Vidyodaya J. of Sci. (2009) Vol. 14, pp 31-39

Use of dried papaya milk in chitosan manufacture

P.A.J.Yapa and A.Silva and W.T.P.S.K.Senarath

Department of Botany, University of Sri Jayawardanepure, Nugegoda, Sri Lanka

Received on : 22-10-2007
Accepted on : 15-12-2008

Abstract

A new method for the production of chitosan from shrimp waste using dried papaya milk (DPM) has been developed. It involves the treatment of demineralized (with 4% HCl) shrimp waste with DPM followed by deproteinization with 3% NaOH and deacetylation with 50% NaOH. The use of DPM brings about considerable reductions in the amount of HCl (20%) and NaOH (40%), which are known to cause environmental pollution problems. Typically, the degree of deacetylation (DD) of resulting chitosan was (67%) comparable to DD of conventional methods. Moisture content (9.12%) and ash content (0.09%) of resulting chitosan were significantly low compared to chitosan obtained by 100% chemical methods.

Introduction

Chitosan is a polysaccharide of very high economic importance. Its potential uses have been reviewed by Li *et al* (1992) and Stevens (1996) in two excellent reviews. This biopolymer is obtained by deacetylating chitin which is the major constituent of the exoskeleton of crustaceans such as shrimps and crabs etc. Basically, the present method of preparation of chitosan involves 3 steps of chemical treatment namely 1) demineralization with HCl, 2) deproteinization with NaOH and 3) deacetylation with 50% NaOH. These chemicals when discharged into environment are known to cause environmental pollution problems. Attempts have been made to reduce the pollutant load through the use of various enzymes such as papain and bacterial proteases (Hall & Ahmed, 1992; Hall, 1996) as well as fungal chitin deacetylase in the deacetylation process (Win *et al*, 2000). However, none of the investigations seem to have gained commercial importance. In Sri Lanka, 4000 – 5000 mt of shrimp waste are produced annually. During the process around1/3 to ¼ of waste get generated.

The objective of the present investigation was to explore the possibility of using dried papaya milk (DPM) for partial or complete replacement of hazardous chemicals in chitosan manufacture and to obtain a product comparable or superior to those obtained by 100% chemical methods.

Materials and Methods

Fresh shrimp waste (heads and shells) was obtained from a shrimp processing plant in Negombo. They were kept frozen until use.

Preparation of dried papaya milk:

Latex was obtained by making incisions on the surface of mature green fruits of papaya by a knife or razor blade. The latex collected was spread on a glass plate and oven dried at 45°C. Dried papaya latex was removed from the plate, powdered and kept in a desiccator. Solutions of papaya milk were prepared by dissolving appropriate amounts in distilled water.

Production of chitosan by standard chemical method

The frozen shrimp waste was thawed and washed with tap water to remove unnecessary materials.

Demineralization: About 250 g of washed shrimp was treated with700 ml of 5% HCl solution in a glass beaker, which was kept in an oven at 50 °C for 24 h. The demineralized shrimp was then filtered and washed out the acid with tap water until wash-water was neutral.

Deproteinization: The demineralized shrimp waste was treated with 500 ml of 5% NaOH in a glass beaker and kept in an oven at 55° C for 24 h. The deproteinized sample was filtered and washed out the alkali with tap water until wash-water was neutral.

Deacetylation: Demineralized and deproteinized shrimp waste was hydrolyzed with 500 ml of 50% NaOH in an oven at 60° C for 24 h. The resulting product, chitosan, was thoroughly washed with tap water until wash-water was neutral. Chitosan was then sun dried for 4 h.

Preparation of chitosan using dried papaya milk.

The demineralization of shrimp waste was carried out as described above with 4% HCl. This was followed by treatment with a solution of dried papaya milk (DPM) at 37°C for 24 h as described in Sri Lanka Patent 13544 (2005). DPM treatment was followed by treatment with 3% NaOH for 24 h at 60°C for deproteinization and finally treatment with 50% NaOH for deacetylation as described above. This procedure is summarized in Fig 1.

Determination of protein content: Protein content was determined by the biuret method (Anon, 1997). Protein was extracted from dried chitosan by soaking 3 g of sample in 30 ml of 4% NaOH in a boiling tube. Mixture was incubated in a water bath for 6 h at 95 ^{0}C to extract the maximum amount of protein. The hydrolysate, after filtration was volumed up to 100 ml with distilled water. Approximately, 10 ml of hydrolysate was further filtered and 4 ml of Biuret reagent was added to 1 ml of filtrate shaking well and left for 30 min. The absorption was read at 570 nm in a spectrophotometer (Model GBC UV/ Vis 911 A).

Determination of percentage of degree of deacetylation (% DD): The % DD was determined by the titration method described by Hayes and Davis (1978).

Properties of chitosan produced by DPM treatment: Ash content and moisture content of chitosan samples were determined by the method of Anon (1997). One gram of the sample was used for each determination.

Results

Effect of DPM treatment on protein content

The effect of different concentrations of DPM on protein content of shrimp waste is given in Table 1. The concentration range investigated was 0.1% - 0.8%. The protein content decreased with increasing concentrations of DPM. The highest concentration of DPM (0.8%) gave the highest reduction in protein content (Table 1).

Effect of DPM treatment on ash content:

The effect of different concentrations of DPM on ash content of shrimp waste is given in Table 1. The concentration range investigated was 0.1% - 0.8%. Ash content was found to slightly increase with increasing concentrations of DPM.

Effect of DPM treatment followed by NaOH treatment

Taking into account the lowest ash and protein contents obtained with different concentrations of DPM, 0.4% was considered best for the shrimp waste treatment. The DPM treated shrimp waste was treated with different concentrations of NaOH for maximum removal proteins. The result of this 'combined treatment' of DPM+ NaOH is given in Table 2. Protein content decreased with increase in concentration of NaOH and 3% concentration was selected for the final process.

Table 1: Effect of DPM treatment on ash and protein contents of shrimp waste

Concentration of DPM %	Ash content %	Protein content %
0	0.64	3.03
0.1	0.69	2.81
0.2	0.61	1.71
0.4	0.66	1.27
0.8	0.67	1.27

n=3

Properties of chitosan produced by DPM treatment:

Moisture content of the chitosan prepared by DPM-treatment was 11.2% (Table 3), which is slightly lower than the moisture content of chitosan prepared by conventional 100% chemical method.

Ash content of chitosan prepared by DPM- treatment of shrimp waste was 0.69% which is slightly lower than the ash content of chitosan prepared by conventional chemical treatment Table 3). This low ash content is reflected in the low moisture content too. Ash content increased with increasing concentrations of DPM. Ash content was found to show a slight fluctuation with the addition of DPM.

The protein content of chitosan prepared by DPM treatment of shrimp waste was 0.49% (Table 3), which is lower than the protein content (0.52%) of chitosan prepared by conventional chemical treatment.

Degree of deacetylation (DD) of chitosan prepared by DPM treatment of shrimp waste was 66.45% compared to 66.77% in chitosan prepared by conventional chemical method (Table 3).

Table 2:Protein content of shrimp waste treated with DPM and NaOH

Treatment	Protein content %
0.4% DPM and 0% NaOH	1.25
0.4% DPM and 1% NaOH	1.13
0.4% DPM and 2% NaOH	0.96
0.4% DPM and 3% NaOH	0.59
0.4% DPM and 4% NaOH	0.51
0.4% DPM and 5% NaOH	0.46

n = 3

Table 3: Properties of chitosan produced by conventional chemical method and DPM treatment of shrimp waste

Property	Chemical method	DPM treatment
Moisture %	12.56	11.2
Ash %	0.72	0.69
Protein %	0.52	0.49
DD %	66.77	66.45

n=3

Table 4: Physicochemical properties of chitosan produced by different methods

Method	Moisture %	Ash %	Protein %	DD%
Chemical	12.56	0.722	0.524	66.77
Combined treatment of 0.4% DPM + 3% NaOH	11.21	0.693	0.486	66.45
Values (ranges) reported in literature	8.5-12.5% Hein et al, 2000	<1% Hein et al, 2000	<1% Hein et al, 2000	50-95% Dee et al, 2001

DPM – dried papaya milk; DD – degree of deacetylation

Shrimp waste

↓

4% HCl at 65 ^{0} C, overnight

↓

wash thoroughly / 1 h soaking in water

↓

treatment with a solution of DPM at 37^0 C, overnight

↓

wash thoroughly & treat with 3% NaOH at 60 ^{0} C, overnight

↓

wash & treat with 50% NaOH at 65 ^{0} C, overnight

↓

wash thoroughly with water until wash water is neutral

↓

chitosan

↓

sun drying

↓

dried CHITOSAN

Figure 1: Schematic procedure for production of chitosan using DPM

Discussion

Chitosan is widely known as a multifunctional marine polymer, which offers a wide range of applications in various areas. At present, production of chitosan is a thermo chemical process. It is highly energy consuming. It produces aggressive wastes, such as hot acids and alkali, which are used to remove minerals, proteins and acetyl groups. They are discharged to environment during the process, usually without prior treatment there by causing various environmental pollution problems.

The objective of the present study was to explore the possibility of developing an more environmentally friendly method for production of chitosan using enzymes such as papain (driedpapaya milk). A range of proteases from plant, animal and marine sources including papain have already been investigated by several workers (Gagne, 1993). But none of them seem to have ended up in commercial application. One of the important differences between the present study and the previous studies is that in the present study we used dried papaya milk where as previous workers have used commercially available purified papain. Dried papaya milk has been successfully used in deproteinized natural rubber (DPNR) manufacture (Yapa & Balasingham, 1974; Yapa, 1976).

In this study, combined treatment of 0.4% DPM followed by 3% NaOH was used to remove proteins from shrimp waste replacing 5% NaOH which is usually used in the conventional chemical method of production of chitosan. Chitosan produced by this way using DPM has comparable physicochemical properties to chitosan produced by conventional chemical method (Table 3). Also, these properties closely resemble those of previous workers (Hein *et al*, 2000 and Dee et al, 2001).

In the selection of the suitable concentration of dried papaya milk to remove proteins, maximum removal of proteins occurred at 0.8% concentration of DPM. In fact it was also the highest concentration of DPM used. However, the use of such high concentrations of DPM is not obviously cost effective. Therefore, 0.4% concentration of DPM was selected, based on the protein content of chitosan in preliminary trials, as a suitable concentration to remove proteins and used for subsequent combined treatment. Ultimately, the use of 0.4% concentration of DPM proved to be correct as evidenced by the physicochemical properties of the final product (Table 3).

Incorporation of a new step of treatment with DPM into the method of manufacture of chitosan resulted in 20% and 40% reductions in the volume of HCl and NaOH used, respectively. The reduction in the use of chemicals would in turn reduce the volume of chemical pollutants released in to the environment making it an environment friendly process. DPM can be easily prepared locally. Low ash content of chitosan produced by DPM method is also expected to be advantageous in some of the industrial applications of chitosan.

Acknowledgements

Authors wish to thank Mr Joe Pinto of Chilaw for providing us with samples of shrimp waste, used in this study. We are also thankful to Mr. Kyaw Nyenin Aye of Bioprocess Technology Division of AIT, Thailand for providing details of the method for determination of degree of deacetylation.

References

Anon (1997) Bioprocess Laboratory Manual, Asian Institute of Technology, Thailand

Bhuwapathapun, S. (1996) Protease enzymes in chitin and chitosan production from shrimp waste products: Proc. of 2nd Asia Pacific Chitin symposium, Bangkok, Nov., 1996, 41 – 49

Hayes, E.R. and Davis, D.M. (1978) Determination of the degree of deacetylation by titration method: Proc. of 1st International Conference on Chitin & Chitosan, Bangi, Malaysia

Dee, G.J., Rhode, O. and Watcher, R. (2001) Chitosan: multifunctional marine polymer, cosmetics and toiletries: **116** (2), 38-43

Gagne, N. (1993) Optimization studies on chitin / chitosan from crustacean waste MSc Thesis, McGill University

Hall, G.M. (1996) Biotechnology approaches to chitin recovery: Proc. of 2nd Asia Pacific Chitin symposium, Bangkok, Nov., 1996, 26 – 33

Hall, G.M. & Ahmed, N.H. (1992) Functional properties of fish protein hydrolysates, In Fish Processing Technology, Ed. GM Hall, Blackie Academic and Professional, Glasgow, 249-274

Hein, S., How, N.G., Chandrkrachang, S. and Stevens, W.F. (2000) A systematic approach to quality assessment system of chitosan: Paper presented at Yamaguchi Conference on Chitosan , 2000 – Preprint

Li, Q., Dunn,T., Grandmaison, E.W. and M.F.A. Goosen (1992) Applications and properties of chitosan : J. of Bioactive Polymers,, October,1992

Silva, A., Yapa, P.A.J. and Senerath, W.T.P.S.K. (2005) Use of dried papaya milk in chitosan manufacture, Sri Lanka Patent No 13544

Stevens, W.F. (1996) Chitosan: A key compound in biology and bioprocess technology, Proc. of 2nd Asia Pacific Chitin symposium, Bangkok, Nov., 1996, 13-21

Win, N.N., Pengju, G. and Stevens, W.F. (2000) Deacetylation of chitin by fungal enzymes: Advan. Chitin Sci. **4**, 55-61

Yapa, P.A.J. & Balasingham, C.G. (1974) Proteolytic action of papain on *Hevea* proteins: R.R.I.C. Quart.J. **56**, 1-12

Yapa, P.A.J. (1976) Preparation and properties of DPNR and viscosity stabilized DPNR: Proc. Internat. Rubber Conf., Colombo, 1976, 508 – 520