A New Process for Deproteinzation of Chitin from Shrimp Head Waste
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A New Process for Deproteinization of Chitin from Shrimp Head Waste

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Abstract

Shrimp (*P.semisulcatus*) head waste is an excellent source of valuable nutrients, containing on the dry basis of the offals about 6% chitin and 66% protein. Chitin is linked to proteins by glycosidic bonds and preparation of chitin necessitates deproteinization process which is commonly accomplished by using alkali digestion. It is known that severe alkali treatment results in degradation of chitin polymer chains and also reduces the quality of the protein extracts. Application of sodium sulfite, Alcalase (a commercial proteinase), Triton X-100 and combination of these reagents was investigated and the effect of each treatment on the quality of chitin was evaluated. The highest protein recovery (64%) was obtained from shrimp head waste when sodium sulfite (200mmol/l) and Alcalase (0.5%) were used. The remaining chitin could be further purified by a mild alkali digestion (0.5% NaOH, 60°C, 0.5hr). The chitin produced by this two-stage process had a good quality, based on its degree of acetylation and content of residual protein.

Keywords: shrimp-head waste, chitin, alkali digestion, deproteinization, Alcalase

1. Introduction

Crustacean wastes are one of the main sources for production of value – added products such as chitin and chitosan (Knorr, 1991; Shahidi, 1991). Chitin is the second most abundant biopolymer on earth. It's annual production in aquatic systems alone is estimated to be 10^6–10^8 tons (Cauchi, 2002).It is a high molecular weight polysaccharide, composed primarily of N- acetylglucosamine units linked to proteins by strong covalent glycosidic bonds (Brine, 1991; Roberts ,1992; Chang,1997).

The main commercial process for chitin extraction from shrimp waste is based upon demineralisation by acid treatment and deproteinization by alkali treatment .In recent years several enzymatic deproteinization processes have been introduced as an alternative treatment for alkali digestion to reduce quality loss of chitin due to depolymerization of it's chain and also produce protein hydrolysate with a well balanced amino acid composition (Gagne, 1993; Synowiecki, 2000; Gildberg, 2001). Microbial proteolytic enzymes and lactic acid fermentation, which were more time consuming treatments, were also applied for chitin production (Shimahara, 1984; Cira, 2002). The main objective of the present research was to use chemical agents such as sodium sulfite and Triton X-100 along with proteolytic enzyme (i.e. Alcalase) for improving protein recovery efficiency from shrimp head waste and investigating the characteristics of produced chitin.

2. Materials & Methods

Alcalase ® food grade (2.4L, *Subtilisin carlsberg*) was provided by Novo Nordisk A/S (Bagsvaerd, Denmark). All other reagents used were of high analytical grade.

Preparation of raw material

Shrimp (*P. semisulcatus*), supplied by Bushehr Fisheries (Bushehr, Iran), were subjected to manual head and carapace separation. Shrimp heads were ground in a National meat grinder (MK-G5NS, Japan) and the resulting smooth paste was used as raw material (SHW). The paste was wrapped in metallized polyethylene packaging material and kept at -20°C for use in further experiments.

Chitin Extraction Procedure

In this research chitin was produced by a two- stage method (Fig 1). At first, different chemical/ enzymatic treatments were used as primary deproteinization process from SHW. Three factors, each at two levels, were chosen as follows: Alcalase (0, 12) AU/Kg, i.e. (0, 0.5% v/w), Triton X-100 ((0, 0.01) g/Kg), sodium sulfite ((0,200) mmol/L). The effect of eight different treatments combinations derived from a (2^3) factorial experiment (using the above mentioned three factors) on the efficiency of deproteinization was compared (Mizani, 2005).The remaining precipitate from the most efficient treatment was further purified by a mild alkali process (60°C,0.5hr) at a ratio of solids to solution of 1:10 (w/v), as the secondary stage of deproteinization. The NaOH concentration varied from 0 to 2%. Optimal concentration was selected as the one giving minimum amount of residual protein and maximum degree of acetylation (DA) in produced chitin.

Chemical Analysis

Chemical analyses of SHW and protein extracts were accomplished by standard methods (Mizani, 2005). The percentage of chitin present in SHW was determined according to the method of Shahidi & Synowiecki (1991).The nitrogenous composition of the precipitate from the first stage was analyzed. Total nitrogen was measured by the Kjeldahl method and residual protein was determined by the Lowry method (Lowry, 1951), using crystalline bovine albumin as standard protein. The results were used to determine chitin nitrogen according to the following formula:

Chitin nitrogen = (total nitrogen – protein nitrogen)= (total nitrogen- (residual protein/6.25))

The quality of chitin produced after the second stage was evaluated and compared with the commercial food grade chitin (Subasinghe, 1999). Moisture and ash were determined according to AOAC method (AOAC, 1980) and the degree of acetylation, as an important structural factor influencing the properties of the chitin was measured from the infrared spectrum recorded on the Michelson series FT-IR spectrometer (Sannan, 1978; Roberts, 1992; Shigemassa, 1996).

3. Results & Discussion

Chemical composition of SHW is given in Table 1 .Eight different deproteinzation processes and the amount of protein recovery obtained by each treatment is summarized in Table 2.The highest protein recovery was obtained by application of both sodium sulfite and Alcalase. The remained precipitate which was rich in chitin , was analyzed to determine it's nitogenous composition(Table 3).According to the findings of Synowiecki & et.al(2000), the enzymatic deproteinzation process has limited value due to residual small peptides directly attached to chitin molecules ranging from 4.4% to 7.9% of total weight. However using chemical agents in combination with proteolytic enzyme in the present research, significantly reduced proteinaceous fraction of precipitate (~3%).It seems that the proteolytic activity of the enzyme makes disulfide bonds of SHW protein more accessible for sodium sulfite. The precipitate obtained from the first stage (A+S) was treated by different alkaline processes. The results showed that increasing NaOH concentration above 0.5%, did not significantly change the protein content and DA of chitin (Table 4).Therefore the mild alkaline treatment (0.5% NaOH, 60°C, 0.5 hr, 1/10 (w/v)) may be sufficient for further protein recovery from the precipitate.Application of lower concentration of alkaline solution in comparison with commercial processes (2-10%) may be considered as an advantage from the economical as well as environmental aspects.It is well known that prolonged alkaline treatments under severe conditions causes depolymerization and deacetylation of chitin(No,1997). According to the results of Table 4 , as the percentage of alkali solution increased, the degree of acetylation of produced chitin decreased . Although these changes were insignificant but it may be reasonably concluded that using higher amounts of alkali (more than 0.5%) is not beneficial to further protein recovery and improving chitin quality. The final characteristics of produced chitin as compared with the commercial food grade product , confirmed it's good quality (Table 5).As it is shown the degree of acetylation of produced chitin was in the acceptable range but it is lower than the amounts reported by other researches (Synowiecki,2000). This difference may be related to the source of the chitin which remarkably influence this factor. Shrimp shell waste, usually used as the main source of chitin, has lower amounts of protein (~40% as compared with 65% in SHW) (Shahidi,1991;Synowiecki, 2000) and more percentage of chitin may be free or loosely bounded to protein . On the other hand the head waste is rich source of protein , and chitin may be strongly linked to protein. Therefore it may be expected that the structure of chitin in the above mentioned sources is slightly different and this differnce may result in different initial degree of acetylation(Blackwell,1984).
This research showed that by using chemical/enzymatic procedures , it may be possible to break the strong covalent bounds in chitin / protein complex more

efficiently in order to facilitate producing chitin with standard quality as a valuable by- product from shrimp head waste processing.

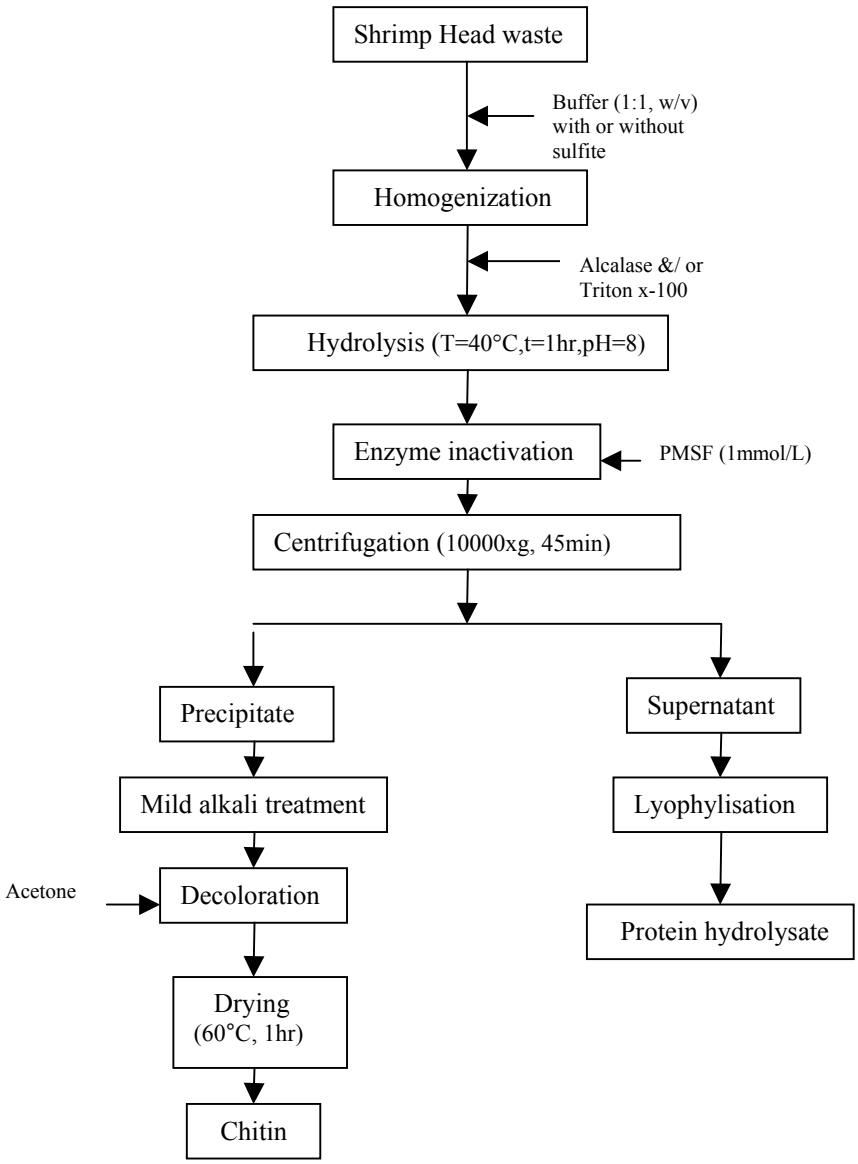

Figure1: The two- stage method for chitin production

Table 1: Chemical Composition of SHW[1]

Component (%)	SHW
Moisture	80.544±0.294
Total nitrogen[2]	10.825±0.443
Crude protein[2]	65.763±2.776
Chitin[2]	5.625±0.903
Ash[2]	23.156±1.355

[1] Results are mean values of four replicates ±S.D.
[2] Dry weight basis,dwb.

Table 2: Protein Recovery (%) Obtained by Using Different Deproteinization Processes[1,2]

Treatment	Protein Recovery (%)
N	37.0
S	48.7
T	38.5
A	45.7
S+T	53.5
S+A	64.3
T+A	39.0
S+T+A	63.2

[1] N=no treatment,T= Triton X-100(0.01 g/kg), S= Sodium Sulfite (200mmol/l), A= Alcalase(12AU/kg, i.e. 0.5% v/w)
[2] The protein recovery is calculated as the percentage of the total protein content of SHW.

Table3: Nitrogenous composition of Precipitate after Deproteinization Processes[1]

Composition (%)	Precipitate
Total nitrogen	6.364±0.101
Chitin nitrogen	5.877±0.110
Protein nitrogen	0.486±0.044
Residual protein	3.042±0.276

[1] Results are mean values of four replicates ±S.D.

Table 4: Effect of NaOH Concentration on the Residual Protein Content and Degree of Acetylation of Chitin Produced by the Two-Stage Method [(1,2)]

NaOH Concentration (%)	0	0.5	1	1.5	2
Residual Protein (%)	3.042±0.276[a]	0.870±0.054[b]	0.865±0.021[b]	0.858±0.007[b]	0.860±0.035[b]
Degree of Acetylation(%)	--	77.670[a]	77.560[a]	76.925[a]	76.830[a]

[1] All of the treatments were accomplished at 60°C with in 0.5 hr.
[2] Values with different superscript letter are significantly different.

Table 5: Specification of Produced Chitin Compared to Food Grade Product[1]

Specification	Chitin	Food Grade
Appearance	white/yellow	white/yellow
Moisture(%)	4.300	<10
Residual Protein(%)	0.870	<1
DA (%) [2]	77.670	70-100
Ash(%)	1.040	<2.5

[1] Commercial food grade chitin (Subasinghe, 1999)
[2] DA was calculated by the following equation(Roberts, 1992; Shigemassa, 1996): $[(A_{1650}/A_{3450}) + (A_{1630}/A_{3450})-0.13]$ x85.5

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