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Application of proteolytic enzyme, papain for the production of chitin and chitosan from shrimp waste

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Abstract

Chitin and chitosan were prepared from shrimp shell waste (*Penaeus indicus*) by enzymatic deproteinisation in combination with chemical treatment and by conventional chemical method and their qualities compared. Proteolytic enzyme, papain was tested for its efficiency in deproteinisation process of shell waste. Papain deproteinised the shell waste (73.1%) in 72 hr digestion process, whereas, chemical method showed 98% deproteinisation. However, the degree of acetylation was higher (19.4%) in the chitin prepared by enzymatic method than that by chemical method (17.2%). Similarly, qualities of chitosan from enzymatic method were superior with respect to deacetylation and viscosity than that of chemical method.

Chitin, poly - βm (1,4) linked N-acetyl D-glucosamine is a highly hydrophobic material that is insoluble in water and most organic solvents. Deacetylation of chitin with strong alkali yields the polymerised 2 amino 2 deoxy D glucosamine which is commonly known as chitosan. Chitosan is insoluble in water but soluble in dilute acids forming the corresponding salts (Pangburn *et al.*, 1984). Chitin and chitosan have immense applications in various fields such as food industry, agriculture, water treatment, biomedicine, biotechnology, textile and paper industry, cosmetics etc. (Knorr, 1984, 1991; Brezeski, 1987; Michihiro *et al.*, 1998; Shahidi, 1994).

The conventional chemical method of preparation of chitin involves decalcification of shell waste by immersing in a diluted hydrochloric acid, deproteinisation by heating in aquous alkali and removal of lipid by extracting with hot ethanol. These processes may cause some modification such as depolymerisation and deacetylation of native chitin resulting in lower molecular weight of the final product with variable and inconsistent physicochemical properties (Brine and Austin, 1981). The desire for producing chitin with more consistent physicochemical properties necessitates the use of milder treatment for removing some of the components associated with shell, such as protein. Some investigators have attempted to deproteinise crustacean waste intended for chitin and chitosan production using different proteolytic ensymes and experimental conditions (Takeda and Abe, 1962; Takeda and Katsuura, 1964; Gagne and Simpson, 1993; Healy *et al.*, 1995; Shirai *et al.*, 1997). The present study was

undertaken to prepare chitin using cheaply available proteolytic enzyme, papain for the deproteinisation process of shrimp shell waste and to compare the quality with that of chemical method.

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Material and methods

Shell waste of white shrimp, *Penaeus indicus* was procured from the fish processing plant, Diamond Seafood located at Tuticorin and brought to the laboratory for processing into chitin and chitosan. One part of the raw material was processed for chitin and chitosan production by chemical method, as per Madhavan and Nair (1974, 1975), with some modifications. Dried shell waste, after thorough washing with tap water, was heated to boiling with 3% aqueous sodium hydroxide at the ration of 2:3 (solid : liquid) in a glass container (3 litre) for 15 minutes. The alkali was drained off and the residual protein was removed by further heating the material to boiling in an equal weight of 3% sodium hydroxide solution. Alkali was then drained off and the shell was washed well and demineralised by treatment with 1.25 N HCl at the ratio of 1:10 (Shell:liquid) for 1hr. The acid was drained off and the chitin thus obtained was bleached with 10% commercial hypochlorite for five minutes (Hall and Reid, 1995). The chitin prepared as above was deacetylated by treatment with 1:1 (w/w) aqueous sodium hydroxide at the rate of 1 : 10 (Solid:liquid) for 2 hours at 90-95°C for conversion to chitosan. After deacetylation, the alkali was drained off and the residue was washed well with tap water, dried at 50°C in hot air oven and pulverised.

Another part of the shrimp shell waste was subjected to enzymatic digestion in combination with chemical treatment to obtain chitin. As per the method described by Gangne and Simpson (1993), the demineralisation of the shrimp shell waste was done using 1.75 N acetic acid at the ratio of waste to solvent 1:15 (w/v) at room temperature for 12hr. The demineralised material was washed with distilled water and dried at 50°C in a hot air oven for 12hr. 25g of demineralised shell was deproteinised with proteolytic enzyme, commercial papain at the ratio of 1:100 (papain to shell). The buffer used for the papain digestion was 0.05 ML-cystein, pH 8.7 (containing 2mg Na_2 EDTA) at the ratio of 1:20 (shell to buffer). The deproteinisation was carried out in stoppered Erlenmeyer flask, incubated for 72hr at room temperature in a reciprocating shaker with 120 agitation/min. Further removal of residual protein in shrimp shell was achieved by treating with 3.5% sodium hydroxide at the ratio of 1(shell) : 10(alkali) at 65°C for 2h (Hall and Reid 1995). A control sample without addition

of enzyme was also subjected to deproteinisation. The bleaching of chitin and further deacetylation to chitosan were carried out as mentioned in chemical method.

Moisture, ash and calcium contents of the sample were determined by AOAC(1995) methods. The protein content of the shell waste was determined by Lowry's method (Lowry *et al.*, 1951). The degree of acetylation was analysed by the method described by Rutherford and Austin (1978) and degree of deacetylation by Muzzarelli and Rachetti (1985). Analysis of variance (ANOVA) technique was followed (Snedecor and Cochran, 1962) to find out whether significant differences exist or not among samples in relation to physical and biochemical characteristics.

Viscosity of chitosan was measured using Haake viscometer. 2 g of chitosan was dissolved in 200ml of 1% acetic acid, placed under bladed propeller for 15 min. until complete dissolution of chitosan and viscosity measured. Solubility of chitosan was determined by dissolving 1g of chitosan in 100 ml of 1% acetic acid.

Results and discussion

The commercial grade enzyme papain was tested for its efficiency in deprotenising the demineralised shrimp shell waste for the production of chitin. Changes in moisture and protein content of the enzyme treated sample are presented in Table 1. There was very little variation in the moisture content of shrimp shell waste during deproteinisation process for 72hr. Steady reduction in protein content was found with increase in digestion period. Enzyme treatment efficiently deproteinised the sample from an initial protein contents of 44.78% to 11.95% after 72hr. Gagne and Simpson (1993) have reported that the residual protein level in the shell waste after papain digestion was 2.8%. However, the residual protein recorded in the present study was quite high (11.95%). Variation in the level of deproteinisation depends on experimental conditions viz., temperature, pH, shell to enzyme ratio, initial protein content of shell waste etc. (Gagne and Simpson, 1993). The shrimp shell waste subjected to chemical method

Table 1. *Changes in moisture and protein contents (DWB) during enzymatic digestion of shell waste*

	Enzyme treated			Control		
Duration(h)	Moisture % in shell	Protein % in shell	Protein % in liquid	Moisture % in shell	Protein % in shell	Protein % in liquid
0	77.20 ± 0.0	44.78 ± 0.2	0.65 ± 0.02	76.95 ± 0.2	43.30 ± 0.3	0.38 ± 0.02
12	77.50 ± 0.4	39.91 ± 0.3	0.91 ± 0.01	ND	ND	ND
24	76.82 ± 0.2	32.93 ± 0.3	1.25 ± 0.03	76.98 ± 0.4	41.36 ± 0.6	0.56 ± 0.3
36	79.96 ± 0.3	26.16 ± 0.2	1.59 ± 0.3	ND	ND	ND
48	76.82 ± 0.6	20.43 ± 0.2	1.88 ± 0.4	77.21 ± 0.5	41.16 ± 0.2	0.57 ± 0.4
60	77.24 ± 0.7	17.28 ± 0.1	2.04 ± 0.3	ND	ND	ND
72	77.29 ± 0.3	11.95 ± 0.1	2.24 ± 0.6	77.52 ± 0.3	40.26 ± 0.4	0.61 ± 0.2

ND-Not done; DWD-Dry weight basis

showed 98% deprotei-nisation whereas in enzymatic method, the deproteinisation was 73.13% and it was only 7% with that of the control sample.

Release of protein from shell waste was reflected in the protein content of liquid in which it increased from 0.65% to 2.24% whereas in the case of control sample (without enzyme addition) reduction in protein content of shell waste was found to be comparatively very less. Protein content decreased from 43.30% to 40.26%. Only a slight increase in protein content was recorded in liquid unlike enzyme treated sample. The raise in liquid protein of control sample (or) decrease in protein content of shell, although very less, could be attributed to the action of proteases of bacterial flora associated with shell waste.

The quality characteristics of chitin prepared by chemical method and enzymatic digestion in combination with chemical treatment are presented in Table 2. The quality of chitin is ascertained based on the standard specification with regard to level of ash content (max.1%) moisture (max. 10%) and color (off white) of the final product (Gopakumar, 1997). In addition, the degree of acetylation, and nitrogen content are expected to be above 20% and below 7% respectively in pure chitin from shrimp source (Rutherford and Austin, 1978). In the present study, the moisture content of chitin produced by both the processes conformed well with the standard. As for ash content, chitin did not conform with the standard. The degree of acetylation was 19.37% in enzymatic method which is almost close to the expected level of 20% whereas in chitin prepared by chemical method it was only 17.2%. The nitrogen contents were 5.08% in both the processes which was well within the acceptable range.

These parameters have been reported to vary with source material (Rutherford and Austin, 1978). The color of chitin from enzymatic and chemical methods was white as required. Various quality characteristics of chitosan produced by both processes are given in Table 3. In addition to the quality parameters meant for chitin, the quality of chitosan is assessed by viscosity, degree of deacetylation and solubility in 1% acetic acid (Filar and Wirick, 1978 and Gopakumar, 1997).

Chitosan prepared from chitin obtained by enzymatic digestion in combination with chemical treatment contained 7.8% moisture, 1% ash, 0.5% calcium and 7.23% nitrogen whereas that of chemical method, the moisture content was 7.54%, ash 0.4%, calcium 0.1% and nitrogen 6.74%. Colour of the product was white

Table 2. *Quality characteristics of chitin prepared by chemical and enzymatic method*

Methods	Moisture (%)	Ash (%)	Calcium (%)	Nitrogen (%)	Degree of acetylation (%)	Color
Chemical Method	6.45	2.40	0.72	5.08	17.20	White
Enzymatic Method	6.45	1.40	0.40	5.08	19.37	White

Table 3. *Quality characteristics of chitosan prepared from chitin processed by chemical and enzymatic method*

Methods	Moisture %	Ash (%)	Calcium (%)	Solubility	Viscosity (CPS)	Degree of deacetylation (%)	Nitrogen (%)	Colour
Chemical Method	7.54	0.40	0.10	CS	110	86.0	6.74	Off White
Enzymatic Method	7.86	1.00	0.30	CS	130	95.2	6.98	Off white

Note : cs - Completely soluble
cps - Centipoise

in both the cases. Both the chitosan were completely soluble in 1% dilute acetic acid and solution was clear. Notable differences were found between the products with respect to viscosity and degree of deacetylation. Viscosity was 130 centipoise (cps) in the chitosan of enzymatic method, whereas it was 110 cps in chemical method, both falling under the medium viscosity category (100-250 cps) suggested by Filar and Wirick (1978). Degree of deacetylation was higher (95.2%) in former and lower (86%) in the later. It suggests that enzymatic digestion conditions the shells for better decetylation process also.

On overall comparison, it was found that chitin and chitosan prepared from enzymatic method in combination with chemical treatment were of better quality. Further, the present study suggests that the less expensive commercial grade papain available in the market could well be used in deproteinisation process of shrimp shell waste in order to obtain chitin and chitosan of superior quality, and the process could reduce the use of strong chemicals and thus eco-friendly.

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