EXTRACTION AND CHARACTERIZATION OF CHITOSAN FROM THE SHELLS OF MUD CLAM (*GELOINA COAXANS*), COCKLE (*Gafrarium tumidum*) AND FRESHWATER MUSSELS (*Lamellidans sp*.)

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INTRODUCTION

Chitosan, a natural polysaccharide is an example of value-added biomaterial. The precursor of chitosan is chitin, one of the three most abundant polysaccharides in nature, in addition to cellulose and starch. It ranks second to cellulose as the most plentiful organic compound on earth (Dutta, 2004). Enormous amounts of chitin can be found in the biosphere, and it is the major component of cuticles of insects, fungal cell walls, yeast or green algae (Einbu, 2007). Nearly 10% of the global landings of aquatic products consists of species rich in chitinous material. These include species such as shrimp, crab, squid, cuttlefish, oyster, clams, mussels, etc. (Subasinghe, 1999). When considering bivalves such as clams, cockels and mussels, their non-edible part of their bodies, the bivalve shells which are available in abundance, have no use and are commonly regarded as waste (Yao, 2014). As shrimp and crab shells, squid pens and cuttlebones that have been widely used after dissolution and deacetylation as a source of chitosan for the preparation of many different materials (Fernandez & Ingber, 2013), these discarding bivalve shells also can be used for extracting valuable biomaterials such as, chitin and chitosan.

The objective of the present study was to extract and characterize the chitosan isolated from the shells of Freshwater Mussels (*Lamellidans* sp.), Mud Clam (*Geloina coaxans*), and Cockle (*Gafrarium tumidum*) collected from the different places in Sri Lanka and emphasizing that these bivalve shells are not a waste but a source of value-added biopolymers such as crustacean exoskeleton and cephalopods internal skeletons (Sanuja et. al., 2017; 2018).

METHODOLOGY

The present study was carried out at the laboratory of the Department of Aquaculture and Aquatic Resources Management, University College of Anuradhapura, Sri Lanka. Freshwater mussel shells were collected from the Nuwara wewa reservoir of the Anuradhapura District. Clams, & cockle shells were collected from the Kinniya bay and Kalpitiya areas respectively. Collected shells were washed with tap water and dried under sunlight for two days until complete drying.

Pre-conditioning, De-mineralization, De-proteinization and De-acetylation steps were followed to isolate chitosan samples from the shells of Freshwater Mussels (*Lamellidans* sp.), Mud Clam (*Geloina coaxans*), and Cockle (*Gafrarium tumidum*)

After the isolation of chitosan samples percentage yield and physicochemical and functional properties as moisture, ash, nitrogen and Water Binding Capacity (WBC) and Degree of De-acetylation (DD%) were analyzed. The DD of chitosan was established using the frequency of 4000-400 cm^{-1}(Figure 1,2 & 3). The DD of the chitosan was calculated using the baseline reported by (Khan et. al., 2002) Infrared Spectroscopy instrument (FTIR Model-Brucker Alpha-T) with frequency. The computation equation for the baseline is given below:

$$DD\ (\%) = 100 - [(A1655 / A3450)\ X\ 100 / 1.33]$$

Where;

A1655 = absorbance at 1655 cm-1 of the amide-I band as a measure of the N-acetyl group content
A3450 = absorbance at 3450 cm-1 of the hydroxyl band as an internal standard to correct for disc thickness
The factor '1.33' denoted the value of the ratio of A1655 / A3450 for fully N-acetylated chitosan (Nessa et al., 2010)

RESULTS AND DISCUSSION

Table 1: Chitosan yield, % and physicochemical and functional properties of chitosan isolated from the shells of Freshwater Mussels (*Lamellidans* sp.), Mud Clam (*Geloina coaxans*), and Cockle (*Gafrarium tumidum*) (dry weight basis)

	Chitosan samples		
	A Mud Clam (*Geloina coaxans*)	**B** Cockle (*Gafrarium tumidum*)	**C** Freshwater Mussels (*Lamellidans* sp.)
Yield %	23.51	36.08	28.81
Physico-chemical and functional Properties			
Moisture %	1.59	1.55	3.40
Ash %	39.27	52.40	45.88
Nitrogen %	23.80	48.96	34.98
DD %	20.82	24.11	24.97
WBC%	219.85	213.17	257.49

The quality and physico-chemical properties of chitosan vary widely with species and methods of preparation and the present study indicates that the Cockle (*Gafrarium tumidum*) shell obtained the highest-quality chitosan considering all above properties. Most studies have reported that, high temperature and high volumes of chemicals are required for production of chitosan. During the present study chitosan was extracted from bivalve shells at ambient temperature and by using low volumes of chemicals. Therefore, this method could be recommended for development of a small-scale industry for chitosan production from discarding bivalve shell waste.

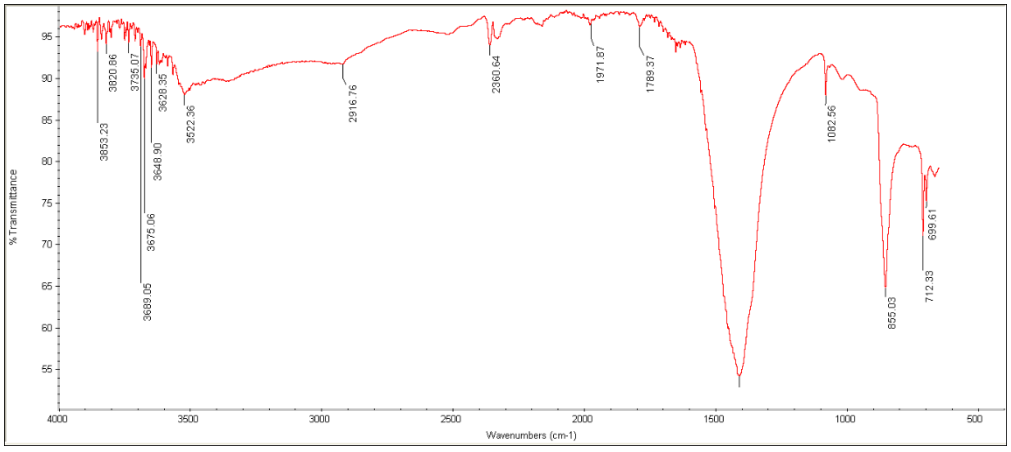

Figure 1 - The FT-IR spectra of chitosan samples from (A) Mud Clam (*Geloina coaxans*)

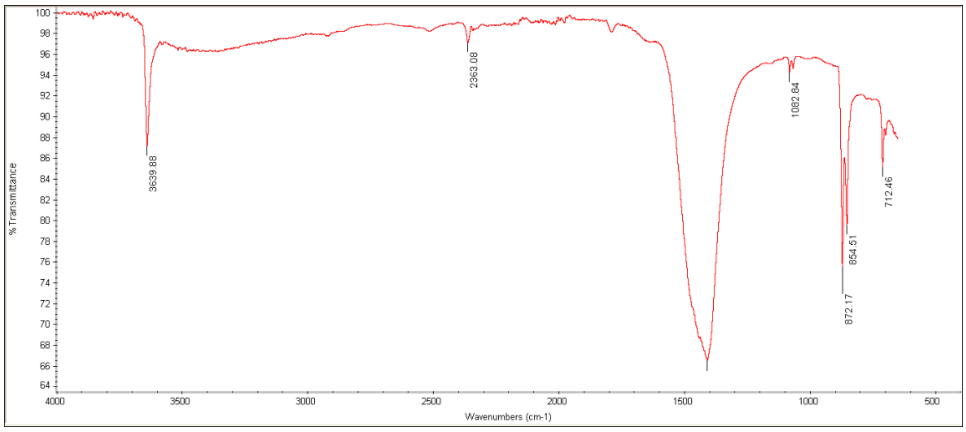

Figure 2 - The FT-IR spectra of chitosan samples from (B) Cockle (*Gafrarium tumidum*)

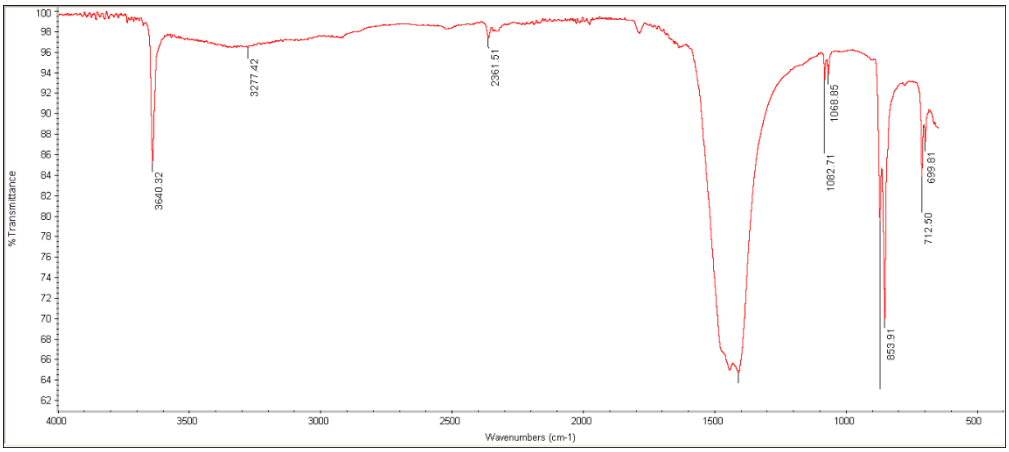

Figure 3 - The FT-IR spectra of chitosan samples from (C) Freshwater Mussels (*Lamellidans* sp.)

CONCLUSIONS/RECOMMENDATIONS

According to the present study the quality and the chemical properties of chitosan extracted from the cockle shells are the best when compared to those of chitosan extracted from clam and mussel shells.

It is better to analyse more physico-chemical and functional properties as Fat Binding Capacity (FBC) using three types of oil as coconut oil, sunflower oil and soybean oil which are available in the market and the solubility of the extracted chitosan, to compare the quality of extracted chitosan samples.

It is better to isolate chitosan using other bivalve shells of different species for more comparison.

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