

Extraction and Purification of Chitin and Chitosan from *Portunus pelagicus* Crab Shell Waste

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Crab (*Portunus pelagicus*) shell waste is highly accumulated in Sri Lankan seafood processing factories, but not many efforts have been taken on utilizing it for commercial level chitin and chitosan extraction with a higher percentage purity. Therefore the objective of this study was to modify and develop a scale-up, simple, and high-yielding chemical method for extraction and purification of chitin and chitosan from locally underutilized *Portunus pelagicus* crab shell waste. A modified process including pre-demineralization (acetic acid), demineralization (citric acid), deproteinization (NaOH), decolourization (*n*-butanol) deacetylation (NaOH), and purification (EDTA and SDS) was optimized to obtain a new combination of treatments. Pre-demineralization was introduced as a new step when developing the process. Citric acid was found as the best alternative organic acid to replace HCl in demineralization. The final product was characterized by X-Ray Diffraction (XRD) Spectroscopy and Fourier Transform Infrared (FTIR) Spectroscopy and various physicochemical and functional properties were analyzed. Control chitin and chitosan samples were produced using a conventional method for comparison. The yields of crude chitin, crude chitosan, and purified chitosan were 32.52±0.68%, 26.28±0.47%, and 21.78±0.34% respectively whereas in the control chitin and chitosan the yields were 20.34±0.72% and 13.79±0.93% respectively ($p < 0.05$). Percentage purity of the final product on a weight basis was 82.54±1.73% with a degree of deacetylation of 85.84±2.45%. The XRD data revealed that chitosan extracted from the developed methodology is a semi-crystalline compound with two characteristic crystalline peaks at $2\theta = 9.05^\circ$ and 19.1° and Crystallinity Index of 67.22%. FTIR analysis revealed that developed chitosan was comparable with control chitosan. Chitosan produced from the developed method showed higher results in physicochemical parameters namely moisture (5.27±0.39%), ash (1.95±0.22%) whiteness index (72.37±0.66%), and functional properties namely water binding capacity (318.74±0.48%), fat binding capacity (351.663±0.69%) DPPH free radical scavenging activity (61.12±0.59%) and ferrous chelating activity (40.19±0.47%) compared with the control ($p < 0.05$). Developed chitosan had no antimicrobial activity for *Salmonella* and *Micrococcus* but showed positive antimicrobial activity against locally isolated *Escherichia coli* at 2.5mg/ml. Thus the developed methodology can be used to obtain high purity and high-quality chitosan with better physicochemical and functional properties from crab shell waste

Keywords: *Portunus pelagicus*; Shell waste; Chitosan; Pre-demineralization; XRD; Percentage purity