Chitin extraction from shrimp shell waste using pineapple crude enzyme

M. Sukanya, B.V.T.T. Bolukandura, L.W.D. Bandara, K.H.I.K. Hewavitharana*

Department of Biosystems Technology, Faculty of Technological Studies, Uva Wellassa University of Sri Lanka, Passara Road, Badulla
* *indika.h@uwu.ac.lk*

1. Introduction

Chitin is the second most abundant biopolymer on the planet after cellulose, consisting of antifungal, antibacterial, antioxidant, anti-inflammatory and anti-hypersensitive properties. It is broadly used in different industries; including food, agriculture, wastewater treatment, cosmetics, and pharmaceuticals (Tolaimate et al., 2003). Chitin can be found in 15-40% of the exoskeleton of crustaceans including shrimp shell waste. Three steps followed in the chitin production are deproteinization, demineralization and decolorization. Deproteinization is the process used to separate proteins from shrimp shells. The pretreatment of deproteinization of chitin uses either partially purified or purified proteolytic enzymes. The cost of chemical methods and commercially available proteolytic enzymes in chitin pretreatment is most of the time unprofitable. As a result, an easily reached, cost-effective and plant-derived crude proteolytic enzyme was used in the pretreatment processing of chitin. Bromelain is an abundant protease enzyme found in pineapples and it breaks down cysteine peptide linkages in proteins. This study determines the efficiency of bromelain of the unpurified pineapple crude enzyme of the commercial cultivar on chitin extraction from shrimp shell waste.

2. Materials and Methods

Three selected pineapple cultivars were purchased and kept under the room temperature. The crude bromelain extraction was done using a method described in Mohan et al. (2016).

Deproteinization and chitin extraction

The clean and sun-dried shrimp cell waste was blended with distilled water in a ratio of 1: 4 (W/V). The deproteinization reaction was performed by mixing the extracted crude bromelain with the blended samples in a ratio of 1: 20 (v/v) at an optimum condition for the enzymatic activity (53 ℃, pH 7) for 24 hours. After the completion of the enzymatic reaction, samples were heated at 90 ℃ for 10 minutes to deactivate the enzyme. The insoluble fraction was separated from the supernatant by centrifugation at 4000 rpm for 45 minutes at 28 ℃.

The precipitate was used to perform the decolorization process by using acetone in a ratio of 1:4 (W/V) for 48 hours in the absence of light, at room temperature. Filtered samples were then demineralized by using 8% HCl solution in a ratio of 1:30 (W/V) for 30 minutes. After demineralization, samples were washed with distilled water until they became neutral. The extracted chitin was dried at 60 ℃ for 18 hours and stored in a desiccator.

The extracted bromelain enzyme and chitin biopolymer were characterized by powder X-ray diffraction (PXRD) and Fourier transform infrared (FT-IR). PXRD and FT-IR data were collected on a Bruker Phaser 11 diffractometer (30kv, 10 mA, Cu K-alpha radiation) and Bruker TENSOR 27 FT-IR spectrophotometer respectively.

3. Results and Discussion

According to the extracted chitin PXRD results (Figure 1), 5 major crystalline reflections were observed at ~9, 19, 20, 23 and 28 and the results are agreed with the α-chitin crystalline reflections observed for chitin extracted from organisms such as crabs, shrimp, and insects in literature (Liu et al., 2012, & Jang et al., 2004).

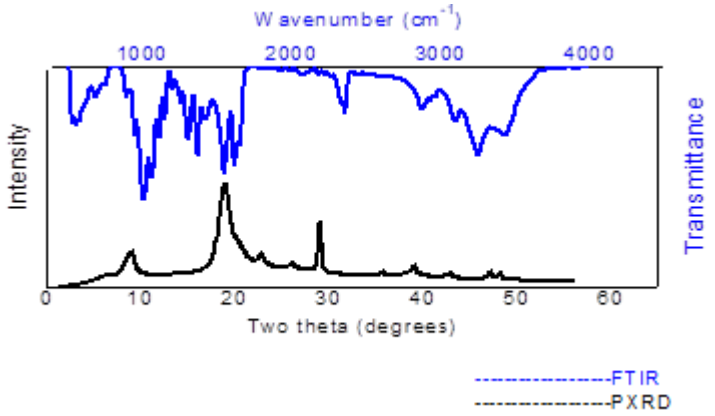

Figure 1. PXRD diffractograms and FTIR spectrum of extracted chitin

FTIR spectra (Figure 1) observed for chitin extracted in the present study has shown doublet at amid 1 band (~ 1620 cm^{-1}) and revealing that the extracted chitin is in the α form as reported in literature (Sajomsang, & Gonil, 2010). Peaks near 1650, 1620, and 1550 cm^{-1} of the chitin polymer correspond to the following functional groups; C=O secondary amide stretching (Amide I), N-H bending, and C-N stretching (Amide II), respectively (Jang et al., 2014). Other peaks observed at 3400 cm^{-1}, 3100-3200 cm^{-1}, 2900 cm^{-1}, 1500 cm^{-1} , 1400 cm^{-1} , 1300 cm^{-1} 1150 cm^{-1}, 1110 cm^{-1}, 1070 cm^{-1}, 1000 cm^{-1}, 950 cm^{-1} and 900 cm^{-1} are the result of O–H stretching, N–H stretching, aliphatic compounds, amide II, CH_2 bending, CH_3 deformation, CH bend and CH_3 sym. deformation, CH_2 wagging, asymmetric bridge oxygen stretching, asymmetric in phase ring stretching mode, saccharide rings, C–O asymmetric stretch in phase ring, along chain and saccharide rings respectively for chitin.

The price of commercially available Sigma Aldrich chitin per kilogram ranges between € 800-807 per kilogram (http://www.sigmaaldrich.com/european-export.html; accessed13th December 2021), whereas the cost of chitin prepared by our enzymatic based method ranges between € 440 - 450 per kilogram and will be an economically important potential method.

4. Conclusions

The present study has shown an effective chitin production method from shrimp shell waste using an unpurified pineapple crude enzyme called bromelain. The FTIR and PXRD analysis has confirmed the extracted chitin in the α form as reported in literature. Hence, it is possible to assess that the chitin extraction method using pineapple crude enzyme is a cost effective and economically important potential method in comparison to other chemical methods available.

5. References

Jang, M. K., Kong, B. G., Jeong, Y. I., Lee, C. H., & Nah, J. W. (2004). Physicochemical characterization of α-chitin, β-chitin, and γ-chitin separated from natural resources. *Journal of Polymer Science Part A: Polymer Chemistry, 42*(14), 3423-3432.

Liu, S., Sun, J., Yu, L., Zhang, C., Bi, J., Zhu, F., & Yang, Q. (2012). Extraction and characterization of chitin from the beetle Holotrichia parallela Motschulsky. *Molecules, 17*(4), 4604-4611.

Mohan, R., Sivakumar, V., Rangasamy, T., & Muralidharan, C. (2016). Optimisation of bromelain enzyme extraction from pineapple (Ananas comosus) and application in process industry. *American Journal of Biochemistry and Biotechnology, 12*(3), 188-195.

Sajomsang, W., & Gonil, P. (2010). Preparation and characterization of α-chitin from cicada sloughs. *Materials Science and Engineering: C, 30*(3), 357-363.

Tolaimate, A., Desbrieres, J., Rhazi, M., & Alagui, A. (2003). Contribution to the preparation of chitins and chitosans with controlled physico-chemical properties. *Polymer, 44*(26), 7939-7952.