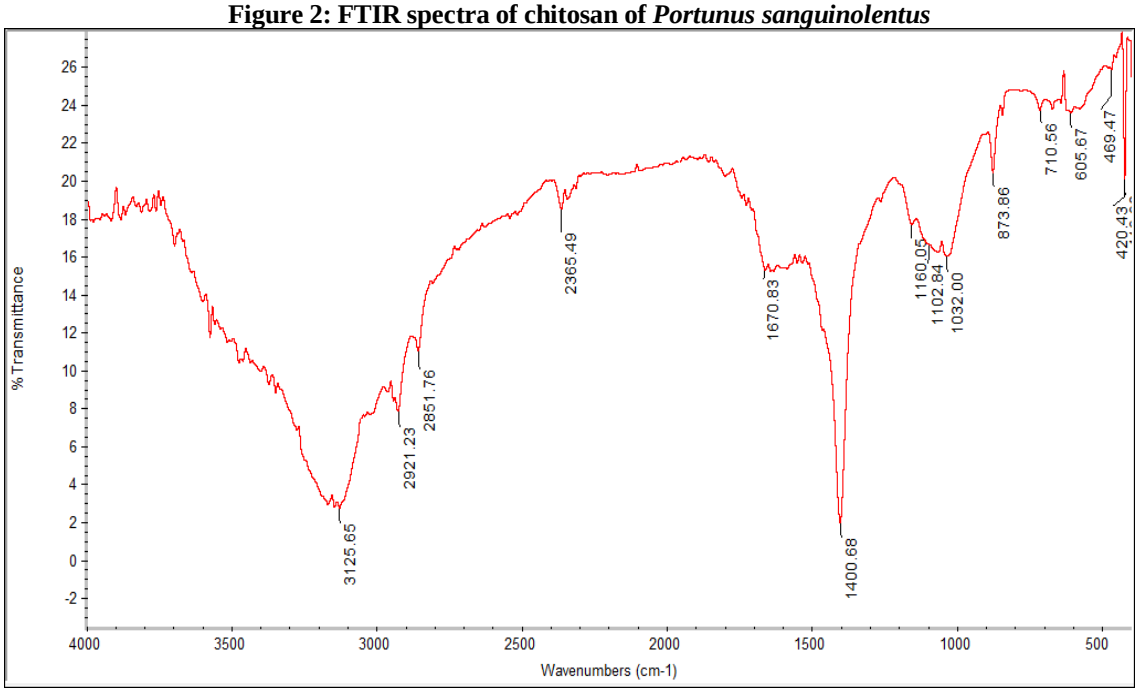

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Figure 3. UV pattern of chitosan of *Penaeus monodon*

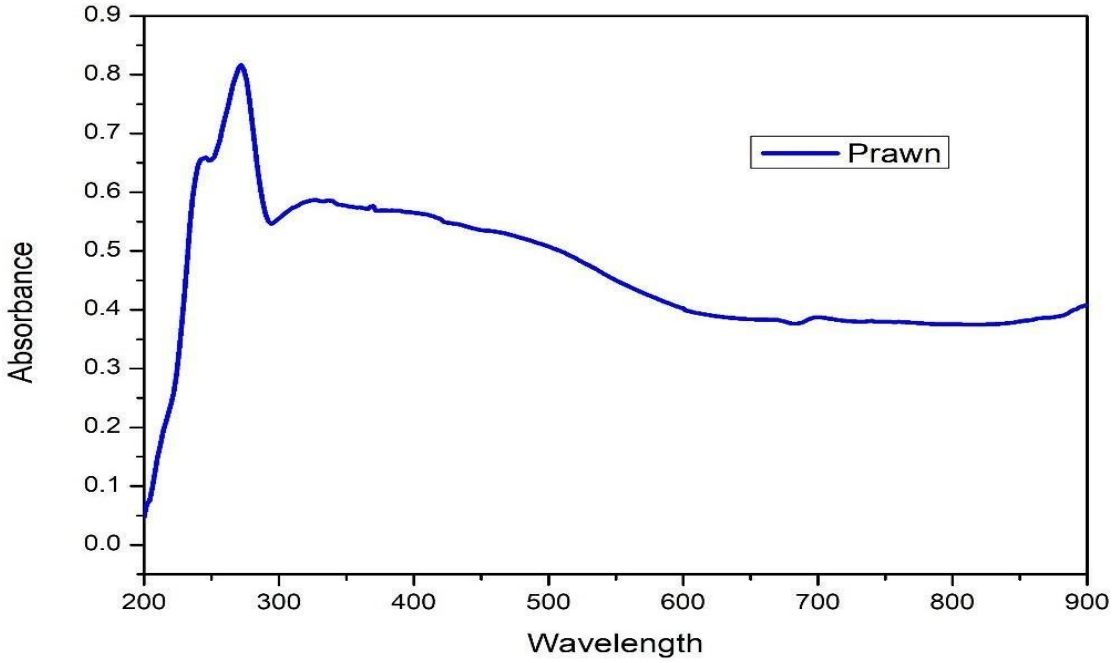
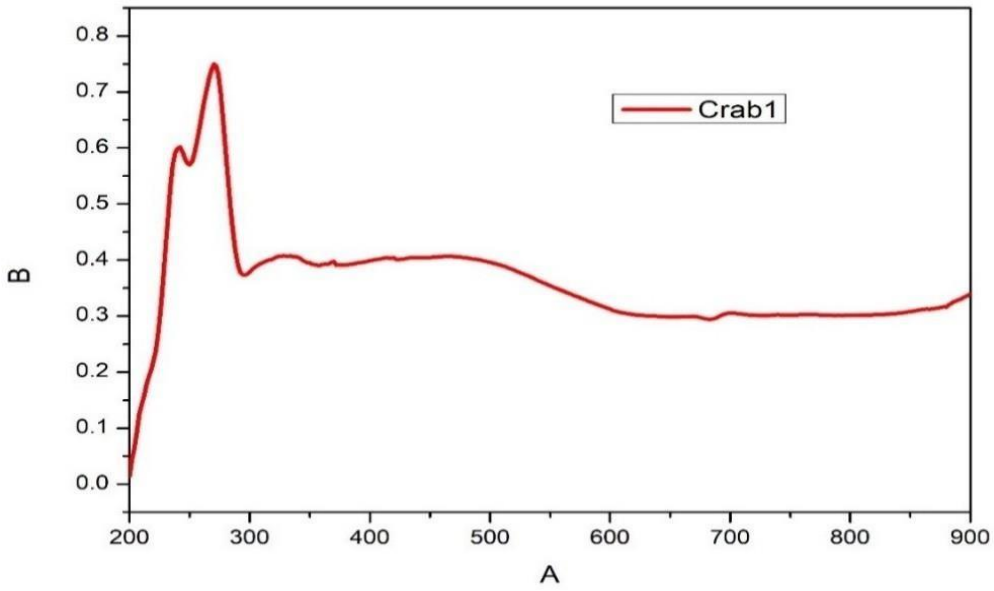


Figure 4: UV pattern of chitosan of *Portunus sanguinolentus*



Physico-chemical properties of chitosan of P. monodon and P. sanguinolentus

i) Moisture content and Ash content of chitosan: In the present study, the moisture content of the chitosan of *Penaeus monodon* was noted as 40% when compared with *Portunus sanguinolentus* as 60%. According to Li *et al.*, 1992, commercial chitosan products contain less than 10% moisture content. Chitosan is hygroscopic and affected by low moisture absorption during storage.

The moisture content of the chitosan extracted from crab and fungi was found to be 64.34% and 66% respectively (Abirami *et al.*, 2021).The moisture contents of all shrimp chitosan samples were not significantly different, ranging from 1.6% to 2.1% being higher for water soluble chitosan (C2). Chitosan is hygroscopic in nature; therefore, it is likely that chitosan samples were affected by small moisture absorption during storage (Fernandez-kim, 2004). Lower the moisture content of chitosan enhances the shelf stability and quality.

The ash content of chitosan is an important indicator of the performance and effectiveness of the demineralization process and affects the chitosan solubility and viscosity. The ash content percentage of chitosan extracted from *Penaeus monodon*

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and *Portunus sanguinolentus* shells ranged from 1% and 17% respectively. It revealed that the higher the reduction of ashcontent, the higher the purity degree of chitosan. The chitosan having 1% ash is a good grade of chitosan.
Ash content is an important parameter that affects chitosan solubility, viscosity and also other important characteristics (Mohanasrinivasan *et al.,* 2013] The ash contents were not significantly varying in commercially available chitosan C1 and C2 as well as laboratory prepared chitosan C3 and were obtained in the range of 3.35% to 4.35%, indicating the effectiveness of the demineralization step in removing minerals.
Ash is a highly-frequency parameter that affects characteristics such as viscosity and solubility. The final process of demineralized chitosan resulted having 31-36% ash. The chitosan having <1% ash is a good grade of chitosan (Divya *et al.,* 2014).

Functional properties of chitosan of *P. monodon and P. sanguinolentus*

i) **Solubility of chitosan :** Chitosan solubility is considered one of the important parameters for determining the quality of chitosan obtained from shrimp and crab shells, the higher solubility indicates higher purity and quality of chitosan. The solubility of *Penaeus monodon* chitosan was recorded as 2% when composed to *Portunus sanguinolentus* as 10%.The chitosan solubility increases proportionally with deacetylation degree. The prepared chitosan from shrimp shellswaste was found to be soluble in 1% acetic acid solution and partially soluble in water (Shilratan walke *et al.,* 2014). The solubility of raw shell, chitin, and chitosan was observed as 33 ± 2.44, 97.3 ± 0.28, and 97.6 ± 0.53 respectively. In a related work, the shells treated with 50 and 60% NaOH solution gave high solubility ranged from 96.01- 97.2%.The chitosan solubility is influenced by temperature, period of deacetylation, concentration of alkaline and yield of chitin, various methods applied to chitin separation and size. Chitosan solubility increases proportionally with deacetylation degree (Hossain and Iqbal, 2014).

ii) Water Binding capacity: Among the functional properties analysed, water binding capacity was significantly different from *Penaeus monodon* chitosan as 294% and *Portunus sanguinolentus* 520% respectively. Water Binding Capacity for acetic acid soluble chitosan; shrimp samples were significantly different and were respectively as 669% and 804%. These results were supported by similar observations made by (Rout 2001) for chitosan samples, wherein, WBC ranged from 581% to 1150% with an average of 702%. Also Cho *et al.,* 1998 reported that WBC ranging from 458% to 805% for five commercial chitosan, Water binding capacity (WBC) of the raw shell, chitin, and chitosan were 170 ± 8.16, 640 ± 16.32, and 580 ± 16.32% respectively. Likewise, the WBC of chitosan ranged from 581 to 1150%.The commercial, shrimp, and crab chitosan wherein the WBC ranged from 458 to 805% (No and Hur, 1998) from shrimp and crab shells.

iii) Fat Binding capacity: The results obtained for fat binding capacity of *Penaeus monodon* and *Portunus sanguinolentus* chitosan samples were different ranged from 78% and 70% respectively. The level of fat binding capacity depends on deproteinization and deacetylation. The results obtained for FBC of chitosan samples were significantly different ranged from 284% to 589%, being higher for laboratory prepared chitosan (C3). Fat binding capacity signifies how the chitosan can easily binding absorb fat. Average fat binding capacity reported by Rout 2001for crawfish chitosan and commercial crab chitosan using soybean oil were 706% and 587% respectively. No *et al.,* 2000 reported this as a physical property of chitosan to hold water or fat held and getting trapped in the structure and swells, thus can be used for drug delivery. Kim and Rajapakse, 2005 pointed that increased advantage of chitosan of fungi made them to receive great attention compared to chitosan extracted from crustacean shell. The FBC of raw shell, chitin, and extracted chitosan were 140 ± 16.32, 420 ± 16.32, and 420 ± 8.16% respectively. In a similar report, FBC in the range from 314 to 535%. The level of FBC depends on deproteinization and deacetylation (Hossain and Iqbal, 2014).

The physico chemical properties of moisture content, ash content, WBC, FBC, and solubility results are presented in Table 2

Table. 2: Proximate analysis of exoskeleton of *P. monodon and P. sanguinolentus*

Name of the Species	Ash Content (%)	Moisture content (%)	Water binding Capacity (WBC) (%)	Fat binding Capacity (FBC) (%)	Solubility (%)
Portunus sanguinolentus	17	60	520	70	10
Penaeus monodon	1	40	294	78	2

4. CONCLUSION

The present study revealed that the physicochemical properties and characteristics of *Portunus sanguinolentus* and *Penaeus monodon* were studied under uniform extraction condition. The yielding capacity of chitosan of both the species was compared. The highest ash content, moisture content, water binding capacity and solubility was observed from *P. sanguinolentus* chitosan. The highest fat binding capacity was observed in P. monodon chitosan. UV and FT IR characterization was done. The importance of chitin and chitosan lies in their biological and physicochemical properties, which have led to their widespread use in agriculture, medicine, pharmacy, food processing, environmental protection and biotechnology.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

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