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Determination of chitin content in shells of selected crab species available in Jaffna

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Abstract—In the post-war context, Jaffna crab industry is developed, exporting live, fresh de-shelled and processed crabs all around the world. Human consumes only 40% of the crab's weight as meat and the rest is thrown into the environment as waste shell. This unutilized waste crab shell contains large amount of chitin which can be extracted and utilized for different applications in cosmetics, agriculture, food, medicine and waste water treatment. The present study was carried out to find out the chitin content in selected, most abundant crabs such as *Portunus pelagicus, Thalamita crenata* and *Portunus sanguinolentus* available in Jaffna peninsula, during the period of July - October, 2017. Crabs were deshelled and the shells were dried, ground and screened through < 0.5 mm sieve. Fine particles of the crab shells samples were deproteinized by treating with 3.5% (w/v) NaOH solution and demineralized by treating with 1 N HCl. The extracted chitin was confirmed via the functional group using Fourier Transmission Infrared Spectroscopy. Higher yield percentage of extracted chitin was observed in the shells of *Portunus sanguinolentus* (23.38±1.11%*)* than *Portunus pelagicus* (15.39±0.73%) and *Thalamita crenata* (15.84±1.30%). The study revealed that the percentage of protein and calcium carbonate in *Portunus sanguinolentus* are 18.94± 1.74% and 62.29±1.92%, in *Portunus pelagicus* are 22.91±2.03% and 61.70±1.97% and in *Thalamita crenata* are 15.24±1.45% and 68.92±1.70%, respectively.

Keywords – Chitin, Crab shell, Demineralization, Deproteinization

I. INTRODUCTION

Every year, around 6 million to 8 million tonnes of waste crab, shrimp and lobster shells are produced globally, about 1.5 million tonnes in South Asia alone (Yan and Chen, 2015). Chitin is the major structural component of the exoskeleton of crab. In crabs, around 40% of the crab's mass is meat and the rest 60% is regarded as waste shell. The waste shells were incinerated, dumped in the coast or used as landfills. Industries, governments and public were still unaware about the uses of crab shells and environmental issues. The amount of chitin with respect to dry weight is the highest in crab shells compared to shrimp or lobster shells. Hence, crab shells are regarded as the main source of chitin for the chemical industry (Sakthivel *et al*., 2015). The main components of crab shells are chitin (15-40%), protein (20- 40%), calcium and magnesium carbonate (20- 50%) (Antonino *et al*., 2017).

Chitin is an insoluble natural biopolymer with a chemical structure of linear β-1, 4-linked polymer of N-acetyl glucosamine, first identified in 1884 (Rinaudo, 2006). Chitin occurs in three different structural forms and they are named as α-, β- and γ- chitin. Among these three structural forms, α- and β- chitin are the most abundant (Aung *et al*., 2018). α-chitin is found in crab shell and it has anti-parallel chitin strands that are tightly joined with both inter- and intra-molecular hydrogen bonding which makes it the strongest structure compared to other two structural chitin.

Chitin can be broadly utilized in water purification, as additives in cosmetics, antibacterial agents, pharmaceutical adjuvants, paper production, textile finishes, heavy metal chelating agents, membranes and biomedical applications such as wound dressings, separation membranes and anti-bacterial coatings, since they are harmless for the human body (Mincea *et al*., 2012). The present study was carried out to find out the amount of extracted chitin content in selected, most abundant crabs available in Jaffna peninsula.

II. MATERIALS AND METHODS

A. Sample Collection

The shells of three different species of crabs *Portunus pelagicus* and *Thalamita crenata* were collected from Kakkaitheevu and shells of *Portunus sanguinolentus* were collected from Point Pedro, from July to October, 2017. The study was intended to concentrate on above species as those are the most abundant species in Jaffna peninsula and the availability is more constant year around.

B. Sample Preparation

Initially, the collected crab shells' waste dried well in an oven at 50 °C until the constant weight was gained. The dried crab shells were ground well, screened through the <0.5 mm sieve and packed well in polythene bags. Then, it was subjected to deproteinization and demineralization and the extracted chitin was analysed by using IR spectroscopy.

C. Extraction of chitin

Powdered shell sample (20 g) was taken into the 250 mL beaker and it was deproteinized by treating with 200 mL of 3.5% (w/v) NaOH solution for 2 hours at 65 °C with constant stirring in orbital shaker. The residue was filtered through the Buchner funnel and washed with deionized water, until the filtrated solution become neutral pH. The neutralized residue was dried well in an oven at 40 °C until the constant weight was obtained (Gaikwad *et al*., 2015).

The 10.0 g of deproteinized dried crab shells powder was demineralized by treating with 150 mL of 1.0 N HCl solution for 15 minutes at room temperature with constant stirring (Younes and Rinaudo, 2015). The residue was filtered through the Buchner funnel and washed with distilled water until the filtrated solution pH become neutral. The neutralized residue was dried in an oven at 40 °C until the constant weight was acquired (Gaikwad *et al*., 2015; Rasti *et al.*, 2017). Finally, the dried sample of chitin was obtained.

D. FTIR Analysis

The vibration of functional groups spectra of chitin and chitosan prepared were analysed using Fourier Transform Infrared Spectrometry (FTIR) (FrontierTM Spectrometer, Perkin Elmer, USA) at the wavelength range of 4000 – 450 cm^{-1} at a resolution of 4 cm^{-1} (Pokhrel *et al*., 2016). Smart iTR was used to collect the horizontal attenuated total reactance (ATR) spectra using a diamond crystal. Samples were pressed with a minigrip device to ensure uniform contact between the samples and ATR crystal. The FTIR spectra were recorded by accumulation of at least 100 scans, using Spectrum 10™ software.

III. RESULTS AND DISCUSSION

Previous study reported that the chitin yield obtained from crab shells was between 10.6-12.73% (Pandharipande and Bhagat, 2016). Another study on extraction of chitin from *Sesarma plicatum* reported that the chitin content was 18.46% (Shakthivel *et al*., 2015). In the present study, the extracted chitin was observed in higher amount from the shells of *Portunus sanguinolentus* (23.38±1.11%) than *Portunus pelagicus* (15.39±0.73%) and *Thalamita crenata* (15.84±1.30%). This reveals that the percentage of chitin content in crab shell is variable, depending on species. Calcium carbonate and proteins are the other valuable constituents of the shell wastes and can serve as a better animal feed supplement (Suryawanshi *et al*., 2019). The percentage of protein in *Portunus sanguinolentus*, *Portunus pelagicus* and *Thalamita crenata* are 18.94±1.74%, 22.91±2.03% and 15.24±1.45% respectively.

Based on their calcium content, crab shells have considerable potential as the basic ingredients of bio-ceramic production. Recently, calcium bio-ceramic utilized from waste shell has gained attention (Haryati *et al*., 2019). The present study showed that the percentage of mineral content in the crab species of *Thalamita crenata* (68.92±1.70%) is comparably higher than *Portunus sanguinolentus* (62.29±1.92%) and *Portunus pelagicus* (61.70±1.97%), the amount of demineralization is related to the percentage of calcium carbonate as the shell composed of the mineral $CaCO_3$. This revealed that the shell of *Thalamita crenata* crab species is considerably harder than other species due to the high yield percentage of $CaCO_3$ present in their shell. For that reason, this crab species is not preferred by people to consume as food.

The functional group of the extracted chitin was confirmed by analysing with FTIR. Figure 1 shows the infrared spectrum of the chitin in the spectrum field 4000 – 450 cm^{-1} at the ATR mode in the form transmittance vs. wave number. The bands are generally large due to the macromolecular character of the compound and because of the numerous intermolecular

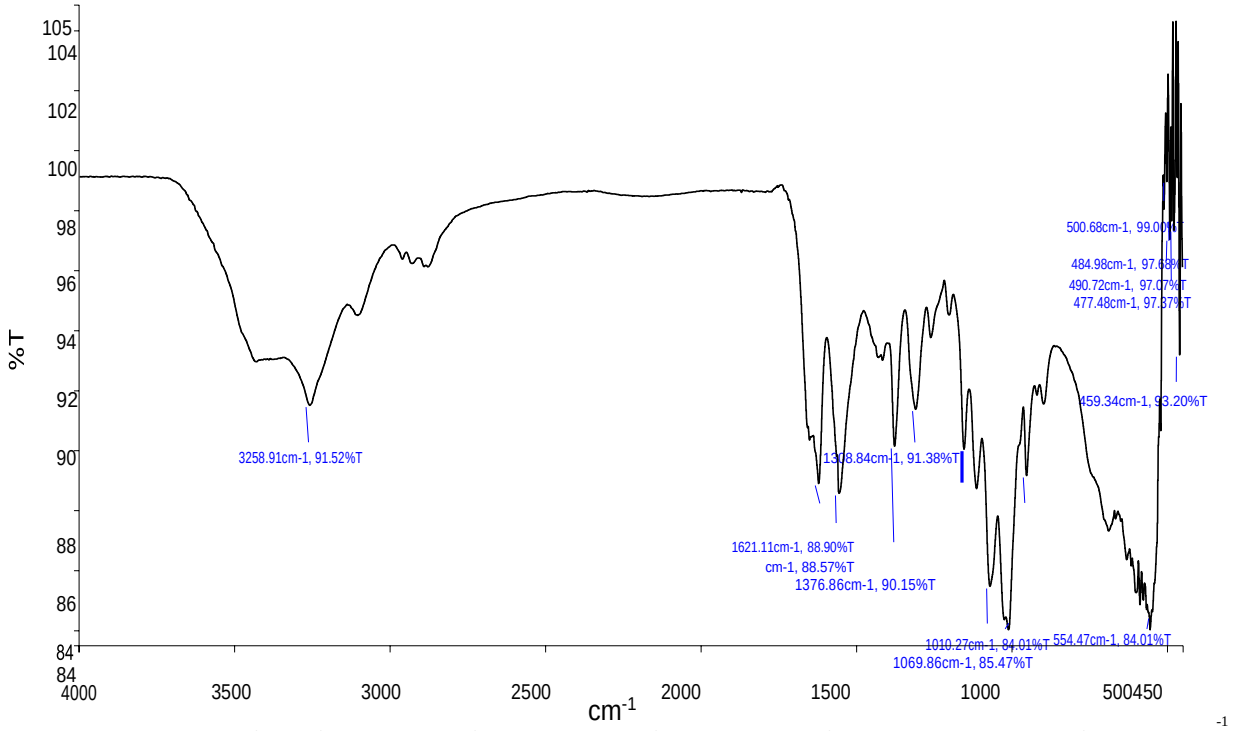

Figure 1: Fourier Transform Infrared Spectrum of the chitin extracted from the crab in the field with wavelength range of 4000 - 450 cm^{-1}

bindings of hydrogen, manifested even in the solid state of the sample (Barbat *et a*l., 2013; Samfira *et al*., 2013).

The highlighted peaks of the FTIR that include amide, carbonyl and hydroxyl groups for the extracted chitin from the crab shells have been identified. The band 3258.91 cm^{-1} is determined by υ(OH) overlapped on υ(N–H). The bands 1621.11 cm^{-1} and 1555.76 cm^{-1} are determined by υ(–C=O) of the proton amide group, and δ(NH3) is determined by the proton amide group. The band 1376.86 cm–1 is determined by δ(–CH3). The bands 1308.84 cm^{-1} and 1317 cm^{-1} are determined by υ(-CH3) third amide with (–CH2) + OH deformation in plane. The band 1153.43 cm^{-1} is determined by υ(C=O) oxygen bridges resulting from the deacetylation of the chitin. The bands 1069.86 cm^{-1} and 1010.27 cm^{-1} are determined by υ(C=O) by the bindings C–O–H, C–O–C and CH_2CO. The band 952.04 cm^{-1} is determined by υ(C–H) from the polysaccharide's structure (Negrea *et al.,* 2015).

The FTIR spectrum of the chitin isolated from the crab shells was compared with the standard chitin, and previous study by Pandharipande and Bhagat (2016). The major peaks of extracted chitin from this research studies were compared with the standard one and previous research study as shown in the Table 1.

Table 1: Comparison of FTIR spectral values for the major functional groups with the standard chitin (Jalal *et al*., 2012) and extracted chitin from Crab shells (Pandharipande and Bhagat, 2016).

Vibration modes	Standard chitin (cm^{-1}) (Jalal et al., 2012)	Extracted Chitin (cm^{-1}) (Pandharipande and Bhagat, 2016)	Extracted Chitin (cm^{-1})
CH_3 wagging alone chain	954.09	951	952.04
CO-stretching	1030.08	1008	1010.27
CO-stretching	1074.41	1067	1069.86
Asymmetric bridge O_2 stretching	1156.73	1112 - 1153	1153.43
Amide III band and CH_2 wagging	1315.04	1307 - 1374	1308.84
CH bending and symmetric CH_3 deformation	1387.67	- -	1376.86
Amide II band	1558.84	1552	1555.76
Amide I band	1653.83, 1634.83	- -	1621.11
OH stretching	3439.58	3430	3258.91

The comparison with earlier studies clearly indicates in the present research study chitin has been successfully extracted from waste crab shells from the three different species of crabs.

IV. CONCLUSION

Chitin is present in higher amount in the shells of *Portunus sanguinolentus* (23.38±1.11%) than *Portunus pelagicus* (15.39±0.73%) and *Thalamita crenata* (15.84±1.30%). This is a fundamental study to extract chitin from crab shell waste. The chitin extracted can be utilized for different applications in cosmetics, agriculture, food, medicine and waste water treatment.

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