Current Research Journal of Biological Sciences 2(1): 72-75, 2010
ISSN: 2041-0778

Submitted Date: October 05, 2009 Accepted Date: October 19, 2009 Published Date: January 05, 2010

Extraction of Chitin from Trash Crabs (*Podophthalmus vigil*) by an Eccentric Method

Sunita Das and E. Anand Ganesh
Centre of Advanced Study in Marine Biology, Annamalai University,
Parangipettai, Tamil Nadu, India

Abstract: The present study was undertaken to extract chitin from trash crab (*Podophthalmus vigil*) in Cuddalore landing center. Demineralization is an important step in chitin purification process from crabs. The chemical method of demineralization includes the use of strong acid (HCl) that harms the physiochemical properties of chitin. In the present study, *Lactobacillus plantarum* produced organic acid was used to substitute the Hydrochloric acid and deproteinization was done by fungus *Aspergillus niger*. The study showed that the effectiveness of using lactic acid bacteria for demineralization of crab's shells was comparable to that of using Hydrochloric acid. Using organic acids for demineralization is a promising concept, since organic acids are less harmful to the environment, which can preserve the characteristics of the purified chitin.

Key words: *Aspergillus niger*, chitin, demineralization, *Lactobacillus plantarum* and *Podophthalmus vigil*

INTRODUCTION

Chitin hold great economic value due to their versatile biological activities and chemical applications, mainly in medical (Murugan and Ramakrishna, 2004; Yadav and Bhise, 2004) and pharmaceutical areas (Takeuchi *et al.,* 2001; Kato *et al.,* 2003). Chitin is a naturally abundant polymer consists of 2-acetamido 2-deoxy-β-D- glucose through a β (1,4) linkage. In spite of the presence of nitrogen, it may be regarded as cellulose with hydroxyl at position C-2 replaced by an acetamide group. Like cellulose, it functions as structural polysaccharides. It is estimated that nearly 10^{11} tons of chitin is produced annually in the biosphere, much of it in the oceans (Poulicek and Jeuniaux, 1991). Chitin occurs in a range of organisms but is particularly important as constituent of arthropods (Muzzarelli, 1977). The crustacean shells are the most important chitin source for commercial use due to its high content and ready availability (Gagné *et al.*, 1993; Subasinghe, 1999). The crustaceans which are considered as trash or not edible can be used as sources of chitin, which adds more values to the bycatch and benefits the fisherman in great economical point of view. Smaller sizes of *Podophthalmus vigil* are used in the present study to extract chitin from their shells in an unusual way.

Hydrochloric acid is the most commonly used chemical in the demineralization of crustacean waste. The use of this strong acid: (a) harms the physiochemical properties of chitin, (b) results in a harmful effluent wastewater and (c) increases the cost of chitin purification process. Percot *et al.* (2003) reported that using HCl for the demineralization of chitin results in detrimental effects on the molecular weight and the degree of acetylation that negatively affects the intrinsic properties of the purified chitin. The importance of the optimization of the extraction process parameters (pH, time, temperature and solids to acid ratio) in order to minimize chitin degradation and bring the impurity levels down to the satisfactory level for specific applications are quite essential. Therefore, a less harmful and cheaper demineralization process is needed for the chitin extraction. *Lactobacillus* sp. has the potential to produce lactic acid and other organic acids. Using organic acids such as lactic and/or acetic acids for the demineralization process is a promising idea since organic acids in order to produce low cost biomass, purified chitin and reduce the harmful to the environment (Jung *et al.*, 2005; Rao *et al.*, 2000). The deproteinization of the shells was done by the use of *Aspergillus niger*, which has the capability of producing proteolytic enzymes (Paranthaman, 2009). The objective of the present study is to evaluate the effectiveness of lactic acid to demineralize the crab shell.

MATERIALS AND METHODS

The study was conducted during January, 2009 in CAS in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu. *Podophthalmus vigil* were procured from heaps of trash fish landed at Mudasalodai fish landing centre, Parangipettai (lat. 11º29′N; long. 79º46′E). The specimens were washed in seawater, drained off and subsequently in a hot air oven for 5 min at 60ºC. The specimens were then shade dried, crushed and kept in airtight containers. The 20 g of crushed powdered was weighed and chitin was extracted from the shells

Corresponding Author: Sunita Das, Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu, India

Table 1: Physio-chemical parameters for extraction process

Parameters	Conventional method (Takaguchi, 1991a)			Unconventional method (lactic acid)		
Conc. of acid	2N HCl	2N HCl	2N HCl	69.5 g/L	69.5 g/L	69.5 g/L
Temperature	RT	RT	RT	40°C	40°C	40°C
Shells to acid ratio	1:15	1:25	1:35	1:15	1:25	1:35
Conc. of deproteinizer	1N NaOH	1N NaOH	1N NaOH	5 g wet weight	5 g wet weight	5 g wet weight
Product appearance	White	Super White	Super White	slightly brownish	White	white
Yield (grams)	5.62	5.47	5.41	4.52	4.17	4.03
Yield (%)	28.1	27.35	27.05	22.6	20.85	20.15
Solubility in water	Insoluble	Insoluble	Insoluble	Partially soluble	Partially soluble	Partially soluble
pH	7.2	7.2	7.2	6.9	6.9	6.9

Conc – Concentration, RT – Room Temperature.

following the method of Takaguchi (1991a) in three different shells to acid ratio (1:15, 1:25 and 1:35 respectively). The extracted chitin was kept for comparison with the unconventional undertaken method. *Lactobacillus plantarum* was fermented and lactic acid was separated from fermentation liquor by electro dialysis method described by Hirata *et al.* (2005). The obtained lactic acid concentration of 69.5 g/L at pH 6.9 was used for demineralization. The crushed shells were placed in the beaker with lactic acid in shells to acid ratio of 1:15, 1:25 and 1:35 respectively. The beakers were kept for 24 hours with constant stirring at 40°C. Then the acid is removed and the sample is washed with distilled water and filtered till the wash liquid showed neutral pH. The filtrate was then dried in oven at 60º C. The proteolytic activity of the fungus *Aspergillus niger* was determined with the azocasein test (Benitez *et al.*, 2001). Deproteinization was carried out according to Mahmoud, 2005 and modified. Briefly, deproteinizing fungus was grown in 250 ml of Martin Rosebengal media for 3 days. The cells were pelleted by centrifuging three times at 5,000 rpm for 5 mins. The pellets were washed with sterilized sea water and 5 g of pellet was mixed in 250 ml sterilized sea water containing 1% (w/w) NaCl and 2.5% (w/w) KH_2PO_4. Fifteen grams of demineralized samples was suspended in 250 ml of the deproteinizing culture. The flasks were kept on a shaker at 110 rpm for 72 h at 37 ºC for deproteinization. The sample was filtered and washed with distilled water; the filtrate was washed till it showed neutral pH. The filtrate was then dried in oven at 40ºC and the resultant product is chitin which is weighed and collected for analysis.

RESULTS

The results of chitin extracted from the trash crab, *Podophthalmus vigil* (small sized crabs) are presented in Table 1. The yield of chitin using Takaguchi (1991a) method was 5.62g, 5.47g and 5.41g for shells to acid ratio of 1:15, 1:25 and 1:35 exhibiting a yield % of 28.1, 27.35 and 27.05 respectively. Whereas the yield of chitin using the modified unconventional method was 4.52, 4.17 and 4.03 g for shells to acid ratio of 1:15, 1:25 and 1:35 exhibiting a yield % of 22.6, 20.85 and 20.15 respectively. Fig. 1 shows a clear comparison of yield percentage between the chemical and lactic acid method. The results illustrate a significant increase in minerals removal when the shell to acid ratio was increased from 1:15 to 1:25 in both methods. A further increase in the shells to acid ratio to 1:35 caused only a minor decrease in the weight. The isolated deproteinating culture of *Aspergillus niger* shows a maximum proteolytic activity in the azocasein test of 67 U ml/1. The yield of crude chitin by this modified method ranged from 4.52-4.03g with a yield percentage of 22.6-20.15%.

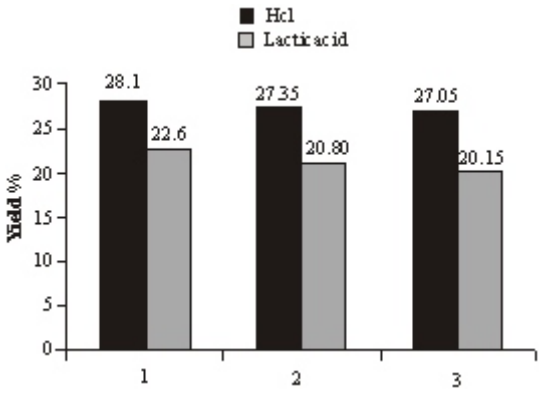

Fig 1: Comparison on yield % between HCl and Lactic acid

DISCUSSION

Crustacean shells contain calcium carbonate and organic macromolecules such as chitin and protein, and in minor amounts, lipids and pigments such as astaxanthin. For an industrial purification process of chitin from crustacean shells, the composition of their main compounds should not vary during the seasons, which was reported to occur with *P. borealis* (Rødde *et al.*, 2008). Marine trash fish are considered as potential resource rather than waste, the production of valuable substances and compounds from bycatch resources gained momentum in recent past (Raffi, 2006). Chitin, valuable bio-polymer which is extracted from the shells of crustaceans exhibits tremendous industrial and pharmaceutical applications (Muzzarelli, 1977; Poulicek and Jeunjaux 1991; Kramer *et al.*, 1995).

During chemical preparation of chitin, high temperatures during strong acid and alkali treatment are used to remove minerals and proteins. However, the use of these chemicals causes some depolymerisation of the chitin and therefore affects its molecular weight and viscosity after solubilisation (Waldeck *et al*., 2006). Biotechnologically produced chitin is at present not commercially available, but offers new perspectives for production of high viscosity chitosan and therefore application in biomedicine and pharmacy. An attempt has been made for extraction of chitin from trash crabs for utilizing the bycatch and to put together a new eccentric method to replace the chemical method, parenthetically comparison was also made to prove the yield efficiency.

The results display an effective yield % in comparison with standard Takaguchi method. The yield percentage ranged from 28.1-27.05% for Takaguchi method and for the lactic acid modified method it ranged from 22.6-20.15%. The production of organic acids by *L. plantarum* decreased the pH and made the environment optimal for demineralization (Hong *et al*., 1999). The effect of acid to shells ratio (1:15, 1:25 and 1:35) investigated using Takaguchi (1991a) method resulted 5.62, 5.47 and 5.41g respectively (after deproteinization). The effect of acid to shells ratio (1:15, 1:25 and 1:35) investigated using lactic acid resulted 4.52, 4.17 and 4.03 respectively (after deproteinization). The yield percentage compared with HCl, 1:25 of shells to acid ratio of lactic acid showed the best efficacy in demineralization since its appearance and demineralization rate was at the finest of all. The crude chitin obtained by chemical method was all insoluble whereas the crude obtained from the lactic acid were partially soluble. The study showed that the effectiveness of lactic acid for the demineralization of crustacean shells was virtually comparable to that of hydrochloric acid. For effective removal of minerals from crab shells using lactic acid, shells to acid ratio of 1:25 and temperature of 40ºC were found satisfactory. *Podophthalmus vigil* exhibited a maximum yield of 22.6% chitin at 1:15 shells to acid ratio using lactic acid as a demineralizing agent whereas HCl used Takaguchi method yielded a maximum quantity of 28% at the same ratio; demineralization was 19% lower in the modified method, where the chemical method was superior. The comparison between the two inorganic and organic is not at wide range, since lactic acid used was at lower pH may be responsible for demineralization rate. According to Mahmoud *et al.* (2007), the solubility, appearance, and rate of sudden decrease in weight at two different shells to acid ratio deflect on the purity of the extracted chitin. In this study, these three criterions are matching the result of 20.85%, at a shell to acid ratio of 1:25 where the crude is pure white and demineralization rate decreases rapidly reflecting on the weight. The *Aspergillus niger* exhibited an activity of 67 U/ml. The proteolytic activity exhibited by the fungi shows the presence of protein degrading enzyme (Mahmoud, 2005).

To determine the physio-chemical properties of chitin, it must be solubilized. Unfortunately, chitin is insoluble in diluted aqueous or common organic solvents due to its strong intra- and inter-hydrogen bonds. The weak solubility of chitin is the reason for the restricted use in biomedicine and biotechnology. The use of chemicals causes some depolymerisation of the chitin and therefore affects its molecular weight and viscosity after solubilisation (Waldeck *et al*., 2006). Since the chitin extracted is partially soluble; this may pierce way through into the Biomedicine and pharmacy industry. These results suggest that lactic acid could provide an alternative to chemical treatment, although a second run or milder acid treatment (such as 0.5 M HCl) may be necessary. It is concluded that the combined lactic acid and *Aspergillus niger* remains cost effective and eco-friendly way for the production of chitin, which has to be examined for viscosity parameters in the future.

REFERENCES

Benitez, J.A., A.J. Silva and R.A. Finkelstein, 2001. Environmental signals controlling production of hemagglutinin/protease in Vibrio cholerae. Infect. Immunol., 69(10): 6549-6553.

Gagné, N. and B.K. Simpson, 1993. Use of proteolytic enzymes to facilitate the recovery of chitin from shrimp wastes. Food Biotechnol., 7: 253-263.

Waldeck,v J., G. Daum, B. Bisping, and F. Meinhardt, 2006. Isolation and molecular characterization of chitinase-deficient *Bacillus licheniformis* strains capable of deproteinization of shrimp shell waste to obtain highly viscous chitin. Appl. Environ. Microbiol., 72(12): 7879-7885.

Jung, W.J., J.H. Kuk, K.Y. Kim and R.D. Park, 2005. Demineralization of red crab shell waste by lactic acid fermentation. Appl. Microbiol. Biotechnol., 67: 851-854.

Kato, Y., H. Onishi, and Y. Machida, 2003. Application of chitin and chitosan derivatives in the pharmaceutical field. Curr. Pharm. Biotechnol., 4(5): 303-309.

Kramer .K.J., T.L. Hopkins and J. Schaefs, 1995. Application of solid NMR analysis of insect sclerotized structures. Insect. Biochem. Mol. Biol., 25: 1067-1080.

Hirata, M., M.T. Gaoa, E. Toorisaka, H. Takanashi and T. Hanoa, 2005. Production of lactic acid by continuous electrodialysis fermentation with a glucose concentration controller. Biochem. Eng. J., 25: 159-163.

Mahmoud, N.S., 2005. Novel biotechnological approach for the production of chitin and de-icing agents. Unpublished Ph.D. Thesis. Halifax, NS: Department of Biological Engineering, Dalhousie University.

Mahmoud, N.S., A.E. Ghaly and F. Arab, 2007. Unconventional approach for demineralization of deproteinized crustacean shells for chitin production. Am. J. Biochem. Biotechnol., 3(1): 1-9.

Muzzarelli, R.A.A., 1977. Chitin. Pergamon. Press, Oxford, pp: 305.

Murugan, R. and S. Ramakrishna, 2004. Bioresorbable composite bone paste using polysaccharide based nanohydroxyapatite. Biomaterials, 25(17): 3829-3835.

Paranthaman, R., K. Alagusundaram and J. Indhumathi, 2009. Production of Protease from Rice Mill Wastes by *Aspergillus niger* in Solid State Fermentation. World J. Agric. Sci., 5(3): 308-312.

Percot, A., C. Viton and A. Domard, 2003. Optimization of chitin extraction from shrimp shells. Biomacromolecules, 4:12-18.

Poulicek, M. and C. Jeunjaux, 1991. Chitin biodegradation in marine environment and experimental approach. Biochem. Syst., 107(19): 385-399.

Raffi, S.M., 2006. Sustainable Utilisation of Bycatch Resources. In: Biodiversity and Conservation of Marine Bioresources. S. Kannaiyan T. Balasubramanian, S. Ajmalkhan and K. Venkataraman (Eds.). National Biodiversity Authority. pp: 107-113.

Rao, M.S., J. Munoz and W.F. Stevens, 2000. Critical factors in chitin production by fermentation of shrimp biowase. Appl. Microbiol. Biotechnol., 54: 808-813.

Rødde, R.H., A. Einbu and K.M. Varum, 2008. A seasonal study of the chemical composition and chitin quality of shrimp shells obtained from northern shrimp (*Pandalus borealis*). Carbohydr. Polym., 71(3): 388-393.

Subasinghe S., 1999. Chitin from shell waste- health benefits over-shadowing industrial areas. Info. Fish Int., 3(99): 58-65.

Takaguchi, Y., 1991a. Physical Properties of Chitinous Material Chitin. In: Advances in Chitin Science, Proceedings from the Asia-Pacific. R.H. Chen and H.C. Chen (Eds), Chitosan-Jikken Manual, Vol. III. Ch. 1, Gihodou Shupan Kabushki Kaisha, Japan, pp: 1-7.

Takaguchi. Y., 1991b. Preparation of Chitosan and Partially Deacetylated Chitin. In: Chitosan Jikkan Manual. A. Otakara and M. Yabuki (Eds.), Ch. 2, Gihodou Shapan. Kabu Shki Kaisha, Japan, pp: 9-17.

Takeuchi, H., H. Yamamoto and Y. Kawashima, 2001. Mucoadhesive nanoparticulate systems for peptide drug delivery. Adv. Drug Deliv. Rev., 47(1): 39-54.

Waldeck, V.J., G. Daum, B. Bisping and F. Mein hardt, 2006. Isolation and molecular characterization of chitinase-deficient *Bacillus licheniformis* strains capable of deproteinization of shrimp shell waste to obtain highly viscous chitin. Appl. Environ. Microbiol., 72(12): 7879-7885.

Yadav, A.V. and B.B. Bhise, 2004. Chitosan a potential biomaterial effective against typhoid. Curr. Sci., 187(9): 1176-1178.